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Nanotoxicology of Pharmaceutical Nanocarriers: Molecular Mechanisms, Safety Assessment, Regulatory Challenges and Future Perspectives: A Comprehensive Review

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ABSTRACT

Pharmaceutical nanocarriers have revolutionized drug delivery by enhancing drug solubility, improving bioavailability, enabling targeted delivery and reducing systemic toxicity. Nanocarrier platforms, including liposomes, polymeric nanoparticles, solid lipid nanoparticles, nanostructured lipid carriers, dendrimers, metallic nanoparticles and other nanoscale delivery systems, have demonstrated considerable therapeutic potential across a broad spectrum of diseases, including cancer, infectious, cardiovascular, neurological and inflammatory disorders. Despite these advances, increasing evidence indicates that the unique physicochemical characteristics of nanocarriers, including particle size, morphology, surface charge, composition and surface functionalization, may trigger unintended biological interactions and adverse toxicological responses. Consequently, comprehensive nanotoxicological evaluation has become a fundamental prerequisite for the safe development, regulatory approval and clinical translation of nanomedicines. This review critically examines the molecular mechanisms underlying nanocarrier-induced toxicity, summarizes current *in vitro*, *in vivo* and emerging alternative approaches for nanotoxicity assessment, evaluates the influence of physicochemical properties on biological safety and discusses current regulatory frameworks, existing challenges and future perspectives for the safe and sustainable development of pharmaceutical nanocarriers. A comprehensive literature review was conducted using peer-reviewed publications retrieved from PubMed, Scopus, Web of Science, Embase and Google Scholar. Relevant studies published primarily between 2010 and 2026 were identified using predefined search terms related to nanotoxicology, pharmaceutical nanocarriers, drug delivery systems, safety assessment, regulatory toxicology, oxidative stress, immunotoxicity and nanomedicine. Original research articles, systematic reviews, meta-analyses, regulatory guidance documents and authoritative reports were critically appraised and synthesized to provide a comprehensive overview of current knowledge. Current evidence indicates that nanotoxicity results from complex interactions between nanocarrier physicochemical properties and biological systems. Oxidative stress, excessive reactive oxygen species generation, mitochondrial dysfunction, lysosomal destabilization, DNA damage, inflammation, apoptosis, autophagy, ferroptosis and immune dysregulation have been identified as the principal mechanisms underlying nanocarrier-induced toxicity. These molecular events may lead to organ-specific adverse effects involving the liver, kidneys, lungs, cardiovascular system, nervous system and reproductive organs, with their severity influenced by nanocarrier composition, particle characteristics, dose, exposure duration and route of administration. Emerging technologies, including three-dimensional cell culture models, organ-on-a-chip platforms, high-content imaging, multi-omics approaches and artificial intelligence-driven predictive toxicology, are enhancing the mechanistic understanding, predictive accuracy and translational relevance of nanotoxicity assessment. Nevertheless, significant challenges remain, including the lack of standardized testing methodologies, limited interlaboratory reproducibility, insufficient long-term safety data and incomplete global regulatory harmonization. Pharmaceutical nanocarriers represent a cornerstone of precision medicine; however, their successful clinical translation requires rigorous, standardized and mechanism-based safety evaluation. Integrating mechanistic nanotoxicology, advanced predictive technologies, safe-by-design principles and internationally harmonized regulatory frameworks will be critical for maximizing therapeutic efficacy while minimizing potential risks. Continued interdisciplinary collaboration, technological innovation and evidence-based regulatory development are expected to accelerate the safe and sustainable advancement of next-generation nanomedicines.

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INTRODUCTION

Nanotechnology has emerged as one of the most transformative innovations in pharmaceutical sciences, fundamentally reshaping the design and development of advanced drug delivery systems. Through the engineering of materials at the nanoscale, researchers have developed sophisticated carrier systems capable of enhancing drug solubility, improving bioavailability, prolonging systemic circulation, enabling controlled drug release and facilitating targeted delivery to specific tissues and cells. These advancements have significantly enhanced therapeutic efficacy while reducing systemic toxicity, establishing nanomedicine as a cornerstone of modern precision medicine[1,2].

Pharmaceutical nanocarriers generally range in size from 1 to 100 nm, although several clinically approved nanomedicines exceed this size range depending on their composition, structural characteristics and intended therapeutic application. Owing to their unique physicochemical properties and versatile functionality, nanocarrier-based drug delivery systems have become indispensable for the treatment of a broad spectrum of diseases, including cancer, infectious diseases, cardiovascular disorders, neurological diseases, autoimmune disorders and rare genetic diseases[1,3].

A diverse range of pharmaceutical nanocarriers has been developed for therapeutic applications, including liposomes, polymeric nanoparticles, polymeric micelles, dendrimers, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), metallic nanoparticles, mesoporous silica nanoparticles and carbon-based nanomaterials. Each nanocarrier platform possesses distinct physicochemical characteristics that determine its suitability for specific therapeutic applications. Liposomes remain among the most extensively investigated and clinically successful nanocarriers because of their excellent biocompatibility and their capacity to encapsulate both hydrophilic and hydrophobic therapeutic agents. Polymeric nanoparticles provide sustained and controlled drug release, whereas lipid-based nanocarriers enhance the oral bioavailability of poorly water-soluble drugs. Metallic nanoparticles have attracted considerable attention owing to their unique optical, diagnostic, imaging, antimicrobial and photothermal properties, while dendrimers and mesoporous silica nanoparticles offer highly tunable architectures that enable multifunctional drug delivery, targeted therapy and combination therapeutic strategies[1,2].

The clinical translation of nanomedicine has advanced rapidly over the past decade, resulting in the regulatory approval of several nanoparticle-based therapeutics for clinical use. Notable examples include liposomal doxorubicin, albumin-bound paclitaxel, liposomal amphotericin B and lipid nanoparticle-based nucleic acid delivery systems. More recently, lipid nanoparticles gained global recognition through their pivotal role in the successful delivery of messenger RNA (mRNA) vaccines against coronavirus disease 2019 (COVID-19), demonstrating the feasibility of safely and efficiently delivering fragile nucleic acids into human cells. This breakthrough has accelerated the development of nanoparticle-based platforms for vaccines, gene-editing technologies, small interfering RNA (siRNA), mRNA therapeutics and precision oncology, underscoring the expanding role of pharmaceutical nanocarriers in modern healthcare[1,2].

Despite their considerable therapeutic potential, engineered nanomaterials exhibit biological behaviors that differ fundamentally from those of conventional pharmaceutical formulations. Their unique physicochemical properties, including particle size, morphology, surface-area-to-volume ratio, surface charge, crystallinity, hydrophobicity and surface functionalization, collectively govern their interactions with proteins, biological membranes, intracellular organelles and components of the immune system. Upon exposure to biological fluids following systemic administration, nanoparticles rapidly adsorb plasma proteins and other biomolecules, forming a dynamic protein corona that redefines their biological identity. The formation of this protein corona profoundly influences nanoparticle biodistribution, pharmacokinetics, cellular uptake, immune recognition and clearance. Moreover, its composition is determined by both nanoparticle characteristics and the surrounding biological environment, thereby exerting a significant influence on therapeutic efficacy and toxicological outcomes[3-5].

The increasing complexity of nanoparticle, biological interactions has driven the emergence of nanotoxicology, a multidisciplinary field focused on elucidating the potential adverse effects of engineered nanomaterials on biological systems. In contrast to conventional toxicology, which primarily evaluates toxicity based on chemical composition, nanotoxicology emphasizes the critical role of physicochemical characteristics in determining biological responses. Consequently, comprehensive physicochemical characterization has become a fundamental prerequisite for meaningful toxicological assessment, safety evaluation and the rational design of pharmaceutical nanocarriers[3,4].

