Biomimetic Cell Membrane-Coated Nanocarriers for CRISPR-Cas9 Delivery: Recent Advances, Challenges and Future Perspectives

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Abstract

The CRISPR-Cas9 system revolutionized genome editing and is a very promising platform for the treatment of cancers, genetic disorders, and infections. Yet the clinical translation of CRISPR-Cas9 is hampered by some major bottlenecks related to delivery issues, such as fast degradation, poor cellular uptake, and immunogenicity. While synthetic nanovehicles like liposomes and metal-organic frameworks enhance the transport of cargos, they may cause systemic toxicity, rapid elimination, and lack specificity in targeting. Cell membrane-coated nanoparticles (CMNPs), which appear to be a biomimetic tool to address this issue, can take advantage of the properties of mammalian membranes, providing these carriers with immune camouflaging, extended circulation time, and targeted delivery. The current review gives an overview of CRISPR-Cas9 delivery challenges, CMNPs fabrication methods, biological benefits, and applications in cancer treatment, inflammation, and targeted gene therapy. At last, we discuss the important aspects that include mass production of CMNPs, membrane orientation, and biosafety.

Keywords: CRISPR-Cas9, Gene editing, Cell membrane-coated nanoparticles, Biomimetic nanotechnology, Drug delivery, Precision medicine, Genome engineering, red blood cell membrane, Cancer cell membrane, Platelet membrane, Macrophage membrane, Lipid nanoparticles, Immune evasion, Targeted delivery, Gene therapy, Nanocarriers, Oncology, Genetic disorders, Inflammatory diseases, Biosafety.

1. Introduction

The discovery of CRISPR-Cas9 technology has revolutionized the field of precision medicine, providing means for DNA editing, mutation correction, and gene regulation. Nevertheless, there is still an issue with the effective delivery of the cargo that should include protection, efficient transfer, and bypassing of the immune response. The first viral vector-based methods were efficient, but were hampered due to the limited capacity, toxicity, and expensive production process; hence, non-viral carriers like lipid nanoparticles, polymer micelles, metal-organic frameworks,

and mesoporous silica nanoparticles were developed. Despite the fact that the latter carriers show improved properties in terms of safety and scalability, they are easily eliminated by the immune system. In order to address this problem, the new approach based on the utilization of cell membrane coated nanoparticles has been introduced as a biomimic method that possesses properties of both the synthetic carrier and natural cell membrane, including immune evasion and targeting.

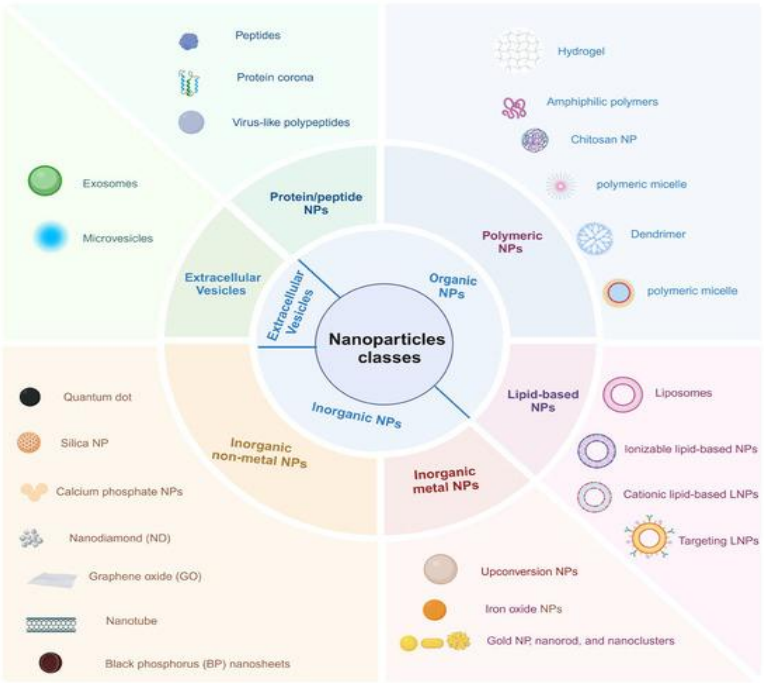

Figure 1: The nanoparticles used for CRISPR- Cas9 delivery [7]

