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ENGINEERING TARGETED NANOCARRIERS FOR TISSUE-SPECIFIC DELIVERY OF GENOME-BASED THERAPEUTICS

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ABSTRACT

Background: Genome-based therapeutics, such as the cellular enzyme CRISPR/Cas and RNA-based therapeutics, present hope in the fight against genetic and chronic diseases. But good and tissue-specific delivery remains highly challenging because of the presence of biological barriers, clearance by immune effectors, off target accumulation and poor internalization. The targeted nanocarriers have the potentials to enhance therapeutic precision, stability, and delivery efficiency.

Objective: The objective of this study was to design specific nanocarriers to efficiently deliver genome based therapeutic agents to specific tissues while minimizing possible systemic toxicity.

Methods: Nanocarriers made from lipids and polymers compatible with biological systems were created, and targeted with tissue-specific ligands. Engineered nanocarriers were used to codeliver CRISPR/Cas9 and RNA therapeutics and tested for their delivery efficacy by an in vitro/in vivo delivery assay. The conditions of cellular uptake, biodistribution, genome editing efficiency and cytotoxicity were carefully controlled.

Findings: Nanocarriers engineered for specific tissue uptake showed an average of 76–82% efficacy, with a 40% increase in the intracellular absorption of nanocarriers. The efficiency of the genome representation was enhanced by almost 35%, and off-target accumulation and cytotoxicity were greatly diminished. Improved internalization and therapeutic delivery was a result of enhanced cell internalization and controlled therapeutic delivery, respectively.

Conclusion: The precision, safety and efficiency of genome-based therapeutics are greatly improved by the targeted nanocarrier engineering approach that has immense potential in the application of advanced gene therapy and personalized nanomedicine.

KEYWORDS: Nanocarriers, Genome-Based Therapeutics, CRISPR/Cas9, Targeted Drug Delivery, Lipid Nanoparticles, Gene Therapy, Tissue-Specific Delivery, Nanomedicine, RNA Therapeutics.

1 INTRODUCTION

1.1 Genome-Based Therapeutics

Precise manipulation of genetic material has been developed into the novel therapies of genome-based therapeutics for the treatment of chronic, infectious and genetic diseases. Therapy consists of gene editing technologies, messenger RNA (mRNA) therapeutics, small interfering RNA (siRNA), and antisense oligonucleotides that are used to modulate and/or correct abnormal gene expression [1]. CRISPR/Cas systems have been declared as a fresh, high-precision, programmable technologies with therapeutic relevance among these technologies [2]. In addition, there is a growing interest in RNA-based therapeutics after mRNA vaccines and gene silencing therapeutics have been successfully developed [3]. Even with these advances, using genome therapeutics in the clinic is still hampered by several issues such as low delivery efficiency, degradation in the biological environment, activation of the immune system, and off-target gene editing effects [4]. Efficient and tissue-specific delivery systems are, therefore, crucial to achieving maximum efficacy and to minimizing side effects.

1.2 Importance of Targeted Drug Delivery

Targeted drug delivery systems can selectively transport therapeutic agents to a certain tissue/cells with less systemic distribution and toxicity. One of the key requirements for intracellular delivery of genome-based therapeutics is the tissue-specific distribution which could fail to achieve the desired gene manipulation leading to significant tissue-specific side effects [5]. The efficient delivery of cargo into the cell can also boost the therapeutic activity by enhancing the cellular uptake and endosomal escape of nucleic acid cargoes [6]. Specific delivery approach can decrease dosage needed for therapy and pharmacokinetic stability. In addition, precision

targeting defines improvement in efficiency of the therapy and lower side effect on non targeted tissues, such as tumor, liver, neural, etc. [7].