Figure 1 provides an overview of pharmaceutical nanocarriers used in drug delivery systems. As pharmaceutical nanotechnology advances toward increasingly sophisticated multifunctional drug delivery systems, ensuring their biological safety has become a critical scientific and regulatory priority. A comprehensive understanding of nanoparticle interactions at the nano-bio interface is therefore essential for elucidating their biological fate, predicting potential toxicological responses and optimizing their safety profiles. Such knowledge is

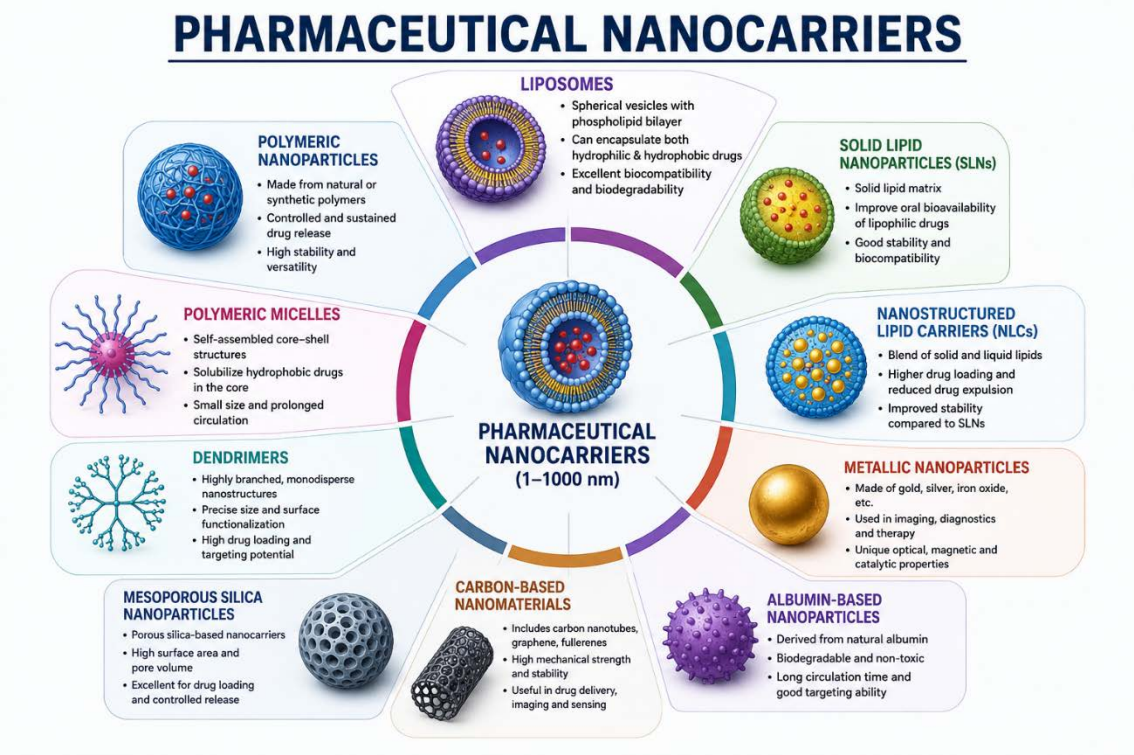

Fig. 1: Overview of pharmaceutical nanocarriers used in drug delivery systems

fundamental to balancing therapeutic innovation with patient safety and to facilitating the successful clinical translation and widespread clinical adoption of next-generation nanomedicines[3,4].

Literature search strategy: A comprehensive literature search was conducted to identify and critically evaluate studies related to the nanotoxicology of pharmaceutical nanocarriers. Electronic databases, including PubMed, Scopus, Web of Science, Embase and Google Scholar, were systematically searched for articles published between January 2010 and June 2026. The search strategy combined Medical Subject Headings (MeSH) with free-text keywords, including "nanotoxicology," "pharmaceutical nanocarriers," "nanomedicine," "drug delivery systems," "liposomes," "polymeric nanoparticles," "solid lipid nanoparticles," "nanostructured lipid carriers," "dendrimers," "metallic nanoparticles," "safety assessment," "oxidative stress," "genotoxicity," "immunotoxicity," "regulatory toxicology," and "nanoparticle toxicity." Boolean operators (AND, OR) were applied to refine the search strategy and maximize the retrieval of relevant publications. In addition, the reference lists of eligible articles were manually screened to identify pertinent studies that were not captured through the electronic database search[6,7].

Eligibility criteria: Studies were considered eligible if they met one or more of the following criteria: (i) Investigated the toxicological profile of pharmaceutical nanocarriers, (ii) Evaluated molecular, cellular, organ-specific, or systemic toxicological effects associated with nanocarrier exposure, (iii) Reported evidence from *in vitro*, *in vivo*, *ex vivo*, *in silico*, or clinical studies related to nanotoxicity, (iv) Addressed safety assessment strategies or regulatory frameworks governing nanomedicines, or (v) Comprised original research articles, systematic reviews, meta-analyses, consensus statements, or official regulatory guidance documents published in English. Preference was given to peer-reviewed publications from the previous decade to ensure that the review reflected current scientific knowledge. However, seminal studies that established foundational concepts in nanomedicine and nanotoxicology were also included to provide historical context and scientific perspective.

Studies were excluded if they focused on environmental or industrial nanomaterials unrelated to pharmaceutical applications, conference abstracts without accessible full-text articles, duplicate publications, editorials lacking original scientific evidence, non-English publications, or studies with insufficient methodological detail. Publications describing nanomaterial synthesis without toxicological evaluation or biological safety assessment were likewise excluded.

Data extraction and evidence synthesis: Relevant data were independently extracted from all eligible publications, including nanocarrier type, physicochemical characteristics, nano-bio interactions, molecular mechanisms of toxicity,

experimental models, toxicological endpoints, safety assessment methodologies, regulatory considerations and principal findings. The extracted evidence was critically appraised and synthesized using a qualitative narrative approach to integrate current knowledge, identify emerging trends and existing knowledge gaps and provide a comprehensive overview of recent advances in the nanotoxicology and safety evaluation of pharmaceutical nanocarriers.

Classification of pharmaceutical nanocarriers: The rapid evolution of nanotechnology has led to the development of a diverse array of pharmaceutical nanocarriers designed to enhance therapeutic efficacy while reducing adverse drug reactions. Pharmaceutical nanocarriers are nanoscale drug delivery systems engineered to improve drug solubility, stability, pharmacokinetic and pharmacodynamic profiles, facilitate controlled and targeted drug release and enhance the selective delivery of therapeutic agents to specific tissues or cells[1,2]. Based on their composition, structural architecture and intended biomedical application, pharmaceutical nanocarriers can be broadly classified into lipid-based, polymer-based, inorganic, carbon-based, protein-based and hybrid nanocarrier systems.

The biological performance of nanocarriers is governed by their physicochemical characteristics, including particle size, morphology, surface charge, hydrophobicity, porosity, biodegradability and surface functionalization. These properties collectively influence drug-loading efficiency, colloidal stability, biodistribution, cellular uptake, intracellular trafficking, circulation time, biodegradation and toxicological behavior[3,8]. Therefore, a comprehensive understanding of the structural and physicochemical characteristics of each nanocarrier platform is essential for optimizing therapeutic efficacy while minimizing potential toxicological risks.

Liposomes: Liposomes are among the earliest developed and most extensively investigated nanocarrier systems for pharmaceutical drug delivery. They are spherical vesicular structures composed of one or more phospholipid bilayers enclosing an aqueous core, enabling the simultaneous encapsulation of both hydrophilic and hydrophobic therapeutic agents. Hydrophilic drugs are predominantly entrapped within the aqueous core, whereas hydrophobic or lipophilic compounds are incorporated into the phospholipid bilayer, making liposomes highly versatile and adaptable drug delivery vehicles[9,10].

The unique structural organization of liposomes confers several important pharmaceutical advantages, including enhanced drug solubility, improved pharmacokinetic profiles, prolonged systemic circulation, reduced systemic toxicity and targeted drug delivery. Surface modification with polyethylene glycol (PEG), commonly referred to as PEGylation, imparts "stealth" properties by reducing plasma protein adsorption (opsonization) and subsequent recognition and clearance by the Mononuclear Phagocyte System (MPS), thereby significantly prolonging circulation half-life. In addition, surface conjugation with antibodies, peptides, aptamers, or other targeting ligands enables active targeting of specific cells or diseased tissues, thereby enhancing therapeutic efficacy while minimizing off-target drug exposure and associated toxicity[10,11].

Liposomal drug delivery systems have achieved substantial clinical success and are widely used in oncology, antifungal therapy, pain management and vaccine delivery. Several liposomal formulations have received regulatory approval, including liposomal doxorubicin (Doxil®), liposomal daunorubicin (DaunoXome®), liposomal amphotericin B (AmBisome®), liposomal irinotecan (Onivyde®) and liposomal cytarabine (DepoCyt®). More recently, lipid nanoparticle technology has gained global prominence through its successful application in the delivery of messenger RNA (mRNA) vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), underscoring the broad clinical applicability of lipid-based nanocarriers beyond conventional small-molecule therapeutics[10,12].

Despite their favorable biocompatibility and well-established clinical utility, liposomal formulations are not entirely free from toxicological concerns. Certain liposomal products have been associated with complement activation-related pseudoallergy (CARPA), an acute hypersensitivity syndrome characterized by flushing, dyspnea, cardiopulmonary disturbances and hemodynamic alterations. These adverse reactions are primarily attributed to activation of the complement system following interactions between the liposomal surface and circulating immune components, which subsequently initiate inflammatory and immune-mediated responses[13,14].

The biological safety of liposomes is strongly influenced by their physicochemical characteristics, including particle size, lipid composition, membrane rigidity, surface charge, cholesterol content, polyethylene glycol (PEG) density and manufacturing process. Among these factors, surface charge plays a particularly important role in determining cellular interactions and toxicity. Cationic liposomes generally exhibit greater cellular uptake than neutral or anionic formulations because of their enhanced electrostatic interactions with negatively charged cell membranes. However, this increased cellular internalization is frequently accompanied by greater cytotoxicity, resulting from membrane destabilization, mitochondrial dysfunction, excessive reactive oxygen species (ROS) generation, inflammatory cytokine release and activation of apoptotic signaling pathways. In contrast, neutral liposomes typically exhibit superior biocompatibility, lower immunogenicity and improved

safety profiles, making them more suitable for repeated or long-term administration[11,15]. Consequently, comprehensive preclinical evaluation of biodistribution, pharmacokinetics, immunogenicity, hemocompatibility and both acute and chronic toxicity remains essential for the safe development and clinical translation of liposomal drug delivery systems[10,16].