2. Challenges in CRISPR

Delivery The ability to deliver CRISPR-Cas9 for successful therapy has a lot to do with overcoming problems related to delivery more than editing efficiency. The unprotected elements, once administered via injection into the bloodstream, are very quickly digested by blood enzymes; therefore, it is necessary to use some kind of nanoparticle protection. In turn, such particles very quickly develop a "protein corona" because of the contact with serum proteins and become a target of the mononuclear phagocyte system, which causes accumulation of such particles not in the target tissue but in the liver and spleen. The Cas9 protein comes from bacteria; therefore, the immune system sees it as an alien element and uses T-cells to eliminate cells where the genes have been edited. Even in case the nanoparticles get into the target cells, they have to escape degrading endosomes to avoid degradation in the acidic lysosomes.

3. Nanocarriers for CRISPR Delivery

To insulate CRISPR-Cas9 from systemic degradation and facilitate cellular entry, various vectors have been engineered. Viral vectors, including AAVs, adenoviruses, and lentiviruses, possess naturally evolved mechanisms for highly efficient cellular transduction.[1] However, their utility in CRISPR delivery is compromised by severe packaging

size constraints, high immunogenicity, and the potential for insertional mutagenesis.[1] Consequently, non-viral synthetic nanocarriers have become the focal point of advanced delivery research.

Lipid Nanoparticles (LNPs) utilise ionizable cationic lipids to condense nucleic acids, facilitating endosomal escape via lipid fusion in acidic environments.[5] Metal-Organic Frameworks (MOFs), particularly zeolitic imidazolate frameworks (e.g., ZIF-8), have emerged as extraordinary carriers due to their ability to co-precipitate around massive Cas9 RNP complexes under mild aqueous conditions, achieving exceptionally high loading efficiencies.[5] Mesoporous Silica Nanoparticles (MSNs) offer tunable pore sizes that can physically accommodate Cas9 RNPs, though they often require complex capping strategies to prevent premature payload leakage.[6] Polymeric nanoparticles, such as those formulated with polyethylenimine (PEI), efficiently condense CRISPR plasmids into tight polyplexes but frequently exhibit dose-limiting cytotoxicity due to their high cationic charge density.[5]

While these synthetic cores provide robust cargo protection and structural tunability, their lack of inherent biological specificity prevents optimal *in vivo* performance. To bridge this gap, researchers have turned to biomimetic strategies, utilizing natural cell membranes to functionalize these synthetic cores.

4. Cell Membrane-Coated Nanocarriers

Cell membrane-coated nanocarriers represent a biomimetic delivery system where natural cell membranes, complete with their native proteins and lipids, are used to encase synthetic nanoparticles.

4.1. Red Blood Cell (RBC) Membrane

It typically travel for around 120 days while evading immune clearance, largely due to the surface protein CD47 that sends a "don't eat me" signal to macrophages. When CRISPR-loaded nanoparticles are coated with RBC membranes, their circulation time is significantly extended, boosting the chances of reaching target tissues. Furthermore, genetically modified RBC membranes can display targeting peptides like NGR for tumor targeting or LPXTG motifs for enzyme-mediated peptide attachment, allowing more accurate delivery and potential treatment of conditions such as autoimmune encephalomyelitis.

4.2. Cancer Cell Membrane (CCM)

It employs homotypic binding, where cancer cells recognize and attach to similar types through adhesion molecules like E-cadherin and EpCAM. CCM-coated nanoparticles leverage this feature to selectively gather in primary tumors and metastatic locations, enhancing CRISPR-Cas9 targeting while minimizing off-target effects on healthy tissues. These membranes also carry tumor-associated antigens, serving as both targeted delivery systems and cancer vaccines. Additionally, immune-evasive proteins like CD47 and PD-L1 help safeguard the nanoparticles from being cleared by the immune system.