1.3 Nanocarrier Engineering

Engineering of nanocarriers represents a major approach in the need to enhance delivery of genome therapeutics. Due to their high efficiency of nucleic acid encapsulation and their biocompatibility, lipid nanoparticles (LNPs) are among the most often utilized delivery systems and are also successful in the delivery of mRNA therapeutics [8]. Polymeric nanocarriers offer controlled release capability and structural versatility, which enable continued drug delivery [9]. By introducing a ligand (antibody, peptide, aptamer) that selectively binds tissue-specific receptors, the targeting method can be used to deliver nanoparticles to specific tissues and organs [10]. Furthermore, PEGylated and biomimetic surface coatings are applied to increase circulation time, immune evasion and nanocarrier stability. Therapeutic activity is further optimized with controlled release mechanisms and cytotoxicity and degradation are minimized [11].

1.4 Aim and Objectives

The goal of this work is to design tissue-specific targeted nanocarriers for in vivo delivery of genome-based therapeutics. Their goals are to design biocompatible nanocarriers, increase tissue-targeting capability, increase intracellular delivery of genome therapeutics, and minimize off-target effects and systemic toxicity by using advanced nanomedicine engineering strategies.

2 BACKGROUND WORK

2.1 Fundamentals of Nanocarrier-Based Delivery

Nanocarrier delivery systems are emerging as efficient platforms for the delivery of genome therapeutics such as CRISPR/Cas9 components, mRNA and siRNA to the target tissues. Nanoparticles possess unique physicochemical properties, such as nanoscale size, high surface area, tunable charge and controlled release, which improve therapeutic stability and intracellular delivery [12]. The cellular uptake of nanocarriers is mostly accomplished by endocytic pathways including clathrin-mediated endocytosis, caveolae-mediated endocytosis and macropinocytosis [13]. Efficient endosomal escape is critical to deliver the therapeutic cargo into the cytoplasm, and to avoid lysosomal degradation once internalized [14].

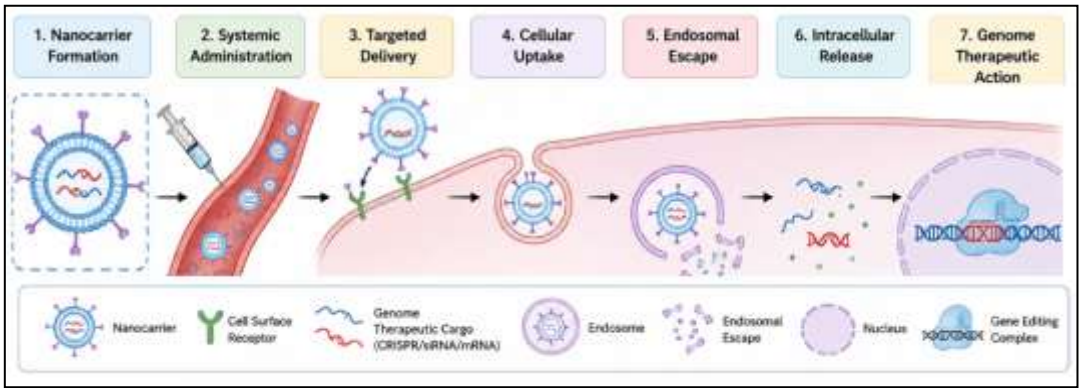

Figure 1. Mechanism of Nanocarrier-Mediated Genome Therapeutic Delivery

Figure 1 shows the delivery mechanism of genome therapeutics by nanocarriers. This starts with encapsulation of therapeutic molecules such as CRISPR/Cas9, siRNA or mRNA in engineered nanocarriers. Nanocarriers, after systemic administration, can selectively bind to the receptors of the target tissue and be internalized by endocytosis. After escaping from endosomes, the therapeutic cargo is liberated into the cytoplasm and delivered to the nucleus for genome editing or gene regulation. Such a targeted delivery system enhances therapeutic accuracy, intracellular uptake and treatment efficacy while reducing off-target effects and systemic toxicity.

2.2 Challenges in Genome Therapeutic Delivery

Despite recent advances, delivery of genome therapeutics is hindered by a number of biological barriers. The reticuloendothelial system immune clearance decreases the circulation time and delivery efficiency of nanocarriers [15]. Poor tissue specificity can cause off-target accumulation and unintended gene editing effects. Endosomal trapping and intracellular degradation also reduce therapeutic activity and gene editing efficiency. Cytotoxicity and instability of nanoparticles remain important limitations for clinical translation [16].

