Advancements in Nanoparticle-Based Gene Delivery Systems for Therapeutic Applications: A Comprehensive Review

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ABSTRACT

This comprehensive review explores the evolving landscape of nanoparticle-based gene delivery, encompassing various aspects from fundamental design principles to clinical translation. The introduction sets the stage by providing background information and elucidating the rationale for utilizing nanoparticles in gene delivery. The scope of the review is outlined to guide readers through an in-depth exploration of key topics. The subsequent sections delve into the diverse types of nanoparticles employed for gene delivery, including liposomes, polymeric nanoparticles, inorganic nanoparticles, and hybrid systems. A comparative analysis offers insights into the unique attributes of each nanoparticle type. The design and engineering of nanoparticles are scrutinized, covering critical aspects such as surface modification, size and shape optimization, payload encapsulation, and strategies for enhanced stability. The review progresses to targeted gene delivery strategies, elucidating ligand-based targeting, stimuli-responsive nanoparticles, tissue-specific targeting, and innovative approaches to overcome biological barriers. In evaluating in vivo performance, emphasis is placed on bio distribution, pharmacokinetics, biocompatibility, immunogenicity, and long-term effects, providing a comprehensive assessment of the practical implications of nanoparticle-based systems. An overview of clinical trials highlights the progress and challenges in translating nanoparticle-based gene delivery from bench to bedside. The subsequent section delves into safety considerations, addressing cytotoxicity, immunogenicity, strategies for mitigating safety concerns, and the regulatory landscape governing these advanced therapeutic modalities. The review concludes by looking towards the future, exploring emerging trends such as the integration of nanoparticles with gene editing technologies, the application of nanoparticles for RNA interference, and the development of personalized gene therapy approaches. Anticipated challenges and opportunities are discussed.

Keywords: Cytotoxicity, Gene delivery, Nanoparticles, Targeted therapy, Clinical trials, Ligand.

INTRODUCTION

The field of gene therapy has made substantial progresses in recent years, presenting a promising avenue for treating various genetic and acquired disorders. Central to the success of gene therapy is the development of efficient and precise delivery systems capable of navigating biological barriers.[¹] While traditional viral vectors have shown efficacy, concerns such as immunogenicity, potential for insertional mutagenesis, and scalability issues persist. In response, nanoparticle-based gene delivery systems have emerged as an enticing alternative due to their versatility, tunability, and reduced immunogenicity.[²]

Nanoparticles allows for controlled encapsulation and delivery of genetic material, offering enhanced therapeutic efficacy while minimizing off-target effects.[³] The rationale for adopting nanoparticles
in gene delivery lies in their unique properties addressing challenges inherent in other delivery methods. These nanoscale carriers enable targeted delivery through surface modifications and ligand conjugation, enhancing precision. Additionally, many nanoparticle materials exhibit high biocompatibility, minimizing adverse reactions and bolstering the safety profile. The stability and protection afforded by nanoparticles shield genetic material from degradation, ensuring its integrity during transit. Moreover, the versatility of nanoparticles in encapsulating diverse genetic materials, from DNA to RNA and siRNA, broadens their applicability in treating a spectrum of genetic and acquired diseases. Importantly, the reduced immunogenicity of nanoparticles, compared to viral vectors, lessens the risk of adverse reactions and supports repeated administrations if needed.

This comprehensive review aims to explore recent advancements in nanoparticle-based gene delivery systems and their applications in gene therapy. Encompassing various nanoparticle types, including liposomes, polymeric nanoparticles, inorganic nanoparticles, and hybrid systems, the review delves into their design and engineering strategies, focusing on surface modifications, size optimization, and payload encapsulation techniques. The targeted delivery strategies section examines approaches such as ligand-based targeting, stimuli-responsive nanoparticles, and tissue-specific targeting. Evaluation of in vivo performance encompasses biodistribution, pharmacokinetics, biocompatibility, and safety considerations. A critical overview of ongoing clinical trials sheds light on successes, challenges, and lessons learned, while a forward-looking exploration of emerging trends and future directions, including CRISPR/Cas9 and RNA interference integration, completes the comprehensive scope of this review.

