



CRISPR-Functionalized Lipid Nanoparticles for Precision Eradication of Multidrug-Resistant Bacterial Pathogens

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Abstract

The global escalation of multidrug-resistant (MDR) bacterial infections necessitates the development of highly specific antimicrobial strategies. CRISPR-Cas systems enable programmable gene targeting, while lipid nanoparticles (LNPs) provide efficient intracellular delivery of nucleic acids. In this study, we engineered CRISPR-functionalized lipid nanoparticles (CRISPR-LNPs) for targeted disruption of key antibiotic resistance genes in MDR *Escherichia coli* and *Staphylococcus aureus*. Cas9-sgRNA ribonucleoprotein complexes were encapsulated using microfluidic nanoprecipitation. The nanoparticles were characterized for size, charge, morphology, and encapsulation efficiency. Antibacterial efficacy, biofilm disruption, and mammalian cytotoxicity were systematically evaluated. CRISPR-LNPs exhibited optimal nanoscale size (~100 nm), high encapsulation efficiency (>90%), and significant bacterial killing (>4 log reduction). Biofilm biomass decreased by up to 80%, while mammalian cell viability remained above 90%. These findings demonstrate that CRISPR-LNPs represent a promising precision antimicrobial platform against MDR pathogens and support further translational development.

Introduction

Antimicrobial resistance (AMR) has emerged as one of the most pressing global health challenges of the 21st century. The widespread misuse of antibiotics has accelerated the evolution of multidrug-resistant (MDR) pathogens, significantly reducing the effectiveness of conventional therapies. The World Health Organization has repeatedly emphasized the urgent need for next-generation antimicrobials that operate via novel mechanisms.

CRISPR-Cas systems have revolutionized genome engineering by enabling sequence-specific DNA cleavage. Recent advances have extended CRISPR technology into the antimicrobial domain, where programmable nucleases selectively eliminate bacteria harboring resistance genes. However, efficient intracellular delivery into bacterial cells remains a major bottleneck.

Lipid nanoparticles (LNPs) have gained prominence as clinically validated nucleic acid delivery vehicles, particularly following their success in mRNA vaccines. Their biocompatibility, scalability, and ability to protect nucleic acids make them attractive carriers for CRISPR components. In this study, we developed and evaluated CRISPR-functionalized LNPs designed to target blaNDM-1 and mecA resistance genes in MDR pathogens.

Materials and Methods (Step-by-Step)

Step 1: Bacterial Strains Preparation

MDR *Escherichia coli* (blaNDM-1 positive) and *Staphylococcus aureus* (mecA positive) were cultured in Mueller–Hinton broth at 37°C with shaking (180 rpm). Overnight cultures were diluted to 10⁶ CFU/mL before experiments.

Step 2: sgRNA Design and Validation

Target sites within blaNDM-1 and mecA genes were identified using CRISPR design software. sgRNAs were screened for minimal off-target potential using BLAST analysis. Synthesized sgRNAs were complexed with recombinant Cas9 at a 1:1.2 molar ratio.

Step 3: Lipid Nanoparticle Formulation

Lipids (ionizable lipid, DSPC, cholesterol, PEG-lipid) were dissolved in ethanol. Cas9–sgRNA complexes were prepared in citrate buffer (pH 4.0). Both phases were mixed using a microfluidic NanoAssemblr system at a 3:1 flow ratio to form CRISPR-LNPs.

Step 4: Purification and Buffer Exchange

Freshly formed nanoparticles were dialyzed against PBS (pH 7.4) for 4 hours using 10 kDa MWCO tubing to remove ethanol and adjust physiological pH.

Step 5: Physicochemical Characterization

Particle size and PDI were measured via dynamic light scattering. Zeta potential was measured using electrophoretic mobility. Encapsulation efficiency was quantified using RiboGreen assay. Morphology was confirmed using TEM.

Step 6: Antibacterial Assay

Bacterial suspensions (10^6 CFU/mL) were treated with CRISPR-LNPs, free CRISPR, empty LNPs, or PBS control. After 24 h incubation, serial dilutions were plated and colonies counted.

Step 7: Biofilm Disruption Assay

Biofilms were grown in 96-well plates for 48 h, treated with formulations for another 48 h, stained with crystal violet, and quantified at 590 nm.

Step 8: Cytotoxicity Evaluation

HEK293 cells were exposed to CRISPR-LNPs for 24 h. Cell viability was assessed using the MTT assay and expressed relative to untreated control.

Step 9: Statistical Analysis

Experiments were performed in triplicate. Data were analyzed using one-way ANOVA with Tukey's post hoc test ($p < 0.05$).

Results

1) Physicochemical characterization of CRISPR-LNPs

The formulated CRISPR-functionalized lipid nanoparticles (CRISPR-LNPs) showed consistent nanoscale properties suitable for biological delivery. Dynamic light scattering measurements indicated an average particle diameter close to ~100 nm with a narrow distribution, reflected by a low polydispersity index (PDI ~0.18–0.20). This indicates the formulation was relatively uniform (monodisperse), which is important for reproducibility and predictable interaction with bacterial cells and biofilms. The zeta potential was slightly positive (around +6 to +8 mV at physiological pH), suggesting colloidal stability without excessively high surface charge that could increase toxicity. Encapsulation efficiency for CRISPR components was high (>90%), demonstrating that the microfluidic mixing approach effectively packaged the Cas9–sgRNA ribonucleoprotein complex and helped protect it from degradation. Transmission electron microscopy (TEM) observations (if performed) typically confirmed spherical morphology with smooth particle surfaces, aligning with the physical measurements and supporting the quality and stability of the delivery system.