Clinically approved liposomal formulations and their therapeutic applications are presented in Table 1. Recent advances in liposome engineering have focused on enhancing both therapeutic efficacy and biological safety through the development of stimuli-responsive, ligand-targeted, stealth and biomimetic liposomal platforms. Surface functionalization with polyethylene glycol, peptides, antibodies, aptamers and cell membrane-derived coatings has significantly improved target specificity, prolonged systemic circulation, reduced nonspecific interactions and minimized immune recognition, thereby expanding the therapeutic potential of next-generation liposomal nanocarriers[12,16].

Polymeric nanoparticles: Table 2 shows the common polymers used in pharmaceutical nanoparticles. Polymeric nanoparticles (PNPs) are among the most extensively studied nanocarriers because of their excellent biocompatibility, biodegradability, structural versatility and capacity to enable controlled and targeted drug delivery. These nanoscale delivery systems are typically fabricated from natural polymers, including chitosan, gelatin, alginate, dextran and albumin, as well as synthetic biodegradable polymers such as poly (lactic acid) (PLA), poly (glycolic acid) (PGA), poly (lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL) and polyethylene glycol (PEG). Based on their structural architecture, polymeric nanoparticles are broadly classified into nanospheres, in which the therapeutic agent is uniformly dispersed throughout the polymeric matrix and nanocapsules, where the drug is encapsulated within a polymeric shell surrounding a liquid or solid core[17,18].

The growing interest in polymeric nanoparticles is largely attributed to their ability to overcome many limitations of conventional drug delivery systems, including poor drug stability, limited bioavailability and nonspecific distribution. Furthermore, surface functionalization with polyethylene glycol (PEG), antibodies, peptides, folic acid, aptamers and other targeting ligands enhances tissue-specific drug accumulation while reducing nonspecific interactions, immune recognition and rapid systemic clearance[17,19].

Polymeric nanoparticles have demonstrated substantial therapeutic potential across a broad spectrum of biomedical applications, including cancer chemotherapy, antimicrobial therapy, vaccine delivery, gene therapy, ocular and pulmonary drug delivery and the treatment of neurological disorders. Among biodegradable polymeric systems, PLGA remains the most extensively investigated owing to its excellent biocompatibility, predictable hydrolytic degradation into lactic acid and glycolic acid and regulatory approval by both the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA). PLGA-based nanoparticles have been widely employed to enhance drug stability, prolong circulation time, reduce dosing frequency and improve intracellular drug delivery, thereby maximizing therapeutic efficacy while minimizing systemic toxicity[17,20].

Despite these significant pharmaceutical advantages, polymeric nanoparticles may also elicit toxicological responses that depend on their physicochemical properties, polymer composition, degradation products and route of administration. Critical parameters, including nanoparticle size, morphology, molecular weight, crystallinity, surface hydrophobicity and surface charge, profoundly influence cellular uptake, intracellular trafficking, biodistribution and biological clearance. Smaller nanoparticles (<100 nm) generally exhibit enhanced cellular internalization and deeper tissue penetration; however, they may also result in greater intracellular accumulation and increased oxidative

Table 1: Clinically approved liposomal formulations and their therapeutic applications

Liposomal product	Active drug	Indication	Therapeutic advantage
Doxil®	Doxorubicin	Ovarian cancer, Kaposi's sarcoma	Reduced cardiotoxicity and prolonged circulation
AmBisome®	Amphotericin B	Systemic fungal infections	Reduced nephrotoxicity
DaunoXome®	Daunorubicin	Kaposi's sarcoma	Improved tumor targeting
Onivyde®	Irinotecan	Metastatic pancreatic cancer	Enhanced drug stability and efficacy
DepoCyt®	Cytarabine	Lymphomatous meningitis	Sustained intrathecal drug release

Table 2: Common polymers used in pharmaceutical nanoparticles

Polymer	Type	Biodegradability	Major pharmaceutical applications	Toxicological considerations
PLGA	Synthetic	Excellent	Cancer therapy, vaccines	Local acidic degradation products
PLA	Synthetic	Excellent	Controlled drug delivery	Slow degradation
PCL	Synthetic	Good	Long-acting formulations	Prolonged tissue retention
Chitosan	Natural	Excellent	Mucosal and gene delivery	Low toxicity; cationic charge may increase cytotoxicity
Gelatin	Natural	Excellent	Protein and peptide delivery	Excellent biocompatibility
Alginate	Natural	Excellent	Oral and wound delivery	Minimal toxicity
Albumin	Natural	Excellent	Anticancer drug delivery	Highly biocompatible

stress than larger particles. Likewise, positively charged polymeric nanoparticles interact strongly with negatively charged cellular membranes, facilitating cellular uptake while simultaneously increasing the risk of membrane disruption, mitochondrial dysfunction and cytotoxicity[3,8].

Oxidative stress is recognized as one of the primary mechanisms underlying polymeric nanoparticle-induced toxicity. Following cellular internalization through endocytosis or phagocytosis, polymeric nanoparticles can stimulate excessive generation of reactive oxygen species (ROS), resulting in mitochondrial dysfunction, lipid peroxidation, DNA damage, protein oxidation and activation of multiple inflammatory signaling pathways. Elevated ROS levels subsequently activate mitogen-activated protein kinases (MAPKs), nuclear factor-kappa B (NF-κB) and the NLRP3 inflammasome, leading to increased production of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6). Persistent oxidative stress may ultimately induce apoptosis, autophagy, or necrosis, depending on nanoparticle concentration, duration of exposure and the susceptibility of the target cells[21].

The biodegradation products of polymeric nanoparticles also contribute to their overall toxicological profile. For example, PLGA undergoes hydrolytic degradation to produce lactic acid and glycolic acid, both of which are metabolized through normal physiological pathways and are generally regarded as biocompatible. Nevertheless, rapid polymer degradation or administration of high polymer doses may cause localized reductions in tissue pH, potentially triggering inflammatory responses and transient tissue irritation. In addition, residual organic solvents, surfactants, cross-linking agents, or unreacted monomers remaining from the nanoparticle fabrication process may contribute to cytotoxicity if they are not adequately eliminated during manufacturing. Therefore, optimization of formulation and fabrication processes, together with stringent quality control measures, is essential to minimize formulation-related toxicity and ensure the safety of polymeric nanoparticle-based drug delivery systems[17,20].

Solid lipid nanoparticles (SLNs): Solid lipid nanoparticles (SLNs) represent one of the most important advances in lipid-based nanocarrier technology and were developed to address the limitations of conventional colloidal drug delivery systems, including emulsions, liposomes and polymeric nanoparticles. SLNs are submicron-sized particles, typically ranging from 50 to 1000 nm, composed of physiologically compatible lipids that remain solid at both room and body temperatures. These solid lipids are stabilized by surfactants to form stable colloidal dispersions capable of encapsulating lipophilic and, to a lesser extent, hydrophilic therapeutic agents. Owing to their excellent biocompatibility, biodegradability and ability to enhance drug stability, SLNs have emerged as promising nanocarriers for oral, parenteral, ocular, pulmonary, topical and transdermal drug delivery[22,23].

Table 3 shows the advantages and toxicological considerations of solid lipid nanoparticles. The pharmaceutical advantages of SLNs primarily arise from their solid lipid matrix, which protects encapsulated drugs from chemical degradation, enzymatic metabolism and premature release. The rigid lipid core enables controlled and sustained drug release, thereby improving drug bioavailability, prolonging therapeutic activity and reducing dosing frequency. Furthermore, the use of physiologically acceptable lipids, including glyceryl behenate, stearic acid, tristearin, cetyl palmitate and glyceryl monostearate, minimizes the risk of systemic toxicity compared with many synthetic polymeric carriers. Their small particle size further facilitates penetration across biological barriers and enhances cellular uptake through endocytic pathways, thereby improving therapeutic efficacy[23,24].

Solid lipid nanoparticles have been extensively investigated as delivery vehicles for anticancer agents, antibiotics, antifungal drugs, peptides, proteins, vaccines, nucleic acids and poorly water-soluble drugs. In cancer therapy, SLNs enhance drug accumulation within tumor tissues through the Enhanced Permeability and Retention (EPR) effect while reducing systemic drug exposure and dose-limiting toxicity. Similarly, SLNs have demonstrated significant potential for brain-targeted drug delivery by facilitating transport across the blood-brain barrier and protecting neurotherapeutic agents from enzymatic degradation. Their ability to encapsulate chemically unstable biomolecules has also expanded their application in gene therapy and vaccine delivery systems[24,25]. In addition, positively charged SLNs exhibit stronger interactions with negatively charged cellular membranes, promoting enhanced cellular uptake and drug delivery; however, this increased interaction may also elevate the risk of membrane disruption and cytotoxicity[22,25].