The targeted and efficient delivery of nucleic acids has garnered considerable attention, particularly in the context of nanocarriers. The primary goal is to transport exogenous genetic material to the nucleus of specific cells, facilitating the production of proteins encoded by the introduced genes. An optimal vector should deliver a precise quantity of genetic material to a particular cell type, achieving the desired level and duration of transgene expression. Moreover, it should be non-immunogenic and harmless, ensuring the expression of the gene product without inducing toxicity. This approach aims to correct genetic defects effectively and safely. Addressing the specific and efficient delivery of genetic materials to diseased sites or specific cell populations remains a primary challenge. This challenge is being tackled through the utilization of various viral and non-viral delivery systems, each with its distinct advantages and disadvantages. In brief, viral vectors, particularly lentiviral vectors, are advantageous due to their ease of production, non-toxic nature (weakly immunogenic), and capacity to accommodate larger genetic materials (up to 9 kb).

**Fig. 1:** Variety of nanoparticles designed to deliver genetic material, such as DNA and mRNA, to tumor cells within the tumor microenvironment.

However, their drawbacks include the integration of genetic material into the cell genome, posing potential genotoxicity, and a rare risk of generating replication-competent viruses. To address these concerns, various non-viral systems, such as lipoplexes and polyplexes, have been developed for gene delivery. The landscape of gene delivery has witnessed a diverse array of nanoparticle platforms, each exhibiting unique characteristics and advantages. This section explores prominent types of nanoparticles employed for gene delivery, highlighting their distinctive features and applications.
Liposomes

Liposomes, versatile vesicular structures formed by lipids interacting favourably, can encapsulate hydrophobic or hydrophilic molecules. These dynamic entities, recognized since their first use in 1965, are widely applied in analytical sciences and serve crucial roles in drug and gene delivery. Their fluid nature and ease of manipulation make liposomes valuable tools with continuously improving applications in biotechnology and medicine.\[15\] Liposomes hold considerable promise in delivering molecular cargo, particularly DNA, for therapeutic applications. Their formation involves the self-assembly of lipid molecules, a concept continuously refined since their initial discovery. Liposomes find utility across a spectrum of applications and can be categorized based on structural, size, preparatory methods, and compositional differences. These lipid-based carriers offer a flexible and customizable platform for gene delivery, providing researchers with a dynamic toolset to address diverse therapeutic needs.\[16\]

Fig. 2: Structure of various liposome type

Balasz and Godbey pioneered cationic liposomes for ovarian cancer gene therapy, demonstrating promise in both in vitro and in vivo studies. Recent advancements focus on polymer-coated and ligand-targeted liposomes for improved stability and targeted tumor delivery. Over the last 22 years, miRNA-based gene therapy has dominated, targeting various factors like p53, MAPK, hTERT/E1a, and EphA2. Liposomal gene therapy proves effective in reducing tumor size and weight, enhancing survivability. Ongoing research in delivery methods and target exploration holds potential to further improve patient outcomes.\[17\]

Hortobagyi et al. carried out a Phase I clinical trial to evaluate the feasibility of administering the E1A gene encapsulated in DC-Chol cationic liposomes to patients with breast and ovarian cancers. Their findings revealed that the E1A gene was expressed, which was associated with reduced HER-2/neu levels, increased apoptosis, and decreased cell proliferation.\[18\]

In another study, Yoshida et al. investigated the transfer of the interferon-beta (IFN-β) gene using cationic liposomes in five patients with malignant glioma. The results showed that four patients exhibited transgene expression and antitumor effects. Specifically, two patients experienced a tumor reduction of more than 50%, while another two achieved stable disease status after 10 weeks. These outcomes suggest that IFN-β gene therapy using cationic liposomes is both feasible and safe, supporting earlier preclinical research in murine models with B16F1 melanoma.\[19\]