4.3. Platelet Membrane

They inherently bind to damaged blood vessels, inflamed tissues, circulating tumor cells, and atherosclerotic plaques through their surface glycoproteins and integrins. Nanoparticles coated with platelet membranes utilize these natural

interactions to deliver CRISPR payloads precisely to areas of vascular injury, inflammation, and tumors. They have shown promise in targeting atherosclerotic plaques by silencing disease-related genes and in transporting gene-editing materials to circulating tumor cells protected within platelet-rich microthrombi, thereby reducing metastasis.

4.4. Macrophage Membrane

It naturally move toward sites of inflammation, infection, and tumors in response to chemokine signals. Nanoparticles coated with macrophage membranes take advantage of this ability for targeted delivery of CRISPR components to inflamed tissues. Their surface receptors can also bind to and neutralize inflammatory cytokines and endotoxins, providing additional anti-inflammatory effects beyond gene editing itself. This dual function positions macrophage membrane-coated nanocarriers as a promising approach for treating inflammatory diseases and cancer.

5. Mechanisms Enhancing Gene Editing Efficiency

The fusion of biomimetic cell membranes with synthetic cores creates synergistic mechanisms that significantly augment the ultimate efficiency of CRISPR-Cas9 gene editing, addressing both extracellular navigation and intracellular processing.[2] While bare synthetic nanoparticles rely on passive, non-specific uptake mechanisms, CMNPs engage in highly efficient receptor-mediated endocytosis.[2] For example, the presence of specific surface antigens on the cell membrane coating facilitates strong binding to complementary receptors overexpressed on target cells.[2] This active biological docking increases the absolute quantity of nanocarriers internalized and directs the vehicles into specific intracellular trafficking pathways.[2] To maximize editing efficiency and minimize off-target temporal exposure, modern CMNPs are often engineered with stimulus-responsive cores that release the CRISPR-Cas9 machinery only upon encountering specific biochemical hallmarks of the target intracellular environment.[1] The acidic microenvironment of tumors and endolysosomes is frequently exploited. MOF cores like ZIF-8 undergo rapid structural collapse in acidic environments, releasing the Cas9 RNP while simultaneously rupturing the endosome via the proton sponge effect.[9] Alternatively, ATP-responsive systems, such as ZIF-90, disassemble selectively in the presence of high intracellular ATP concentrations, ensuring cargo release exclusively within the cytoplasm of metabolically active cells.[5] Exogenous triggers are also utilized; biomimetic platforms paired with upconversion nanoparticles (UCNPs) respond to deep-tissue penetrating near-infrared (NIR) light, breaking photolabile bonds to trigger spatiotemporally controlled payload release.[3]

6. Applications

The integration of CRISPR-Cas9 with cell membrane-coated nanocarriers and endogenous vesicle platforms has catalyzed significant preclinical breakthroughs across multiple disease paradigms.[1]

6.1. Cancer Therapy

The main strategy for CRISPR-Cas9 gene therapy in cancers is to inhibit oncogenes or other relevant genes. In the

case of pancreatic cancer, exosomes derived from mesenchymal stem cells (MSCs) were loaded with CRISPR-Cas9 for the treatment of KRAS G12D mutation and were found to inhibit tumor progression by blocking the activation of ERK pathway. In the case of breast cancer, a cancer cell membrane-encapsulated MOF system was reported to be highly efficient in homotypic targeting and editing of the target genes in MCF-7 cancer cells. For the treatment of HNSCC, a dual-responsive cancer cell membrane-encapsulated system was developed to co-deliver CRISPR-Cas9 along with telaglenastat which blocks glycolysis and glutaminolysis.

6.2. Genetic Disorders and Inflammatory Diseases

Cell membrane-coated nanoparticles (CMNPs) have demonstrated great potential in CRISPR-Cas9 delivery, but they face challenges in manufacturing and biosafety. The production process includes cell culture, membrane isolation, purification and coating with nanoparticles, which is expensive and difficult to standardise for large-scale manufacturing. Batch consistency and the complete removal of residual DNA, RNA and intracellular components are still major problems. Correct orientation of the membrane is critical to keep proteins such as CD47 and integrins for efficient immune evasion and targeting. In addition, membranes derived from cancer cells may contain oncogenic or immunosuppressive molecules, and allogeneic blood cell membranes must be blood-group compatible. Clinical translation needs strict quality control and safety assessment.