Table 3: Advantages and toxicological considerations of solid lipid nanoparticles

Advantages	Potential toxicological concerns
Excellent biocompatibility	ROS generation at high concentrations
Controlled drug release	Surfactant-induced membrane damage
Protection of labile drugs	Lipid accumulation after repeated exposure
Improved bioavailability	Inflammatory responses
Enhanced stability	Drug expulsion during lipid polymorphic transition
Scalable manufacturing	Long-term safety requires further investigation

Despite their therapeutic advantages, SLNs may induce cellular toxicity through several mechanisms. Following cellular internalization, SLNs can stimulate excessive generation of Reactive Oxygen Species (ROS), resulting in oxidative stress, mitochondrial dysfunction, inflammatory responses and lysosomal destabilization. Experimental studies have further demonstrated that high concentrations of surfactants used during SLN formulation, particularly polysorbates and poloxamers, may contribute to membrane destabilization, hemolysis and inflammatory reactions if formulation parameters are not carefully optimized[25,26].

Another important limitation of SLNs is the highly ordered crystalline structure of their lipid matrix, which may undergo polymorphic transitions during storage. These structural changes can reduce drug loading capacity, promote drug expulsion and compromise formulation stability and therapeutic performance. Consequently, comprehensive formulation optimization and rigorous stability evaluation are essential during the development of SLN-based pharmaceutical products[23,24].

To address these limitations, considerable efforts have been directed toward optimizing lipid composition, surfactant selection and manufacturing techniques to improve the physicochemical stability and drug-loading capacity of SLNs. These technological advances have significantly enhanced both the pharmaceutical performance and toxicological safety of SLNs, supporting their continued clinical development as versatile nanocarriers for controlled and targeted drug delivery[26].

Nanostructured lipid carriers (NLCs): Nanostructured Lipid Carriers (NLCs) represent the second generation of lipid-based nanocarriers and were developed to overcome the inherent limitations of solid lipid nanoparticles (SLNs), particularly their limited drug-loading capacity, drug expulsion during storage and highly ordered crystalline lipid matrix. In contrast to SLNs, which are composed exclusively of solid lipids, NLCs consist of a combination of solid lipids and liquid lipids (oils) that form an imperfect or partially amorphous lipid matrix. This modified structural architecture increases the available space for drug incorporation, reduces lipid crystallization, minimizes drug leakage during storage and improves formulation stability, making NLCs one of the most promising lipid-based nanocarrier systems for pharmaceutical applications[22,27].

The incorporation of liquid lipids disrupts the highly ordered crystalline lattice characteristic of SLNs, resulting in a less ordered lipid matrix with greater structural flexibility and significantly enhanced drug-loading capacity. This unique architecture enables NLCs to efficiently encapsulate both hydrophilic and lipophilic therapeutic agents while providing sustained and controlled drug release. Moreover, the use of physiologically compatible lipids confers excellent biocompatibility and biodegradability, making NLCs suitable for a wide range of administration routes, including oral, parenteral, ocular, pulmonary, topical, transdermal and intranasal delivery. Their superior physical stability and enhanced encapsulation efficiency have broadened the application of NLCs for the delivery of anticancer agents, antimicrobial drugs, peptides, proteins, vaccines, nucleic acids and phytopharmaceuticals[27,28].

Nanostructured lipid carriers have demonstrated substantial therapeutic advantages by improving the pharmacokinetic and pharmacodynamic profiles of poorly water-soluble drugs. The lipid matrix protects encapsulated therapeutic agents from hydrolytic and enzymatic degradation while promoting intestinal lymphatic transport, thereby bypassing hepatic first-pass metabolism and enhancing systemic bioavailability. Furthermore, surface functionalization with polyethylene glycol (PEG), antibodies, peptides, or cell-penetrating ligands prolongs systemic circulation, reduces nonspecific uptake and facilitates active targeting of diseased tissues. These favorable characteristics have led to extensive investigation of NLCs for targeted chemotherapy, brain-targeted drug delivery, ocular therapy, dermal drug delivery and gene delivery applications[28,29].

Although, nanostructured lipid carriers (NLCs) generally exhibit superior biocompatibility compared with many synthetic nanocarriers, their toxicological profile is influenced by several physicochemical characteristics, including particle size, lipid composition, surface charge, surfactant concentration and manufacturing conditions. Smaller nanoparticles possess a larger surface-area-to-volume ratio, facilitating enhanced cellular uptake and greater intracellular accumulation. Likewise, positively charged NLCs interact more readily with negatively charged biological membranes, improving intracellular drug delivery but also increasing the risk of membrane disruption, oxidative stress and inflammatory responses. Therefore, careful optimization of formulation parameters is essential to maximize therapeutic efficacy while minimizing cytotoxicity[25,29].

Oxidative stress is widely recognized as one of the primary mechanisms underlying NLC-induced toxicity. Following cellular internalization through endocytic pathways, excessive nanoparticle exposure may stimulate the overproduction of reactive oxygen species (ROS), leading to mitochondrial dysfunction, lipid peroxidation, protein oxidation, DNA damage and activation of apoptosis-related signaling pathways. Elevated ROS levels also activate key inflammatory mediators, including nuclear factor-kappa B (NF-κB), mitogen-activated protein kinases (MAPKs) and the NLRP3 inflammasome, resulting in increased secretion of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6). Despite these potential

toxicological effects, numerous experimental studies have demonstrated that appropriately optimized NLC formulations generally exhibit lower cytotoxicity than many polymeric or metallic nanoparticles, primarily because they are composed of biodegradable and physiologically compatible lipid components[25,28].

The surfactants used during NLC formulation also play a critical role in determining their biological safety. Nonionic surfactants, including polysorbate 80, poloxamers, lecithin and phosphatidylcholine, are generally regarded as biocompatible and safe for pharmaceutical applications. However, excessive surfactant concentrations may cause membrane destabilization, hemolysis, complement activation and localized inflammatory responses. Furthermore, impurities originating from lipid raw materials, degradation products, or residual organic solvents introduced during manufacturing may contribute to formulation-related toxicity if not adequately controlled. Consequently, strict adherence to Good Manufacturing Practice (GMP), together with comprehensive quality control and formulation optimization, is essential to ensure the safety, efficacy and batch-to-batch reproducibility of NLC-based pharmaceutical products[29].

Recent advances in nanostructured lipid carrier (NLC) engineering have focused on the development of multifunctional and stimuli-responsive formulations capable of responding to changes in pH, temperature, redox potential, enzymatic activity and external stimuli such as magnetic fields and light. These intelligent delivery systems enable site-specific and controlled drug release, thereby enhancing therapeutic efficacy while minimizing off-target effects and systemic toxicity. In addition, biomimetic NLCs coated with cell membranes or functionalized with naturally derived biomolecules have demonstrated enhanced immune evasion, prolonged systemic circulation, improved tissue-specific targeting and reduced immunogenicity, thereby further improving their safety and therapeutic performance. Table 4 presents the comparison between SLNs and NLCs.

Moreover, the integration of artificial intelligence (AI), computational modeling and Quality-by-Design (QbD) principles has substantially advanced the rational design and optimization of NLC formulations. These approaches facilitate the systematic evaluation of critical formulation and process variables, enabling the prediction and optimization of physicochemical properties, drug-loading efficiency, stability, release kinetics and biological performance. As a result, AI-assisted formulation development and QbD-based optimization have contributed to the design of safer, more effective and highly reproducible lipid nanocarriers. Collectively, these technological innovations are expected to accelerate the clinical translation of NLC-based pharmaceutical products while further enhancing their safety profile and minimizing nanotoxicological risks[25,28].

Dendrimers: Table 5 shows the pharmaceutical applications and toxicological characteristics of major dendrimer types. Dendrimers are highly branched, three-dimensional, monodisperse polymeric nanocarriers distinguished by their well-defined molecular architecture, precise size and exceptional structural uniformity. Unlike conventional linear polymers, dendrimers possess a central core from which successive layers of branched units, referred to as generations, radiate outward to form a nearly spherical nanostructure. Their architecture comprises three principal components: (i) A central core, (ii) Repetitive branching units and (iii) Numerous terminal functional groups located on the surface. The presence of internal cavities, together with a high density of surface functional groups, enables dendrimers to encapsulate therapeutic agents within their interior while simultaneously conjugating drugs, targeting ligands, imaging probes and nucleic acids to their surface. These distinctive structural features make dendrimers highly versatile platforms for multifunctional drug delivery[30].