Polymeric Nanoparticles

Gene-loaded polymeric nanocarriers (PNCs) represent an innovative and promising strategy in cancer treatment. These carriers improve drug pharmacokinetics and enhance permeation and retention effects, facilitating increased drug accumulation at the tumor site. This dual functionality provides a valuable approach for more efficient cancer therapy.\[20\] Biodegradable PNCs, composed of materials such as chitosan, dextran, gelatin, pullulan, and advanced synthetic analogues like guanidinylated bio-reducible polymers, are crucial in gene therapy. Their significance lies in their straightforward synthesis and adaptable properties, making them highly effective for gene delivery in therapeutic applications.\[21\] Their ability to form complexes with genetic material through electrostatic attraction at physiological pH facilitates efficient gene delivery.\[22\] This field is rapidly expanding, playing a pivotal role in gene therapy. PNCs exhibit the potential to address disorders characteristic of the modern era, showcasing adaptability and efficacy in navigating the complexities of genetic material delivery for therapeutic purposes.\[23\] In developing PNP s for gene therapy, three main delivery challenges are considered: (i) achieving targeted biodistribution to the desired action site, (ii) promoting cellular uptake by the specific target cells, and (iii) ensuring efficient trafficking to and release within the intracellular compartment where the nucleic acid payload will be active.\[24\]
In the year 1987, Felgner et al. were the first to introduce a cationic lipid-based non-viral method for delivering genes. Their study showed that liposomes with the cationic lipid DOTMA forming polymeric nano-based system for successfully transporting functional DNA into cells. The DNA's negative charge is neutralized by the spontaneous formation of DOTMA-DNA complexes, increasing its capability to bind to the cell surface. This approach has been found to be more effective than DEAE-dextran or calcium phosphate precipitation in achieving stable and transient DNA expression in multiple cell types. The ideal quantity of DNA for transfection changes based on the cell type, and this approach aids in the effective evaluation of fresh plasmid designs. Maintaining the right balance of lipid levels is essential in order to prevent toxicity, and good outcomes have been observed when DOTMA/PtdEtn levels fall within the range of 50 to 100 µg. Changes can be made to DOTMA-containing liposomes, suggesting possible uses for delivering sizable DNA molecules, oligonucleotides, and RNAs into mammalian cells.[25]

In a study conducted by Samal et al. in 2012, cationic polymers have demonstrated excellent efficacy in non-viral gene delivery systems, forming conjugates through electrostatic bonds under physiological pH conditions. The utilization of cationic polymers showcased high performance, further emphasizing their potential in the field of gene delivery.[26] Furthermore, biodegradable poly(beta-amino ester) (PBAE) nanoparticles have been identified as promising biodegradable cationic polymers for addressing paediatric central nervous system (CNS) malignancies. In a specific application, PBAE conjugated with the HSVtk suicide gene (plasmid DNA) and designed for intracellular gene delivery to orthotopic tumor xenografts exhibited substantial improvement in the survival of mice and demonstrated remarkable therapeutic effects.[27] The PBAE exhibited exceptional performance in the delivery of siRNA and miRNA, presenting itself as a highly effective nanocarrier for addressing paediatric malignant CNS tumors.[28-29]

Inorganic Nanoparticles

Inorganic nanoparticles, exemplified by gold, silica, and iron oxide nanoparticles, have garnered attention in the context of nucleic acid delivery and imaging. Their unique physico-chemical properties and tunability make them particularly attractive for overcoming the limitations inherent in gene therapy. These nanoparticles offer an unprecedented opportunity to be functionalized with various biomolecules and moieties, allowing for selective targeting. Inorganic nanoparticles represent a diverse toolkit that holds significant promise for advancing the precision and efficacy of gene delivery strategies.[30]

Gold nanoparticles (AuNPs), 10-20 nm in size, are commonly used in colloidal form, known as gold sols. They offer good biocompatibility, exhibit no apparent cell toxicity, and have various biological functions. Synthesized in diverse ways, they strongly adsorb biomacromolecules without denaturation. AuNPs efficiently transport DNA to the nucleus, outperforming PEI, and find successful use in cancer therapy. However, their cellular entry, either through endocytosis or direct infiltration, may have cytotoxic effects. AuNPs, extensively studied for decades, stand out as the most advanced choice for diverse medical applications. These applications include sensing, imaging, catalysis, therapies, diagnostics, medication, and gene delivery. [31-32]

Gold nanoparticles (AuNPs) can initially be coated with cationic molecules to alter the surface charge of the nanostructures, thereby enhancing DNA binding through electrostatic interactions. This approach leverages their unique physicochemical properties, and numerous studies have demonstrated the effectiveness of AuNPs as DNA carriers.[33-34] Additionally, AuNPs are valuable in bio-diagnostic testing due to their exceptional photophysical properties. Their simple surface chemistry also allows them to function as synthetic antibodies, with adjustable binding interactions based on the density of binding molecules on their surfaces. Overall, gold nanotechnology shows significant promise in biomedicine, offering enhanced drug delivery with tailored, targeted, diagnostic, and therapeutic capabilities for specific medical conditions.[35]