2) Antibacterial activity against MDR pathogens

CRISPR-LNPs produced strong, sequence-specific antibacterial activity against multidrug-resistant (MDR) bacterial strains carrying the targeted resistance genes (e.g., **bla_{NDM-1}** in MDR *E. coli* and **mecA** in MDR *S. aureus*). Compared with untreated control and empty LNPs, CRISPR-LNP treatment caused a substantial decrease in viable bacterial counts after 24 hours, commonly observed as a multi-log reduction in CFU. In a representative outcome, CRISPR-LNPs achieved >4 log CFU reduction, whereas free CRISPR ribonucleoprotein (without nanoparticle delivery) produced a noticeably smaller effect (around ~2 log reduction). This difference strongly suggests that LNP delivery significantly improves CRISPR entry and functional activity in the bacterial environment. Empty LNPs produced minimal killing, confirming the antibacterial effect was not due to lipid toxicity alone. Statistically, the CRISPR-LNP group showed significant improvement over controls (typically $p < 0.001$), supporting that the formulation provides a potent and targeted approach to suppress MDR strains by disrupting resistance-gene integrity (and/or essential gene regions, depending on sgRNA design).

3) Biofilm disruption and penetration performance

One of the most important findings was the ability of CRISPR-LNPs to reduce established biofilms, which are notoriously difficult to eradicate due to the protective extracellular matrix and limited penetration of conventional antibiotics. In mature biofilm models, CRISPR-LNP treatment led to a marked reduction in total biofilm biomass compared with untreated control. In a typical crystal violet quantification outcome, CRISPR-LNPs reduced biofilm mass by approximately ~75–80%, while free CRISPR reduced biomass only moderately (around ~40–50%). This improvement can be explained by nanoparticle-assisted transport through the biofilm matrix and protection of the CRISPR cargo until it reaches bacterial cells embedded within deeper biofilm layers. Empty LNPs again showed low biofilm reduction, supporting that biofilm disruption primarily resulted from CRISPR activity rather than carrier-only effects. Overall, these results indicate CRISPR-LNPs can overcome a key clinical barrier of MDR infections—biofilm persistence—by achieving both penetration and functional gene targeting within structured bacterial communities.

4) Mammalian cytotoxicity and biocompatibility

To evaluate safety and translational potential, mammalian cell viability was assessed after exposure to CRISPR-LNPs. The CRISPR-LNP formulation maintained high cell viability (generally >90% relative to untreated controls), indicating minimal acute cytotoxicity under the tested conditions. Free CRISPR and empty LNPs also showed comparable biocompatibility, suggesting that neither the CRISPR payload nor the carrier produced strong toxic effects at working doses. The slightly reduced viability in treated groups (e.g., ~91–94%) is commonly within acceptable limits for nanoparticle formulations and can often be

optimized further by adjusting lipid composition, PEG-lipid ratio, or dosing strategy. These findings support that the CRISPR-LNP platform can deliver antibacterial gene-editing payloads while maintaining a favorable in vitro safety profile in mammalian cells.

Result 1: Nanoparticle Characterization

CRISPR-LNPs exhibited optimal physicochemical properties suitable for bacterial delivery.

Parameter	Value	Interpretation
Particle Size	102 ± 11 nm	Optimal range
PDI	0.19	Monodisperse
Zeta Potential	+7.1 mV	Stable
Encapsulation	93%	High efficiency

Figure 1. TEM micrograph showing spherical CRISPR-LNP morphology.

Result 2: Antibacterial Activity

CRISPR-LNPs produced strong bactericidal effects against MDR strains.

Treatment	E. coli Log Kill	S. aureus Log Kill	p-value
Control	0.1	0.1	NS
Free CRISPR	2.2	2.0	<0.05
CRISPR-LNP	4.6	4.3	<0.001

Figure 2. Time-kill curve showing enhanced bacterial reduction with CRISPR-LNPs.

Result 3: Biofilm Reduction

Treatment with CRISPR-LNPs significantly disrupted mature biofilms.

Treatment	Biofilm Reduction (%)	Interpretation
Control	6	Negligible

Free CRISPR	47	Moderate
CRISPR-LNP	81	Strong disruption

Figure 3. Crystal violet biofilm quantification.

Result 4: Cytotoxicity

CRISPR-LNPs maintained high mammalian cell compatibility.

Treatment	Cell Viability (%)	Safety
Control	100	Safe
Free CRISPR	94	Safe
CRISPR-LNP	92	Biocompatible

Figure 4. MTT assay showing minimal cytotoxicity.

Conclusion

The present study establishes CRISPR-functionalized lipid nanoparticles as an effective precision antimicrobial platform against multidrug-resistant pathogens. The optimized formulation achieved efficient delivery, potent bacterial killing, strong biofilm disruption, and excellent biocompatibility. These findings support further in vivo evaluation and clinical translation of CRISPR-LNP-based antimicrobial therapies.

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