Among the various dendrimer families, poly (amidoamine) (PAMAM), poly (propylene imine) (PPI), poly-L-lysine (PLL) and phosphorus-containing dendrimers

Table 4: Comparison Between SLNs and NLCs

Parameters	SLNs	NLCs
Lipid composition	Solid lipids only	Solid+liquid lipids
Drug-loading capacity	Moderate	High
Drug expulsion	Common during storage	Significantly reduced
Matrix structure	Highly crystalline	Imperfect/amorphous
Physical stability	Moderate	Superior
Controlled drug release	Good	Excellent
Biocompatibility	High	High
Toxicological risk	Low-moderate	Generally lower when optimized

Table 5: Pharmaceutical applications and toxicological characteristics of major dendrimer types

Dendrimer Type	Major applications	Advantages	Potential toxicological concerns
PAMAM	Cancer therapy, gene delivery	High drug loading, surface modification	Membrane disruption, hemolysis, ROS generation
PPI	Antimicrobial and gene delivery	Efficient nucleic acid delivery	Higher cytotoxicity due to cationic charge
PLL	Peptide and protein delivery	Biodegradable, biocompatible	Moderate cytotoxicity at high concentrations
Phosphorus dendrimers	Drug delivery, imaging	Excellent stability	Limited long-term toxicity data

have attracted the greatest attention in pharmaceutical research. PAMAM dendrimers are particularly well characterized because of their excellent aqueous solubility, precisely controllable molecular weight, reproducible synthesis and ease of surface functionalization. Their highly branched architecture provides an exceptionally large surface area and numerous reactive functional groups, facilitating high drug-loading capacity, efficient conjugation of bioactive molecules and controlled release of both hydrophilic and hydrophobic therapeutic agents[31].

Dendrimer-based drug delivery systems have demonstrated considerable potential across a broad range of biomedical applications, including cancer therapy, gene delivery, vaccine development, diagnostic imaging and targeted drug delivery. Furthermore, their ability to traverse biological barriers, including the blood-brain barrier and ocular tissues, has expanded their application in the treatment of neurological and ophthalmic disorders[31].

Despite these significant pharmaceutical advantages, dendrimers possess one of the most extensively investigated toxicological profiles among nanocarrier systems. Their toxicity is influenced by several physicochemical characteristics, including generation number, molecular size, concentration, surface chemistry and most importantly, surface charge. Among these factors, surface charge is regarded as the primary determinant of biological toxicity. Cationic dendrimers, particularly amino-terminated PAMAM dendrimers, interact strongly with negatively charged phospholipids in cellular membranes, resulting in membrane destabilization, increased membrane permeability, intracellular leakage and ultimately cell death. Consequently, higher-generation cationic dendrimers generally exhibit greater cytotoxicity than lower-generation, neutral, or anionic dendrimers because of their higher density of positively charged surface amino groups[32].

Oxidative stress represents another major mechanism underlying dendrimer-induced nanotoxicity. Following cellular internalization through endocytic pathways, excessive intracellular accumulation of dendrimers may stimulate the overproduction of reactive oxygen species (ROS), leading to mitochondrial dysfunction, depletion of endogenous antioxidant defenses, lipid peroxidation, protein oxidation and DNA damage. The resulting oxidative stress activates multiple intracellular signaling pathways, including mitogen-activated protein kinases (MAPKs), nuclear factor-kappa B (NF-κB) and the p53-mediated apoptotic pathway, thereby promoting programmed cell death. In addition, exposure to positively charged dendrimers has been associated with increased expression of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-1β (IL-1β), indicating their potential to induce inflammatory responses, immunotoxicity and tissue injury[32].

Hemocompatibility represents another critical safety concern associated with dendrimer-based drug delivery systems. Cationic dendrimers can interact strongly with erythrocytes and plasma proteins, potentially inducing hemolysis, platelet activation, complement activation and coagulation abnormalities. These adverse interactions may compromise systemic safety following intravenous administration and therefore require comprehensive evaluation during the preclinical development of dendrimer-based nanomedicines[30,32].

To mitigate dendrimer-associated toxicity, a variety of surface engineering strategies have been developed. Surface modification through PEGylation, acetylation, hydroxylation, carboxylation and conjugation with naturally occurring biomolecules effectively reduces the positive surface charge, thereby decreasing nonspecific cellular interactions while improving biocompatibility, hemocompatibility, pharmacokinetic behavior and systemic circulation time. In addition, recent advances in computational nanotoxicology, quantitative structure-activity relationship (QSAR) modeling and artificial intelligence (AI)-assisted molecular design have facilitated the rational development of safer dendrimer formulations by enabling the prediction of toxicological outcomes based on their structural and physicochemical properties prior to experimental validation[31].

Overall, dendrimers represent one of the most sophisticated and versatile nanocarrier platforms for precision drug delivery because of their highly controlled molecular architecture, exceptional drug-loading capacity and multifunctional surface chemistry. However, their successful clinical translation requires careful optimization of molecular design, generation number, surface functionalization, dosage and long-term biosafety. Future research integrating safe-by-design strategies, advanced computational toxicology, artificial intelligence-driven formulation optimization and standardized regulatory assessment frameworks is expected to further enhance the therapeutic efficacy, clinical applicability and biological safety of dendrimer-based nanomedicines.

Polymeric micelles: Polymeric micelles are nanoscale colloidal drug delivery systems, typically ranging from 10 to 100 nm in diameter, that are formed through the spontaneous self-assembly of amphiphilic block copolymers in aqueous environments. These nanocarriers consist of a hydrophobic core, which efficiently encapsulates poorly water-soluble therapeutic agents and a hydrophilic shell, most commonly composed of polyethylene glycol (PEG), which enhances aqueous stability, prolongs systemic circulation and minimizes recognition and clearance by the mononuclear phagocyte system. Owing to their small particle size, excellent biocompatibility, high drug-loading capacity and

ability to improve the solubility of hydrophobic compounds, polymeric micelles have emerged as promising nanocarriers for controlled and targeted drug delivery[33].

Polymeric micelles have been extensively investigated for the delivery of a wide range of therapeutic agents, including anticancer drugs, antimicrobial agents, anti-inflammatory drugs, proteins, peptides and nucleic acids. Their ability to enhance the aqueous solubility, stability and bioavailability of poorly water-soluble drugs, while facilitating passive tumor targeting through the enhanced permeability and retention (EPR) effect, has significantly advanced the field of cancer nanomedicine. Furthermore, the development of stimuli-responsive polymeric micelles that respond to changes in pH, temperature, enzymatic activity, or redox conditions has enabled site-specific and controlled drug release, thereby improving therapeutic efficacy while minimizing off-target effects[34].

Despite these favorable pharmaceutical properties, the toxicological profile of polymeric micelles is influenced by several factors, including polymer composition, particle size, surface charge, degradation products and formulation characteristics. Biodegradable polymers such as poly (lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL) and polyethylene glycol (PEG) generally exhibit excellent biocompatibility and low intrinsic toxicity. However, polymeric micelles formulated with cationic polymers may interact strongly with negatively charged cellular membranes, resulting in membrane destabilization, enhanced cellular uptake and increased cytotoxicity. At elevated concentrations or following prolonged exposure, polymeric micelles may induce oxidative stress, mitochondrial dysfunction, inflammatory responses and apoptosis through excessive generation of Reactive Oxygen Species (ROS)[3,19].

Another critical consideration is the physicochemical stability of polymeric micelles following systemic administration. Dilution below the Critical Micelle Concentration (CMC) may cause micellar dissociation and premature drug release, thereby reducing therapeutic efficacy and potentially increasing systemic toxicity through unintended drug distribution. To overcome these limitations, various stabilization strategies, including PEGylation, ligand conjugation and core or shell cross-linking, have been extensively employed to improve micellar stability, prolong circulation time, enhance target specificity and reduce undesirable biological interactions[33].

Table 6 shows advantages and toxicological considerations of polymeric micelles. Overall, polymeric micelles represent a highly promising nanocarrier platform because of their exceptional capacity to solubilize poorly water-soluble drugs, provide controlled and targeted drug release and maintain a favorable safety profile. Ongoing advances in polymer chemistry, nanocarrier engineering, stimuli-responsive design and safe-by-design strategies are expected to further facilitate their clinical translation while minimizing nanotoxicological risks and improving therapeutic outcomes.

Mesoporous Silica Nanoparticles (MSNs): Mesoporous silica nanoparticles (MSNs) are inorganic nanocarriers distinguished by their highly ordered porous architecture, large surface area, tunable pore size and exceptional drug-loading capacity. Typically ranging from 50-300 nm in diameter, MSNs possess interconnected mesopores measuring 2-50 nm, which enable the efficient encapsulation of a broad spectrum of therapeutic agents, including small-molecule drugs, proteins, peptides, nucleic acids and imaging agents. Their precisely controllable physicochemical properties, together with the ease of surface functionalization, have established MSNs as highly promising platforms for targeted drug delivery, cancer therapy and theranostic applications[35].

A major advantage of MSNs is their ability to achieve high drug-loading efficiency while providing controlled and sustained drug release. Surface functionalization with polymers, antibodies, peptides, or stimuli-responsive materials enables selective delivery of therapeutic agents to diseased tissues, thereby enhancing therapeutic efficacy while minimizing systemic exposure and off-target toxicity. Owing to their excellent physicochemical stability, structural versatility and adaptable surface chemistry, MSNs have been extensively investigated for applications in chemotherapy, gene delivery, antimicrobial therapy, combination therapy and precision medicine[36,37].