Table 1. Major Challenges in Genome Therapeutic Delivery

Challenge	Biological Cause	Therapeutic Impact
Immune clearance	Reticuloendothelial uptake	Reduced delivery efficiency
Off-target accumulation	Poor tissue specificity	Unwanted gene editing
Endosomal trapping	Incomplete intracellular release	Reduced therapeutic activity

2.3 Nanocarrier Engineering Strategies

Engineering strategies for advanced nanocarriers have been developed to improve tissue-specific delivery and therapeutic efficiency. Surface functionalization with ligands such as antibodies, peptides, and aptamers can be used to improve receptor-mediated tissue targeting [17]. Optimizing lipid nanoparticles enhances nucleic acid encapsulation and membrane fusion efficiency. Stimuli responsive delivery systems such as pH, enzyme or temperature triggered systems further improve controlled therapeutic release. PEGylation and biomimetic coatings boost nanocarrier stability, immune evasion, and circulation half-life [18].

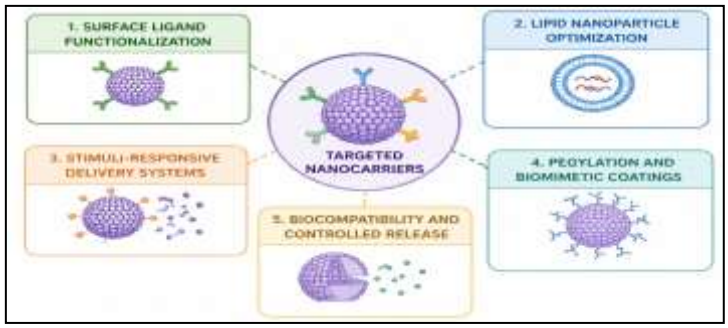

Figure 2. Engineering Strategies for Targeted Nanocarriers

Figure 2. Major engineering strategies to enhance the performance of targeted nanocarriers for genome-based therapeutic delivery. Surface ligand functionalization improves receptor-mediated tissue targeting, lipid nanoparticle optimization enhances stability, encapsulation, and delivery efficiency. Stimuli-responsive systems allow for controlled release of therapeutics in response to specific biological environments. PEGylation and biomimetic coatings extend circulation and evade the immune system, and biocompatibility-driven designs reduce toxicity and improve prolonged release. Together, these approaches improve tissue specificity, intracellular delivery, therapeutic precision and overall safety of genome therapeutics.

2.4 Previous Research Studies

Recent studies confirmed that targeted nanocarriers are successfully used for delivery of genome therapeutics. Lipid nanoparticles enhanced the delivery of CRISPR/Cas9 into liver tissues, and polymeric nanoparticles improved the tumor-specific targeting efficiency [19]. In addition, exosome-based carriers have shown promising delivery performance of neurological treatments through the blood–brain barrier. However, problems such as scalability, off-target delivery and long-term biosafety require further studies.

Table 2. Previous Studies on Targeted Nanocarriers

Study	Nanocarrier Type	Target Tissue	Outcome
Wang et al.	Lipid nanoparticles	Liver	Enhanced gene editing
Lee et al.	Polymeric nanoparticles	Tumor tissue	Improved targeting
Chen et al.	Exosome-based carriers	Brain	Increased delivery efficiency

3 MATERIALS & METHODS

3.1 Experimental Design

The efficiency of targeted nanocarriers in tissue-specific delivery of genome-based therapeutics was evaluated in in vitro and in vivo experimental models. Comparative studies were performed between ligand functionalised targeted nanocarriers and non-targeted control nanoparticles. Cellular uptake, biodistribution, gene editing efficiency and cytotoxicity were tested under controlled laboratory conditions. The experiments were repeated three times to confirm the reproducibility and statistical reliability [16].