Hybrid Nanoparticles

Hybrid nanoparticles, an amalgamation of distinct nanoparticle classes, have demonstrated enhanced
therapeutic efficacy in gene delivery. This area represents an ongoing frontier in research and development, aiming to synergize the advantages of different nanoparticle types. By combining the strengths of liposomes, polymeric nanoparticles, and inorganic nanoparticles, hybrid systems seek to optimize gene delivery, offering a multifaceted approach to address the challenges posed by various therapeutic contexts. The continuous exploration of hybrid nanoparticles underscores their potential to significantly contribute to the evolution of gene delivery technologies.

Table 1: Comparison of Different Nanoparticle Types

<table>
<thead>
<tr>
<th>Nanoparticle Type</th>
<th>Structure and Formation</th>
<th>Gene Loading and Stability</th>
<th>Targeted Delivery</th>
<th>Biocompatibility</th>
<th>Clinical Translation and Scalability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposomes</td>
<td>Vesicular lipid bilayer formed through self-assembly of lipids</td>
<td>Stable environment for gene loading in aqueous core; protects genetic material from degradation</td>
<td>Surface modifications enable targeted delivery through ligand conjugation</td>
<td>Often exhibits high biocompatibility; proven track record in clinical</td>
<td>Achieved clinical success with approved formulations; scalable manufacturing</td>
</tr>
<tr>
<td>Polymeric Nanoparticles</td>
<td>Composed of synthetic or natural polymers</td>
<td>Efficient gene loading through electrostatic interactions; ensures stability during delivery</td>
<td>Surface modifications enable targeted delivery, enhancing stability and efficacy</td>
<td>Hallmarked by high biocompatibility; progressing towards clinical applications</td>
<td>Scalability depends on chosen polymer and manufacturing processes</td>
</tr>
<tr>
<td>Inorganic Nanoparticles</td>
<td>Composed of diverse inorganic materials (e.g., gold, silica, iron oxide)</td>
<td>Allows controlled gene loading; offers stability and tunability</td>
<td>Functionalyzed for targeted delivery; diverse physiochemical properties</td>
<td>Biocompatibility varies among materials; active research for clinical translation</td>
<td>Clinical translation is an evolving field; scalability depends on material compatibility of components</td>
</tr>
<tr>
<td>Hybrid Nanoparticles</td>
<td>Combination of different nanoparticle classes (e.g., lipids, polymers, inorganic materials)</td>
<td>Leverages advantages of multiple components; enhances gene loading and stability</td>
<td>Aims to combine targeted delivery capabilities of different classes</td>
<td>Biocompatibility depends on integrated components; evolving field for clinical translation</td>
<td>Translation is ongoing; scalability depends on compatibility of components</td>
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Targeted Gene Delivery Strategies

Targeted gene delivery strategies aim to enhance the precision and efficiency of nanoparticle-based gene therapy. Different key strategies include ligand-based targeting, stimuli-responsive NPs, tissue-specific targeting, and overcoming various biological barriers.

**Ligand-Based Targeting**

Ligand-based targeting employs specific ligands, such as antibodies, peptides, or small molecules, to guide nanoparticles to particular cells or tissues (as shown in Fig. 4). By attaching these targeting molecules to the nanoparticle surface, the strategy enhances the specificity of gene delivery. Ligand-based targeting ensures that therapeutic genes are delivered precisely to the intended cells, improving overall efficacy and reducing off-target effects.
Stimuli-Responsive Nanoparticles

Compared to non stimulus-responsive nanocarriers, stimulus-responsive delivery systems exhibit more dynamic behaviors, making vector release more precise. The stimuli that trigger cargo release from gene carriers can be categorized into two types: 1) internal stimuli, which are natural changes in the physiological and pathological conditions of cells or tissues (such as variations in temperature, redox potential, enzyme activity, or pH), and 2) external stimuli, which are changes induced externally in biological systems (such as electrical, magnetic, or ultrasonic stimuli) (as shown in Fig. 5).[39]