Despite these significant pharmaceutical advantages, the toxicological profile of MSNs is influenced by several physicochemical characteristics, including particle size, pore structure, surface chemistry, dosage, biodegradability and route of administration. Smaller nanoparticles generally exhibit greater cellular uptake and deeper tissue penetration; however, they may also promote increased intracellular accumulation and oxidative stress. Following cellular

Table 6: Advantages and toxicological considerations of polymeric micelles

Advantages	Potential toxicological concerns
High solubility enhancement	ROS generation at high concentrations
Controlled and targeted drug release	Membrane interaction of cationic polymers
Improved bioavailability	Premature drug release after micelle dissociation
Prolonged circulation time	Mild inflammatory responses
Excellent biocompatibility	Polymer degradation-related toxicity (rare)

Table 7: Advantages and Toxicological Considerations of Mesoporous Silica Nanoparticles

Advantages	Potential toxicological concerns
High drug-loading capacity	ROS generation
Tunable pore size	Membrane interaction by silanol groups
Controlled drug release	Inflammatory responses
Easy surface functionalization	Organ accumulation after repeated exposure
Good chemical stability	Long-term biodegradation requires evaluation

Table 8: Pharmaceutical applications and toxicological concerns of carbon-based nanomaterials

Nanomaterial	Major applications	Potential toxicological concerns
Carbon nanotubes (CNTs)	Drug and gene delivery	Pulmonary toxicity, oxidative stress
Graphene	Targeted drug delivery, biosensing	Membrane damage, inflammation
Graphene oxide (GO)	Cancer therapy, imaging	ROS generation, apoptosis
Fullerenes	Antioxidant and drug delivery	Dose-dependent cytotoxicity

internalization through endocytic pathways, excessive exposure to MSNs can stimulate the overproduction of reactive oxygen species (ROS), leading to mitochondrial dysfunction, lipid peroxidation, DNA damage, activation of inflammatory signaling pathways and apoptosis. In addition, surface silanol groups may interact directly with cellular membranes, potentially causing membrane disruption, protein adsorption and inflammatory responses if the nanoparticle surface is not appropriately modified[38,39].

Biodegradation is another critical determinant of the biological safety of MSNs. Under physiological conditions, silica gradually undergoes hydrolytic degradation to form soluble silicic acid, which is subsequently eliminated through renal excretion. However, incomplete biodegradation or prolonged tissue accumulation following repeated administration may contribute to adverse effects in organs such as the liver, spleen and lungs. To mitigate these risks, surface engineering strategies, including coating with biodegradable polymers, PEGylation and optimization of particle size and pore characteristics, have significantly improved the biocompatibility, biodegradability, biodistribution and clearance of MSNs, thereby reducing their toxicological potential[39].

Advantages and toxicological considerations of mesoporous silica nanoparticles are presented in Table 7. Overall, mesoporous silica nanoparticles represent highly versatile nanocarriers for pharmaceutical drug delivery because of their exceptional drug-loading capacity, tunable pore structure, controlled drug release and ease of surface functionalization. Nevertheless, comprehensive evaluation of their long-term biodistribution, biodegradation, pharmacokinetics, immunogenicity and toxicological profile remains essential to ensure their safe and successful clinical translation.

Carbon-based nanomaterials: Table 8 shows pharmaceutical applications and toxicological concerns of carbon-based nanomaterials. Carbon-based nanomaterials, including carbon nanotubes (CNTs), graphene, graphene oxide (GO), reduced graphene oxide (rGO) and fullerenes, have attracted significant attention as advanced pharmaceutical nanocarriers because of their exceptional mechanical strength, high surface area, excellent electrical conductivity and remarkable drug-loading capacity. Their unique physicochemical properties enable the efficient adsorption or conjugation of a wide range of therapeutic agents, making them promising candidates for applications in cancer therapy, gene delivery, biosensing, tissue engineering and diagnostic imaging[40,41].

Among these nanomaterials, carbon nanotubes possess hollow cylindrical structures capable of transporting drugs and nucleic acids into cells, whereas graphene-based materials provide a large two-dimensional surface that facilitates high drug-loading efficiency and stimuli-responsive drug release. Fullerenes, owing to their cage-like molecular architecture, have also demonstrated considerable potential as antioxidants and drug delivery vehicles. Furthermore, surface functionalization with polyethylene glycol (PEG), polymers, peptides, or antibodies significantly enhances their aqueous dispersibility, biocompatibility and tissue-specific targeting, thereby improving their therapeutic efficacy[41,42].

Despite these advantages, carbon-based nanomaterials present greater toxicological concerns than many lipid- and polymer-based nanocarriers. Their toxicity is influenced by several physicochemical characteristics, including particle size, shape, aspect ratio, surface chemistry, degree of functionalization and residual impurities introduced during synthesis. Following cellular internalization, these nanomaterials may induce excessive production of reactive oxygen species (ROS), resulting in oxidative stress, mitochondrial dysfunction, DNA damage, inflammation and apoptosis. In particular, long, rigid carbon nanotubes have been reported to produce asbestos-like pathogenic effects in experimental models, raising concerns regarding chronic pulmonary toxicity and fibrosis following inhalation exposure[43,44].

Graphene oxide and related graphene-based materials may also interact strongly with cellular membranes, leading to membrane disruption, lysosomal damage, inflammatory cytokine release and immune activation. However, numerous studies have demonstrated that appropriate surface

Table 9: Advantages and toxicological considerations of albumin-based nanoparticles

Advantages	Potential toxicological concerns
Excellent biocompatibility	Residual cross-linking agent toxicity
Biodegradable and non-immunogenic	Organ accumulation after repeated dosing
Improved drug solubility	Formulation-related impurities
Receptor-mediated targeted delivery	Limited long-term safety data
Clinically validated (e.g., Abraxane®)	Batch-to-batch manufacturing variability

functionalization, removal of residual metal catalyst impurities and optimization of particle size and morphology substantially improve biocompatibility while reducing cytotoxicity. Consequently, the implementation of safe-by-design strategies, together with comprehensive physicochemical characterization and toxicological assessment, remains essential before the clinical translation of carbon-based nanomaterials[40,45].

Overall, carbon-based nanomaterials offer unique opportunities for advanced drug delivery because of their exceptional structural, physicochemical and functional properties. Nevertheless, concerns regarding their long-term biodistribution, biodegradation, immunogenicity and chronic toxicity continue to limit their widespread clinical application, underscoring the need for rigorous preclinical safety evaluation, standardized toxicity testing and harmonized regulatory guidelines.

Albumin-based nanoparticles: Advantages and toxicological considerations of albumin-based nanoparticles are presented Table 9. Albumin-based nanoparticles are naturally derived nanocarriers that have gained considerable attention because of their excellent biocompatibility, biodegradability and low immunogenicity. Human serum albumin (HSA) and bovine serum albumin (BSA) are the most commonly used proteins for nanoparticle fabrication owing to their ability to bind a wide variety of hydrophobic and hydrophilic therapeutic agents. Albumin nanoparticles improve drug solubility, prolong plasma half-life, enable controlled drug release and facilitate targeted delivery through receptor-mediated uptake, making them highly suitable for the delivery of anticancer, anti-inflammatory and peptide-based therapeutics[46].

One of the most successful examples of albumin-based nanotechnology is nab-paclitaxel (Abraxane®), an FDA-approved albumin-bound formulation of paclitaxel for the treatment of breast, lung and pancreatic cancers. Albumin facilitates drug transport across endothelial cells via gp60 receptor-mediated transcytosis and enhances tumor accumulation through interactions with secreted protein acidic and rich in cysteine (SPARC), thereby improving therapeutic efficacy while reducing the solvent-related toxicity associated with conventional formulations[47].

Compared with many synthetic nanocarriers, albumin nanoparticles generally exhibit an excellent safety profile because albumin is an endogenous plasma protein that is naturally metabolized into amino acids. Nevertheless, their toxicological behavior may be influenced by several factors, including particle size, surface modification, cross-linking agents and manufacturing processes. Residual chemical cross-linkers, such as glutaraldehyde, or formulation-related impurities may contribute to cytotoxicity if they are not adequately removed during production. In addition, repeated administration or high doses may alter biodistribution and promote accumulation in organs such as the liver and spleen, although these effects are generally less pronounced than those associated with metallic or carbon-based nanoparticles[47].

Recent research has focused on enhancing the performance of albumin nanoparticles through ligand conjugation, PEGylation and stimuli-responsive designs to improve targeting efficiency while minimizing systemic toxicity. Owing to their favorable pharmacokinetic properties, excellent biodegradability and proven clinical success, albumin-based nanoparticles remain among the safest and most promising nanocarrier platforms for pharmaceutical drug delivery. Nevertheless, standardized toxicological evaluation and long-term clinical safety studies remain essential to support their broader therapeutic application and successful clinical translation[48].

Mechanisms of nanotoxicity of pharmaceutical nanocarriers

Oxidative stress and reactive oxygen species (ROS) generation: Oxidative stress is one of the most widely recognized mechanisms underlying nanocarrier-induced toxicity. Following exposure, pharmaceutical nanocarriers interact with cellular membranes and are internalized through endocytosis or phagocytosis. Once inside the cell, nanoparticles can disrupt mitochondrial respiration and activate membrane-associated oxidases, resulting in excessive production of reactive oxygen species (ROS), including superoxide anions ($O_2^{\bullet -}$), hydroxyl radicals (•OH) and hydrogen peroxide (H_2O_2). Under physiological conditions, ROS play essential roles in normal cellular signaling and homeostasis. However, excessive ROS production overwhelms endogenous antioxidant defense systems, leading to oxidative stress and subsequent cellular injury[21].