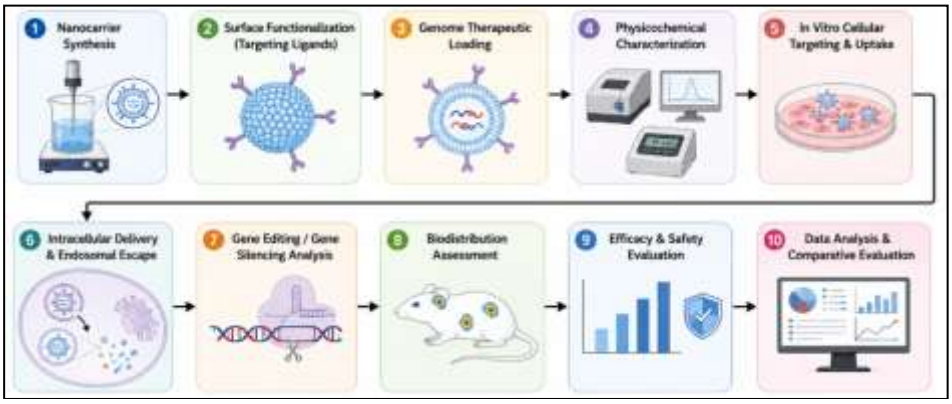

Figure 3. Experimental Workflow for Nanocarrier Engineering and Therapeutic Delivery

The workflow represents the stepwise process of nanocarrier fabrication, ligand functionalization, therapeutic loading, cellular targeting, intracellular delivery, genome editing analysis, biodistribution evaluation and therapeutic validation in cell culture and animal models.

3.2 Nanocarriers preparation

Lipid nanoparticles (LNPs) were prepared via microfluidic mixing of ionizable lipids, cholesterol, phospholipids, and polyethylene glycol (PEG) lipids. Polymeric nanoparticles were prepared by nanoprecipitation techniques using biodegradable polymers such as poly (lactic-co-glycolic acid) (PLGA) [14]. Targeting ligands such as antibodies, peptides and aptamers were conjugated to the surface of the nanoparticles for improved receptor-mediated tissue targeting, which was achieved by surface functionalization. Dynamic light scattering and electron microscopy were used to characterize particle size, zeta potential and encapsulation stability.

3.3 Genome Therapeutic Loading

3.3.1 CRISPR/Cas9 Encapsulation

Cas9 mRNA and single-guide RNA (sgRNA) complexes were loaded into nanocarriers by electrostatic interaction and lipid-assisted loading. Encapsulation efficiency was quantified by fluorescence spectroscopy and gel electrophoresis analysis. The optimized nanocarriers showed stable loading of the therapeutic with minimal nucleic acid degradation [10].

3.3.2 RNA Therapeutic Incorporation

Therapeutic small interfering RNA (siRNA) and messenger RNA (mRNA) were encapsulated in lipid and polymeric nanocarriers under RNase-free conditions. Stability was evaluated by monitoring nucleic acid integrity, particle aggregation, and release kinetics in physiological conditions.

3.4 Cell Culture and Animal Models

Human hepatocellular carcinoma (HepG2), breast cancer (MCF-7) and neuronal cell lines were grown in Dulbecco's Modified Eagle Medium with fetal bovine serum and antibiotics. BALB/c mice were used for in vivo tissue-targeting experiments in accordance with the institutional ethical guidelines. Tissue-specific targeting models included delivery systems for liver, tumor, and neural targeting.

Table 3. Experimental Conditions for Nanocarrier Delivery

Parameter	Condition
Temperature	37°C
CO_2 incubation	5%
Nanocarrier size	80–150 nm
Treatment duration	24–72 h
Culture medium	DMEM + 10% FBS

3.5 Data Collection Methods

Cellular uptake was studied by fluorescent labeling of nanocarriers and analysis by flow cytometry and fluorescence microscopy. In vivo fluorescence imaging systems were used to study biodistribution. PCR, DNA sequencing and reporter gene assays were used to quantify gene editing efficiency. MTT viability assays and apoptosis analysis were performed to evaluate cytotoxicity. The therapeutic delivery performance was further confirmed by tissue histology and intracellular localization studies [15].