6.3. Infectious Diseases

HBV infection is chronic due to the presence of covalently closed circular DNA (cccDNA) in the nuclei of infected liver cells. Current treatments of HBV are effective in suppressing the replication of virus, but not able to eradicate the reservoir. Therefore, a biomimetic nanosystem based on NIR-responsive nanoparticles and Cas9 RNPs against the genome of HBV, which was enclosed by the membrane from the host cells for specific delivery to the liver, has been established. The cleavage and inactivation of latent HBV cccDNA can be achieved through NIR-induced release of Cas9 RNPs.

7. Challenges and Limitations

Cell membrane coated nanoparticles (CMNPs) for CRISPR-Cas9 delivery hold great promise in therapeutics but suffer from significant manufacturing and biosafety challenges. The production process includes large-scale cell culture, membrane isolation and several purification steps and is therefore labor intensive, expensive and difficult to scale with batch-to-batch consistency. Residual DNA, RNA, and intracellular contaminants must be completely removed to avoid unwanted immune or biological responses. It is also important to preserve the right-side-out orientation of the membrane, as improper coating can mask important surface proteins like CD47 and integrins, which reduces immune evasion and targeting efficiency. Furthermore, cancer cell-derived membranes may carry oncogenic, pro-angiogenic or immunosuppressive molecules, raising safety concerns. Likewise, allogeneic red blood cell or platelet membranes must be carefully tested for blood group compatibility to avoid immune reactions. Therefore, rigorous quality control and comprehensive safety evaluation are essential for successful clinical translation.

8. Future Perspectives

Combining biomimetics and synthetic biology is likely to lead to an improvement in the delivery of CRISPR technology using cell membrane-coated vectors. Genetic engineering of the donor cells as well as hybrid membrane production is likely to increase accuracy, effectiveness, and evasion of the immune system. To surmount the hurdles associated with large-scale production, sophisticated manufacturing methodologies, such as microfluidic electroporation and automated extrusion systems, are under development. These advanced techniques aim to ensure consistent membrane coating, appropriate protein orientation, and reproducible product quality. Furthermore, engineered extracellular vesicles (EVs) and exosomes present a compelling alternative for CRISPR delivery. Platforms like safeEXQ, for instance, produce RNA-depleted exosomes that can be loaded with Cas9 ribonucleoproteins (RNPs) and subsequently functionalized with tissue-specific targeting molecules. This approach enables efficient and precise genome editing. Collectively, these advancements are poised to accelerate the clinical application of biomimetic CRISPR nanocarriers, paving the way for safer, more targeted, and highly effective gene-editing therapies applicable to a broad spectrum of diseases.

9. Conclusion

The integration of the CRISPR-Cas9 genome editing system with biomimetic cell membrane-coated nanocarriers represents one of the most sophisticated and promising frontiers in modern molecular medicine. By expertly masking the immunogenic and structurally cumbersome CRISPR machinery within synthetic cores—such as metal-organic frameworks and responsive polymers—and subsequently cloaking these cores with the native membranes of red blood cells, platelets, macrophages, or cancer cells, researchers have successfully navigated the stringent biological barriers that have historically stifled gene therapy. These bio-inspired platforms offer unprecedented solutions to the drug delivery dilemma, simultaneously maximizing cargo protection, ensuring precise tissue homing, and mitigating catastrophic off-target mutagenesis. From the targeted knockout of oncogenes in aggressive malignancies to the resolution of chronic inflammation and the eradication of latent viral DNA, the therapeutic applications of this technology are exceptionally broad. While significant challenges regarding manufacturing scalability, membrane orientation control, and strict regulatory safety oversight remain, continuous advances in microfluidic fabrication, genetic engineering of source cells, and hybrid membrane fusion are rapidly dismantling these final hurdles. Ultimately, biomimetic CRISPR-Cas9 delivery systems stand poised to transition from the realm of preclinical innovation to transformative clinical realities, unlocking the full curative potential of precision genetic medicine.

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