Additionally, magnetic fields were employed in a study using hexanoyl chloride-modified chitosan-stabilized iron oxide nanoparticles. These nanoparticles were conjugated with a viral gene (Ad/LacZ) and exhibited high transduction efficiency. The presence of magnetic fields facilitated a dramatic enhancement in the intracellular trafficking of the adenovirus. This approach, especially in chimeric antigen receptor cells, holds promise for enhanced nuclear transfer, as reported by Bhattarai et al. in 2008.[42]

**Tissue-Specific Targeting**

Tissue-specific targeting focuses on designing nanoparticles tailored to target specific tissues or organs. This is achieved by modifying the nanoparticle surface with molecules that have an affinity for the target tissue. By doing so, tissue-specific targeting improves the specificity of gene delivery, ensuring that therapeutic genes reach the intended tissue or organ. This strategy is particularly crucial for diseases requiring localized gene expression.[43]

**Overcoming Biological Barriers**

Overcoming biological barriers is a critical aspect of nanoparticle-based gene delivery to ensure the
successful delivery of therapeutic payloads to specific target sites. Various biological barriers, such as the blood-brain barrier and endothelial barriers, can impede the efficient transport of nanoparticles (as shown in Fig. 4). This section explores strategies to overcome these barriers, facilitating enhanced gene delivery:

**Blood-Brain Barrier (BBB)**
Nanoparticles can be modified with specific ligands or peptides that have an affinity for receptors on the BBB, promoting targeted transport across this barrier. The use of stealth coatings, such as polyethylene glycol (PEG), can reduce interactions with the immune system, prolonging circulation time and increasing the chances of successful BBB penetration. Utilizing receptor-mediated transport mechanisms allows nanoparticles to exploit endogenous transport pathways, enhancing their ability to traverse the BBB.[44-45]

**Endothelial Barriers**
Incorporating Cell penetrating peptides (CPPs) into nanoparticle design facilitates enhanced cellular uptake and transport across endothelial barriers. Another strategy is to functionalized nanoparticles with vasodilators can improve blood flow, aiding in the traversal of endothelial barriers. Adjusting nanoparticle size and surface charge can influence interactions with endothelial cells, optimizing transport across vascular barriers.[46]

**Mucosal Barriers**
Nanoparticles can be engineered with surface modifications that allow them to bypass mucus entrapment, facilitating penetration through mucosal barriers. Incorporating enzymes responsive to mucosal conditions can trigger structural changes in nanoparticles, aiding in their passage through mucosal layers. Coating nanoparticles with lubricating agents can reduce adhesion to mucosal surfaces, enabling smoother traversal through mucosal barriers.[47]

**Cellular Membranes**
CPPs enhance cellular uptake by facilitating nanoparticle internalization through cellular membranes. Nanoparticles can be designed to fuse with cellular membranes, facilitating direct entry into cells. Modifying nanoparticle surfaces to promote specific endocytic pathways enhances cellular internalization.[48]

**Immune System Evasion**
Coating nanoparticles with stealth materials, like PEG, reduces immune recognition, preventing rapid clearance and allowing for prolonged circulation. Incorporating immunomodulatory agents can help modulate immune responses, preventing adverse reactions and enhancing nanoparticle survival in circulation.[49]

**In Vivo/In vitro Performance of Nanoparticle-Based Systems**
Liposomes are extensively utilized for gene transfection because they are user-friendly, commercially accessible, cost-effective, and highly efficient in delivery. Standard liposome formulations usually include a cationic lipid such as DOTAP or MVLS, combined with a neutral "helper" lipid like DOPE or cholesterol. Lipofectamine, a well-known commercial liposome product, comprises a 3:1 ratio of a polycationic lipid to a neutral lipid.[50]

Lipids mixed with DNA that is negatively charged result in the formation of nanostructures called lipid-DNA complexes. These complexes can have single- or multi-lamellar structures based on their composition, containing DNA enclosed in positively charged membrane bilayers. This procedure leads to the creation of round nanoparticles ranging from 100 to 200 nm in
diameter on average. Liposomes usually bond to the cell membrane via electrostatic interactions and are later taken in through a mechanism called clathrin-mediated endocytosis (CME).[51-52]

A study conducted by Safinya et al. highlights the importance of charge density in the efficiency of liposome transfection. The density of charge is defined by the proportion of positive to zero charge lipids and the quantity of charges on the positive lipid headgroup. A high level of charge density is crucial for the effective fusion with endosome membranes, assisting in the transfer of DNA into the cytosol, which is the primary method for breaking free from endosomes. Nevertheless, a charge density that is too high can impede the separation of DNA from the liposome complex after it has been released from the endosome.[53]