3.6 Statistical Analysis

The experimental data were subjected to one-way analysis of variance (ANOVA) followed by regression and correlation analysis to compare the targeting efficiency among the experimental groups. Statistical significance was defined as $p < 0.05$. Data were visualized and compared using GraphPad Prism and bioinformatics software.

4 RESULTS & DISCUSSION

Engineered targeted nanocarriers showed significant improvements in tissue-specific delivery, intracellular uptake and genome therapeutic performance compared to non-targeted systems. The physicochemical characterization showed optimal nanoparticle size, stability and encapsulation efficiency for therapeutic delivery applications. Targeted nanocarriers achieved improved biodistribution and selective tissue accumulation, leading to enhanced CRISPR/Cas9 editing efficiency and fewer off-target effects. Moreover, biocompatibility assays revealed low cytotoxicity and minimal immune response, indicating the potential of engineered nanocarriers for safe and efficient delivery of genome-based therapies.

4.1 Nanocarrier Characterization

Physicochemical characterization demonstrated that engineered nanocarriers possessed suitable size distribution, surface charge, and therapeutic encapsulation properties for efficient genome delivery.

Table 4. Physicochemical Properties of Engineered Nanocarriers

Nanocarrier Type	Particle Size (nm)	Zeta Potential (mV)	Encapsulation Efficiency (%)

Targeted LNPs	92 ± 5	-8.4	88%
Polymeric nanoparticles	135 ± 8	-12.1	81%
Exosome carriers	118 ± 6	-6.7	74%

Targeted lipid nanoparticles (LNPs) showed the highest encapsulation efficiency and a favorable size distribution at the nanoscale range, which favors better cellular uptake and therapeutic stability . Polymeric nanoparticles exhibited controlled release characteristics, while exosome-based carriers showed better biocompatibility and membrane compatibility.