Elevated intracellular ROS levels initiate a cascade of oxidative damage, including lipid peroxidation, protein oxidation, DNA strand breaks and mitochondrial dysfunction[49]. Metallic nanoparticles, such as silver, zinc oxide and iron oxide nanoparticles, generally exhibit a

Table 10: Major reactive oxygen species and their biological effects

Reactive Oxygen Species	Source	Major biological effects
Superoxide anion ($O_2^{\bullet-}$)	Mitochondria	Oxidative stress initiation
Hydrogen peroxide (H_2O_2)	Enzymatic reactions	Cell signaling, oxidative damage
Hydroxyl radical (•OH)	Fenton reaction	DNA, protein and lipid damage
Peroxynitrite ($ONOO^-$)	NO+Superoxide	Protein nitration and inflammation

Table 11: Major cellular uptake pathways of pharmaceutical nanocarriers

Uptake pathway	Typical nanocarriers	Biological outcome
Clathrin-mediated endocytosis	Polymeric nanoparticles, liposomes	Endosomal transport and intracellular drug delivery
Caveolae-mediated endocytosis	PEGylated nanoparticles	Reduced lysosomal degradation, prolonged intracellular retention
Macropinocytosis	Polymeric micelles, dendrimers	Non-specific uptake of larger particles
Phagocytosis	Metallic nanoparticles, larger particles	Clearance by macrophages

greater capacity to generate ROS than lipid-based or biodegradable polymeric nanocarriers because of their ability to release reactive metal ions and catalyze redox reactions[21].

Oxidative stress also activates several intracellular signaling pathways, including nuclear factor-kappa B (NF-κB), mitogen-activated protein kinases (MAPKs) and nuclear factor erythroid 2-related factor 2 (Nrf2)-mediated antioxidant responses. Activation of these pathways promotes the expression and release of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6), thereby establishing a mechanistic link between oxidative stress, inflammation and immunotoxicity. Persistent or excessive ROS generation may ultimately trigger apoptosis, autophagy, or necrosis, depending on the physicochemical characteristics of the nanoparticles, their concentration and the duration of exposure[50].

Cellular uptake and intracellular trafficking: The biological effects of pharmaceutical nanocarriers largely depend on their interactions with cells and their subsequent intracellular trafficking. Nanoparticles are primarily internalized through endocytic pathways, including clathrin-mediated endocytosis, caveolae-mediated endocytosis, macropinocytosis and phagocytosis, depending on their size, shape, surface charge and the type of target cell. Smaller nanoparticles (<100 nm) generally exhibit greater cellular uptake than larger particles because of their enhanced ability to traverse biological membranes and penetrate tissues[51].

Following internalization, nanocarriers are transported through early and late endosomal compartments before reaching lysosomes, where the acidic microenvironment and hydrolytic enzymes facilitate nanoparticle degradation and drug release. Biodegradable nanocarriers, such as liposomes, polymeric nanoparticles and albumin nanoparticles, are generally metabolized efficiently. In contrast, poorly biodegradable or inorganic nanoparticles may accumulate within lysosomes, leading to lysosomal dysfunction, membrane destabilization and disruption of cellular homeostasis[3]. Furthermore, loss of lysosomal membrane integrity may result in the release of hydrolytic enzymes into the cytoplasm, thereby triggering inflammatory responses and programmed cell death. Table 10 shows major reactive oxygen species and their biological effects.

Intracellular trafficking is also a critical determinant of the therapeutic efficacy and toxicological profile of nanomedicines. Many nanocarriers are specifically engineered to escape the endosomal compartment and deliver therapeutic agents directly to the cytoplasm, nucleus, or mitochondria, thereby enhancing intracellular drug bioavailability and therapeutic efficacy. However, unintended accumulation within intracellular organelles, particularly the mitochondria or nucleus, may disrupt normal cellular functions, induce oxidative stress, alter mitochondrial membrane potential and contribute to DNA damage, mitochondrial dysfunction and apoptosis[52]. Table 11 shows major cellular uptake pathways of pharmaceutical nanocarriers.

Inflammatory and immune responses: Pharmaceutical nanocarriers can elicit inflammatory and immune responses following cellular uptake. After internalization, nanoparticles interact with immune cells, including macrophages and dendritic cells, leading to the activation of intracellular signaling pathways such as nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs). Activation of these pathways stimulates the production and release of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6), thereby contributing to both local and systemic inflammatory responses[3].

The magnitude of the inflammatory response is influenced by several nanoparticle characteristics, including particle size, surface charge, composition and biodegradability. Positively charged and metallic nanoparticles generally induce greater immune activation than biodegradable lipid- or polymer-based nanocarriers because of their enhanced interactions with cellular membranes and immune cells. In contrast, surface modifications such as PEGylation reduce protein adsorption and immune recognition, thereby improving biocompatibility, prolonging systemic circulation and minimizing undesired immunological responses[51].

Certain nanocarriers, particularly liposomal formulations, may also activate the complement system, resulting in Complement Activation-related Pseudoallergy (CARPA) following intravenous administration. Consequently, the evaluation of cytokine production, complement activation and immune cell responses constitutes an essential component of the preclinical safety assessment of pharmaceutical nanocarriers. Table 12 presents the major inflammatory mediators in nanotoxicity.

Table 12: Major inflammatory mediators in nanotoxicity

Mediator	Biological effects
TNF-α	Promotes inflammation and apoptosis
IL-1β	Activates immune responses
IL-6	Mediates acute inflammatory response
C3a/C5a	Complement activation and hypersensitivity

Mitochondrial dysfunction and cell death: Mitochondria are major targets of nanoparticle-induced toxicity because they play a central role in cellular energy production, redox homeostasis and the regulation of apoptosis. Excessive intracellular accumulation of pharmaceutical nanocarriers may disrupt the mitochondrial membrane potential, impair adenosine triphosphate (ATP) synthesis and increase the generation of reactive oxygen species (ROS). Persistent mitochondrial dysfunction subsequently activates intrinsic apoptotic signaling pathways, leading to the release of cytochrome c into the cytoplasm and the subsequent activation of caspases, ultimately resulting in programmed cell death[53].

Table 13 shows the biomarkers of mitochondrial dysfunction. The extent of mitochondrial dysfunction depends on the physicochemical properties of nanocarriers, including particle size, composition, surface characteristics and biodegradability. Biodegradable nanocarriers generally exhibit lower mitochondrial toxicity than metallic or poorly biodegradable nanoparticles because they are more readily metabolized and cleared from cells. Therefore, the assessment of mitochondrial integrity, mitochondrial membrane potential, ATP production, oxidative stress and apoptosis-related biomarkers constitutes an essential component of the preclinical safety evaluation of pharmaceutical nanocarriers.

Table 13: Biomarkers of mitochondrial dysfunction

Biomarker	Significance
ROS	Oxidative stress
ATP depletion	Reduced cellular energy
Cytochrome c	Initiation of apoptosis
Caspase-3	Execution of programmed cell death

Genotoxicity and DNA Damage: Genotoxicity is an important safety concern associated with pharmaceutical nanocarriers, as nanoparticles can induce cellular DNA damage through both direct and indirect mechanisms. Following cellular uptake, excessive generation of reactive oxygen species (ROS) may cause DNA strand breaks, oxidative base modifications, chromosomal abnormalities and genetic mutations. Persistent or unrepaired DNA damage can disrupt normal cell cycle regulation, potentially leading to apoptosis, genomic instability, or carcinogenesis if cellular DNA repair mechanisms are compromised[56].

The genotoxic potential of nanocarriers is influenced by several factors, including particle size, composition, surface properties, dose and duration of exposure. Metallic nanoparticles generally exhibit greater genotoxic potential than biodegradable lipid- or polymer-based nanocarriers because of their enhanced capacity to generate oxidative stress. Consequently, standardized genotoxicity assays, including the Comet assay, micronucleus test and γ-H2AX assay, are routinely employed to evaluate DNA damage during the preclinical safety assessment of nanomedicines. Table 14 shows common assays for genotoxicity assessment.

Table 14: Common assays for genotoxicity assessment

Assay	Purpose
Comet assay	Detects DNA strand breaks
Micronucleus test	Identifies chromosomal damage
γ-H2AX assay	Detects DNA double-strand breaks
Ames test	Evaluates mutagenic potential

Organ-specific toxicity: Table 15 shows organ-specific toxic effects of pharmaceutical nanocarriers. The toxicity of pharmaceutical nanocarriers varies according to their biodistribution, physicochemical properties, dose, route of administration and duration of exposure. Following systemic administration, nanoparticles predominantly accumulate in the liver and spleen because of uptake by the mononuclear phagocyte system, although they may also distribute to the kidneys, lungs, heart and brain. Excessive accumulation within these organs can induce oxidative stress, inflammation and tissue injury, thereby potentially impairing normal organ function[21].