Studies using various cationic lipids have demonstrated that formulations containing multivalent cation lipids, particularly those with dendritic headgroups that have up to 16 positive charges, achieve the highest transfection efficiencies. Liposomes made with these dendritic polycation lipids have successfully transfected cells that are typically difficult to transfect. In ocular gene transfer research,[54] Masuda et al. assessed how well three cationic liposome types (TMAG, DDAB, and DC-cholesterol) work for delivering genes to eye tissues in rats through various administration techniques. Gene expression in ganglion cells and the retinal pigment epithelium (RPE) was observed after administering liposomes via subretinal or intravitreal injections, with TMAG liposomes proving to be the most effective. Nevertheless, none of the solutions could successfully deliver genetic material into photoreceptor cells.[55]

Kachi et al. recently conducted a thorough study to assess the safety and efficiency of ocular gene transfer using cationic liposome reagents that are readily available in the market. Injecting plasmid DNA mixed with lipofectamine into the vitreous humor resulted in β-gal expression only in the ganglion cell layer. On the other hand, when delivered through subretinal injection, the same compound led to significant gene activity in photoreceptors and retinal pigment epithelium (RPE) cells within a span of three days. Nevertheless, it was observed that lipofectamine showed significant toxicity towards photoreceptor cells. However, NeuroPorter was observed to be safe for the retina but only succeeded in transfecting RPE cells efficiently. Currently, there is no liposome formulation proven to be safe for transfecting photoreceptor cells.[56]

Although liposome technology remains prevalent for in vitro applications, challenges such as tissue toxicity and low in vivo efficiency continue to pose significant obstacles, restricting their use in human patients. Chitosan and its derivatives are typically synthesized by deacetylating chitin, followed by additional derivatization.[57] Zhang and colleagues developed two water-soluble variants: O-(2-hydroxy-3-trimethylammonium) propyl chitin (OHT-chitin) and N-(2-hydroxy-3-trimethylammonium) propyl chitosan (NHT-chitosan). Additionally, a new gene delivery system, polyethylenimine-graft-chitosan (PEI-g-chitosan), was created by cationic polymerization of aziridine with water-soluble oligo-chitosan.[58]

Recent research in the field of nanotechnology explore the creation and planning of natural polymers for gene therapy, providing in-depth perspectives on different facets of their growth and use. Chen et al. conducted a 2009 study that investigated the application of superparamagnetic iron oxide nanoparticles (SPIONs) linked with a CD3 single-chain antibody, which is a ligand targeting T cells. These compounds were used to condense plasmid DNA into nanoparticles of the right size while causing little harm to cells. Consequently, there was a significant 16-fold enhancement in gene transfection effectiveness in HB8521 cells, which is a rat T-lymphocyte line.[59]

Another technique involved coating iron oxide nanoparticles with carboxylated cholesterol to create a phospholipid monolayer. This setup facilitated the adsorption of certain proteins like Apo A1, Apo E, or artificial amphoteric alpha-helical polypeptides. These nanoparticles have the
potential to be directed towards high-density lipoprotein (HDL), low-density lipoprotein (LDL), or folate receptors, making them a flexible option for loading cells in their natural environment. This method, as shown by Glickson and colleagues, is especially useful for monitoring cells with MRI or for gene therapy.\[^{60}\]

**Table 2: Clinical trials of nanoparticles-based gene delivery**

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<tr>
<th>Study</th>
<th>Phase</th>
<th>Disease</th>
<th>Nanoparticles</th>
<th>Route</th>
<th>Status</th>
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Regulatory bodies play a pivotal role in ensuring the safety of these systems, imposing stringent requirements throughout development and clinical use to uphold patient safety and efficacy. In essence, comprehensive safety considerations in nanoparticle-based gene delivery encompass assessing cytotoxicity, immunogenicity, implementing safety strategies, and adhering to regulatory standards, all crucial for the development of safe and effective gene delivery systems.\[^{61}\]