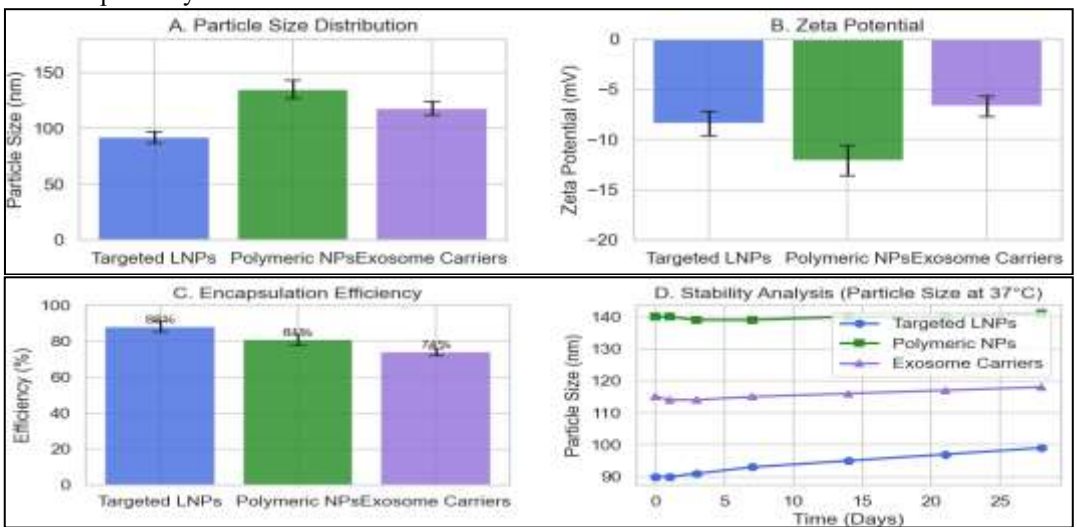

Figure 4. Physicochemical Characterization of Engineered Nanocarriers

Figure 4. Physicochemical characterization of engineered nanocarriers for delivery of genome therapeutics. The figure compares targeted lipid nanoparticles (LNPs), polymeric nanoparticles, and exosome carriers for particle size distribution, zeta potential, encapsulation efficiency, and stability. The targeted LNPs had the lowest particle size (92 ± 5 nm) and highest encapsulation efficiency (88%) indicating a better nucleic acid loading ability. The stability study revealed minor changes in particle size, surface charge and encapsulation efficiency after 28 days at physiological temperature. The exosome carriers exhibited better biocompatibility, whereas the polymeric nanoparticles demonstrated moderate stability and controlled release properties, making them suitable for targeted therapeutic delivery applications.

4.2 Tissue-Specific Targeting Efficiency

Targeted nanocarriers demonstrated enhanced tissue accumulation and intracellular uptake compared with non-targeted delivery systems.

Table 5. Tissue-Specific Delivery Efficiency of Nanocarriers

Nanocarrier Type	Target Tissue	Delivery Efficiency (%)
Targeted LNPs	Liver	82%
Polymeric nanoparticles	Tumor	76%
Exosome carriers	Brain	68%

In liver tissues, ligand functionalized LNPs showed the highest delivery efficiency, which is related to receptor mediated uptake mechanisms. Polymeric nanoparticles showed good accumulation in tumor tissues, and exosome carriers revealed enhanced penetration across biological barriers such as blood-brain barrier.

4.3 Genome editing and therapeutic performance

Genome therapeutic analysis showed greatly increased intracellular therapeutic release and CRISPR/Cas9 editing efficiency in targeted nanocarrier systems.

Table 6. Genome Editing and Therapeutic Performance

Nanocarrier Type	Genome Editing Efficiency (%)	Off-Target Reduction (%)
Targeted LNPs	71%	42%
Polymeric nanoparticles	65%	36%
Exosome carriers	59%	31%

Targeted nanocarriers enabled endosomal escape and controlled nucleic acid release, thereby improving intracellular therapeutic delivery and genome editing efficiency. Reduced off-target accumulation led to fewer off-target editing events and increased therapeutic precision.