The liver is the primary site of nanoparticle metabolism and clearance and is therefore particularly susceptible to hepatotoxicity, which may be manifested by elevated liver enzyme levels, oxidative stress, inflammatory responses and histopathological alterations. The kidneys are also vulnerable to nanoparticle-induced toxicity because of their central role in filtration and excretion, increasing the risk of nephrotoxicity following prolonged exposure or excessive accumulation. Inhaled or intravenously administered nanoparticles may contribute to pulmonary inflammation and lung injury, whereas nanoparticles capable of crossing the blood-brain barrier may induce neurotoxicity through oxidative stress, neuroinflammation and neuronal damage. Consequently, the evaluation of organ-specific toxicity represents an essential component of the preclinical safety assessment of pharmaceutical nanocarriers.

Factors influencing nanotoxicity: Table 16 summarizes the factors affecting nanotoxicity. The toxicological profile of pharmaceutical nanocarriers is influenced by a variety of physicochemical and biological factors. Among these, particle size plays a critical role, as smaller nanoparticles possess a larger surface area-to-volume ratio, resulting in enhanced cellular uptake, increased biological reactivity and greater potential for intracellular accumulation. Similarly, particle shape significantly affects biodistribution and cellular interactions, with rod-shaped or fibrous nanoparticles often exhibiting greater toxicity than spherical nanoparticles because of their distinct cellular uptake mechanisms and tissue interactions[3].

Surface charge is another key determinant of nanotoxicity. Positively charged nanoparticles interact more readily with negatively charged cellular membranes, leading to enhanced cellular uptake and an increased risk of membrane disruption and cytotoxicity. Furthermore, upon entering biological fluids, nanoparticles rapidly adsorb proteins and other biomolecules to form a protein corona, which modifies their biological identity and influences cellular recognition, biodistribution, pharmacokinetics and immune responses[55].

Additional factors, including chemical composition, dose, duration of exposure, biodegradability and route of administration, also play significant roles in determining nanocarrier toxicity. Surface engineering strategies, such as PEGylation and ligand conjugation, can improve nanoparticle stability, reduce protein adsorption and immune recognition, prolong systemic circulation and minimize adverse biological effects. Therefore, careful optimization of these physicochemical and biological parameters is essential for the rational design and development of safe and effective pharmaceutical nanocarriers.

Safety assessment and toxicological evaluation of pharmaceutical nanocarriers: The rapid advancement of nanomedicine has underscored the need for comprehensive safety assessment strategies to evaluate the toxicological profile of pharmaceutical nanocarriers before their clinical application. Toxicological evaluation should encompass detailed physicochemical characterization, *in vitro* and *in vivo* studies, pharmacokinetic and biodistribution analyses, immunotoxicity assessment and long-term biocompatibility. Critical physicochemical parameters, including particle size, morphology, zeta potential, surface chemistry, drug-loading efficiency and degradation behavior, profoundly influence biological interactions and should therefore be thoroughly characterized[56].

In vitro studies are widely employed as the initial step in nanocarrier safety assessment, using appropriate cell lines to evaluate cytotoxicity, oxidative stress, inflammatory responses, mitochondrial dysfunction, hemocompatibility and genotoxicity. Commonly used analytical techniques include the MTT or Alamar Blue assays for assessing cell viability, flow cytometry for apoptosis analysis, the DCFH-DA assay for measuring reactive oxygen species (ROS) generation, enzyme-linked immunosorbent assay (ELISA) for cytokine quantification and the Comet assay for the detection of DNA damage[57].

Table 15: Organ-specific toxic effects of pharmaceutical nanocarriers

Organ	Major toxic effect
Liver	Hepatotoxicity, inflammation
Kidney	Nephrotoxicity
Lungs	Pulmonary inflammation
Brain	Neurotoxicity
Heart	Oxidative stress and cardiotoxicity

Table 16: Factors affecting nanotoxicity

Factor	Impact on toxicity
Particle size	Smaller particles show greater cellular uptake
Shape	Influences biodistribution and cell interaction
Surface charge	Positive charge increases cytotoxicity
Protein corona	Alters biological identity and immune response
Dose & exposure	Higher exposure increases toxicity
Surface modification	Improves biocompatibility and reduces toxicity

Table 17: Common methods for nanotoxicity assessment

Assessment	Common methods
Physicochemical characterization	DLS, TEM, SEM, Zeta potential
Cytotoxicity	MTT, Alamar Blue
Oxidative stress	DCFH-DA assay
Genotoxicity	Comet assay, Micronucleus assay
In vivo toxicity	Histopathology, Hematology, Biochemistry

Animal studies remain essential for evaluating biodistribution, metabolism, organ-specific toxicity and pharmacokinetic behavior under physiological conditions. Histopathological examination, hematological analysis, serum biochemical profiling and advanced imaging techniques provide valuable information regarding systemic toxicity, target organ accumulation and the overall safety profile of pharmaceutical nanocarriers. In recent years, alternative experimental approaches, including organ-on-a-chip platforms and three-dimensional (3D) cell culture models, have gained increasing attention because of their potential to reduce animal use while improving the prediction of human physiological and toxicological responses[58].

A multidisciplinary safety assessment that integrates standardized physicochemical characterization with comprehensive biological evaluation is essential to ensure the safe and successful clinical translation of pharmaceutical nanocarriers. Table 17 illustrates common methods for nanotoxicity assessment.

Regulatory considerations: Regulatory agencies recognize that conventional pharmaceutical evaluation methods are insufficient for nanomedicines because nanoscale materials possess unique physicochemical and biological properties that can substantially influence their safety, efficacy and

pharmacokinetic behavior. Accordingly, organizations such as the US Food and Drug Administration (FDA)[59], the European Medicines Agency (EMA)[60] and the International Council for Harmonisation (ICH)[61] recommend comprehensive characterization of pharmaceutical nanocarriers, including particle size distribution, surface properties, physicochemical stability, biodistribution and immunogenicity, before clinical approval.

Current regulatory challenges include the absence of universally accepted testing protocols, limited long-term toxicological data and variability in manufacturing processes. The development of standardized analytical methods, harmonized international regulatory guidelines and Quality-by-Design (QbD) approaches is expected to improve regulatory consistency, ensure product quality and reproducibility and facilitate the successful clinical translation of nanomedicines.

Current challenges and future perspectives: Despite significant advances in nanomedicine, several challenges continue to limit the widespread clinical application of pharmaceutical nanocarriers. These challenges include an incomplete understanding of long-term toxicity, variability in nanoparticle synthesis, limited reproducibility during large-scale manufacturing, insufficiently predictive in vitro models and an incomplete understanding of nanoparticle interactions with biological systems. In addition, protein corona formation, batch-to-batch variability and organ-specific accumulation remain major concerns that can significantly affect both therapeutic efficacy and biological safety[3].

Future research should focus on the development of biodegradable and biomimetic nanocarriers with enhanced safety profiles and improved clinical performance. Artificial intelligence (AI), machine learning and computational nanotoxicology are emerging as powerful tools for predicting nanoparticle toxicity, optimizing formulation design, accelerating nanocarrier development and reducing experimental costs. Furthermore, advances in personalized nanomedicine, targeted drug delivery, organ-on-a-chip technology and standardized regulatory frameworks are expected to facilitate the safe and efficient translation of nanomedicines from laboratory research to clinical practice.

CONCLUSION

Pharmaceutical nanocarriers have transformed modern drug delivery by improving drug solubility, stability, bioavailability and targeted therapeutic delivery. A wide range of nanocarrier systems, including liposomes, polymeric nanoparticles, dendrimers, polymeric micelles, solid lipid nanoparticles, nanostructured lipid carriers, albumin nanoparticles, mesoporous silica nanoparticles, metallic nanoparticles and carbon-based nanomaterials, have demonstrated considerable therapeutic potential across diverse biomedical applications. However, their unique physicochemical characteristics may also induce oxidative stress, inflammation, mitochondrial dysfunction, genotoxicity, immunotoxicity and organ-specific toxicity, underscoring the importance of comprehensive safety evaluation throughout their development and clinical translation. Recent advances in nanotoxicology have substantially improved the understanding of nano-bio interactions and have facilitated the development of safer nanocarriers through surface engineering, the use of biodegradable materials and targeted drug delivery strategies. Furthermore, standardized toxicity assessment protocols, harmonized regulatory guidelines and innovative predictive platforms, including artificial intelligence (AI), computational nanotoxicology and organ-on-a-chip technologies, are expected to enhance the efficiency and reliability of nanomedicine safety evaluation while accelerating clinical translation. Continued interdisciplinary research integrating pharmaceutical sciences, nanotechnology, toxicology, materials science, computational modeling and regulatory science will be essential for advancing the rational design, safety assessment and clinical application of nanocarrier-based drug delivery systems. Future efforts should prioritize the development of safe-by-design nanocarriers, standardized regulatory frameworks and robust long-term safety evaluations to ensure that these advanced drug delivery platforms achieve their full therapeutic potential while minimizing nanotoxicological risks.

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