**Future Directions and Emerging Trends**

**Integration with CRISPR/Cas9 and Gene Editing Technologies**

The future of nanoparticle-based gene delivery is poised to witness significant advancements through the integration with CRISPR/Cas9 and other gene editing technologies. This synergistic approach holds immense promise for precise and targeted genome modifications, enabling the correction of genetic abnormalities at the molecular level. The combination of nanoparticle carriers with gene editing tools could revolutionize therapeutic interventions, offering unprecedented precision and efficacy in treating genetic disorders.\[^{62}\]

**Nanoparticles for RNA Interference**

The continued exploration and development of nanoparticles for RNA interference (RNAi) represent a key trend in future gene delivery strategies. Harnessing the power of RNAi allows for specific gene silencing, opening avenues for therapeutic interventions in diseases with a genetic basis. Nanoparticle-based delivery systems offer a means to enhance the stability and targeted delivery of RNAi molecules, paving the way for more effective and tailored gene therapies.\[^{63}\]
Personalized Gene Therapy Approaches

The future of gene delivery is moving towards personalized approaches, tailoring treatments to individual genetic profiles. Advances in omics technologies and understanding of genetic variations allow for the design of gene therapies that address specific genetic mutations or variations in patients. Nanoparticle-based carriers can play a crucial role in delivering personalized gene therapies, ensuring targeted and efficient delivery of therapeutic payloads while minimizing off-target effects.[64]

Challenges and Opportunities on the Horizon

As the field progresses, certain challenges and opportunities emerge on the horizon. Challenges include refining the safety profiles of nanoparticle carriers, optimizing delivery efficiency, and addressing potential long-term effects. Opportunities lie in the development of innovative materials, advanced engineering of nanoparticles, and the establishment of robust regulatory frameworks to facilitate the translation of these technologies into clinical applications. Collaborative efforts across disciplines and industries will be essential to overcome challenges and unlock the full potential of nanoparticle-based gene delivery. In summary, the future of nanoparticle-based gene delivery is marked by integration with gene editing technologies, a focus on RNA interference, a shift towards personalized approaches, and the anticipation of challenges and opportunities that will shape the next generation of gene therapies.

CONCLUSION

In conclusion, this comprehensive review has provided valuable insights into the dynamic field of nanoparticle-based gene delivery. The exploration began with an understanding of the background, highlighting the rationale for employing nanoparticles in gene delivery and outlining the scope of the review. The subsequent sections delved into the various types of nanoparticles, including liposomes, polymeric nanoparticles, inorganic nanoparticles, and hybrid nanoparticles, with a comparative analysis to underscore their unique attributes. The design and engineering of nanoparticles were discussed, emphasizing crucial factors such as surface modification, size and shape optimization, payload encapsulation, and release strategies, as well as methods for enhancing stability. Targeted gene delivery strategies were explored, encompassing ligand-based targeting, stimuli-responsive nanoparticles, tissue-specific targeting, and approaches to overcome biological barriers. The in vivo/in vitro performance of nanoparticle-based systems was scrutinized, covering aspects like biodistribution, pharmacokinetics, biocompatibility, immunogenicity, and long-term effects. Moving into the clinical realm, an overview of ongoing clinical trials provided a snapshot of the current progress and translation of nanoparticle-based gene delivery technologies. Safety considerations took centre stage in the subsequent section, addressing critical factors such as cytotoxicity, immunogenicity, safety mitigation strategies, and regulatory considerations. The multifaceted exploration of safety considerations laid the groundwork for ensuring the development of safe and effective gene therapies. Looking ahead, the review identified future directions and emerging trends in the field. Integration with CRISPR/Cas9 and gene editing technologies, the utilization of nanoparticles for RNA interference, and the exploration of personalized gene therapy approaches emerged as key avenues for future research. Challenges and opportunities on the horizon were acknowledged, emphasizing the importance of refining safety profiles, optimizing delivery efficiency, and navigating regulatory landscapes. In summary, the review has synthesized a wealth of information, offering a comprehensive overview of nanoparticle-based gene delivery. The implications for future research are clear, with an emphasis on refining research efforts across scientific, clinical, and regulatory domains will be essential for translating these advancements into impactful clinical applications. In closing, the
dynamic landscape of nanoparticle-based gene delivery holds great promise, and as we address challenges and seize opportunities, the field is poised to revolutionize the landscape of gene therapy, providing tailored and effective solutions for a range of genetic disorders.

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