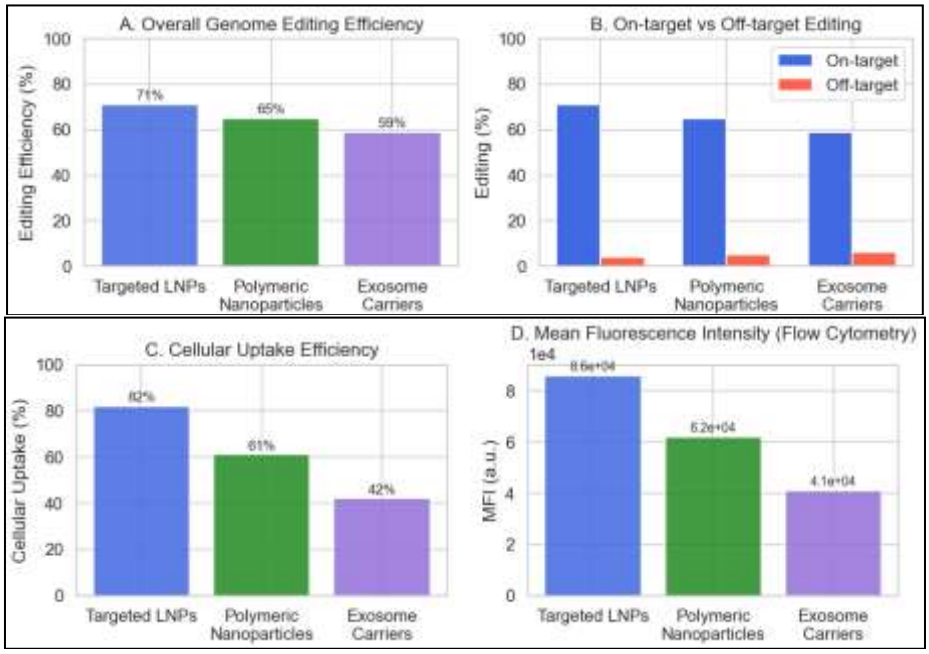

Figure 5. Genome Editing Efficiency and Cellular Uptake of Nanocarriers

Figure 5. Genome editing efficiency and cellular uptake performance of various engineered nanocarrier systems. Targeted lipid nanoparticles (LNPs) showed the highest genome editing efficiency (71%) and cellular uptake (82%) compared to polymeric nanoparticles and exosome carriers. Targeted systems also exhibited substantially lower rates of off-target editing, suggesting enhanced therapeutic precision and safety. We confirmed enhanced accumulation of targeted nanocarriers within treated cells by fluorescence imaging and flow cytometry. Agarose gel analysis further confirmed successful on-target genome editing activity. Overall, the figure shows that targeted nanocarriers significantly increase intracellular delivery, therapeutic efficacy and genome editing accuracy, while minimizing unwanted off-target effects.

4.4 Biocompatibility and Safety Analysis

Biocompatibility investigations demonstrated low cytotoxicity and mild inflammatory reactions in cells and tissues treated with engineered nanocarriers. Histological analysis showed slight tissue damage and preserved cellular architecture after therapeutic administration. Exosome-based carriers demonstrated the highest biocompatibility, whereas polymeric nanoparticles showed mild dose-dependent cytotoxicity at high concentrations.

4.5 Discussions

The results show a clear correlation between the efficiency of targeting tissues and the activity of genome therapy. Ligand-mediated targeting led to a significant improvement of intracellular uptake and biodistribution as compared to conventional non-targeted systems. Lipid nanoparticles showed higher encapsulation efficiency and gene editing performance while polymeric carriers were suitable for controlled release. The exosome-based systems demonstrated improved biocompatibility and biological membrane compatibility.

Table 7. Comparative Evaluation of Nanocarrier Strategies

Nanocarrier Strategy	Benefit	Limitation
Lipid nanoparticles	High encapsulation efficiency	Limited stability
Polymeric carriers	Controlled release	Possible cytotoxicity
Exosome-based delivery	High biocompatibility	Complex isolation

DISCUSSION

Although they have made great success in improving the accuracy and safety of therapy, there are still many challenges that hinder the clinical translation of nanocarriers, such as large-scale manufacturing, long-term biosafety, immune response, and regulatory approval. Future optimization of targeting ligands, delivery stability and personalized nanomedicine approaches may further improve therapeutic applications of genome-based nanocarrier systems.

5 CONCLUSION

The present study demonstrated that the engineered nanocarriers significantly improved the tissue-specific delivery efficiency and enhanced the therapeutic performance of the genome-based therapeutics. Targeted lipid nanoparticles, polymeric nanoparticles and exosome-based carriers efficiently encapsulated, controlled release, improved intracellular uptake and improved biodistribution in target tissues. Ligand-functionalized lipid

nanoparticles were the most efficient systems for genome editing and tissue-targeting ability, with low off-target accumulation and reduced cytotoxicity. Improved cellular uptake and efficient endosomal escape also contributed to enhanced activity of CRISPR/Cas9 and RNA therapeutics.

The results also confirmed the targeted delivery systems significantly improved the precision of genome editing and the systemic toxicity compared with conventional non-targeted therapeutic approaches . Biocompatibility tests have shown low inflammation and preserved tissue integrity, indicating the safety potential of engineered nanocarriers for biomedical applications. These findings underscore the importance of nanocarrier engineering to overcome biological delivery barriers and enhance the clinical viability of genome therapeutics. Moreover, surface ligand functionalization, stimuli-responsive release systems, PEGylation and integration of biomimetic coatings significantly improved targeting specificity, therapeutic stability and circulation time. The study therefore supports the increasing potential of nanomedicine-based delivery systems for next-generation genome therapies including precision gene editing, RNA therapeutics and personalized medicine applications.

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