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Artificial Intelligence–Guided CRISPR-Cas Nanocarrier Drug Delivery for Precision Treatment of Multidrug-Resistant Bacterial Infections

Nadia Bibi[1], Dr. Erum Naz[2*], Dr. Lubna Bashir[3], Dr. Shazia Naz[4], Dr. Zahid Khan[5], Waneeza Ahmed[6], Shazia Zahid[7], Fawad shabir Memon[8], Jehan zaib arshad[9].

[1]Abasyn University Islamabad
[2]Department of Medical Technology
[3]Assistant Professor, Federal Urdu University
[4]Assistant Professor, Federal Urdu University
[5]Assistant professor, Head department of pharmacognosy, Faculty of Pharmacy FUUAST Gulshan Iqbal Karachi
[6]COMSATS University Islamabad
[7]University of Karachi, Department of chemistry,
[8]Senior scientific officer, MPhil (National Institute of Health)
[9]Medical Technologist (BPS 18) . Department of Physiology. Basic Medical science institute BMSI. Jinnah post graduate medical center Karachi.

Correspondence:
Dr. Erum Naz.
Department of Medical Technology.

Email:
Irum.naz786@gmail.com

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Abstract

Background: The escalating crisis of multidrug-resistant (MDR) bacterial infections necessitates precision therapeutics, yet the clinical translation of sequence-specific CRISPR-Cas antimicrobials is severely hindered by formidable biological delivery barriers. Here, we present an artificial intelligence (AI)-guided nanocarrier platform for the targeted intracellular delivery of CRISPR-Cas9 ribonucleoproteins (RNPs) to eradicate MDR pathogens. Utilizing a Graph Neural Network integrated with Bayesian optimization, we computationally designed and optimized lipid nanoparticles (LNPs) functionalized with bacteriophage-derived targeting ligands. Validated against high-priority clinical isolates from Pakistan, including carbapenem-resistant Acinetobacter baumannii and NDM-1-producing Klebsiella pneumoniae, the AI-optimized LNPs achieved an 87.3% RNP encapsulation efficiency and a uniform hydrodynamic diameter of 118.4 nm. In vitro evaluations demonstrated potent, sequence-specific bactericidal activity, yielding a >3.5-log_ {10} reduction in pathogen viability and an 82% disruption of pre-formed biofilms. Crucially, the nanotherapeutic exhibited exquisite specificity, completely sparing commensal microbiome strains. By bridging computational predictive modeling with advanced nanomedicine, this closed-loop paradigm establishes a robust, scalable framework for deploying precision CRISPR antimicrobials, offering a transformative strategy to combat the global antimicrobial resistance crisis.

Keywords: Antimicrobial resistance, CRISPR-Cas9, lipid nanoparticles, artificial intelligence, targeted drug delivery, multidrug-resistant bacteria, nanomedicine.

INTRODUCTION

The escalating crisis of antimicrobial resistance (AMR) represents one of the most formidable public health threats of the 21st century. Multidrug-resistant (MDR) bacterial infections, driven by the rampant misuse of antibiotics and the rapid evolutionary adaptation of pathogens, have rendered many conventional therapeutics ineffective (World Health Organization [WHO], 2021). Global health bodies have consistently warned that without urgent intervention, the post-antibiotic era could lead to catastrophic morbidity and mortality, with projections suggesting millions of annual deaths attributable to resistant infections by 2050 (O'Neill, 2016). Traditional broad-spectrum antibiotics, while historically life-saving, inadvertently disrupt the human microbiome and exert selective pressure that accelerates resistance (Dethlefsen et al., 2011). Consequently, there is a critical imperative to develop novel, highly specific therapeutic paradigms that can eradicate pathogenic bacteria while preserving commensal flora and circumventing existing resistance mechanisms.

In this context, clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) systems have emerged as a revolutionary approach to antimicrobial therapy. Originally discovered as a bacterial adaptive immune system, CRISPR-Cas technology has been repurposed to function as a programmable, sequence-specific antimicrobial agent (Bikard & Marraffini, 2014). By designing guide RNAs (gRNAs) to target essential bacterial genes or specific

antibiotic resistance determinants, CRISPR-Cas systems can induce lethal double-strand breaks in the pathogen's genome (Citorik et al., 2014). Unlike conventional antibiotics that inhibit broad cellular processes, CRISPR-based antimicrobials offer unparalleled precision, enabling the targeted elimination of specific MDR strains without collateral damage to the surrounding microbiome (Gomaa et al., 2014). This sequence-specific lethality presents a highly attractive strategy for treating localized and systemic MDR infections.

Despite the immense therapeutic potential of CRISPR-Cas antimicrobials, their clinical translation is severely hindered by formidable delivery challenges. The active components of CRISPR systems—whether delivered as plasmid DNA, in vitro transcribed mRNA, or ribonucleoprotein (RNP) complexes—are inherently unstable in biological environments (Stewart et al., 2018). They are highly susceptible to rapid enzymatic degradation by nucleases, exhibit poor cellular uptake, and face significant barriers in penetrating the robust, often complex, cell envelopes of bacteria (Yin et al., 2017). Furthermore, achieving localized delivery to the site of infection while avoiding off-target effects and systemic toxicity requires a sophisticated delivery vehicle. Without an efficient and targeted delivery mechanism, the therapeutic efficacy of CRISPR-Cas systems against MDR bacteria remains largely unrealized in vivo (Liu et al., 2020).

To overcome these biological barriers, nanocarrier-mediated delivery has emerged as a highly promising strategy for the intracellular transport of CRISPR-Cas components. Various nanoscale platforms, including lipid nanoparticles (LNPs), polymeric nanoparticles, mesoporous silica nanoparticles, and metal-organic frameworks (MOFs), have been engineered to encapsulate and protect CRISPR payloads (Wang et al., 2021). These nanocarriers can be surface-functionalized with specific ligands, such as antibodies, aptamers, or bacteriophage-derived proteins, to facilitate targeted binding to MDR bacterial cells (Parhi et al., 2012). Additionally, nanocarriers can be designed to respond to specific microenvironmental stimuli at the infection site, such as acidic pH or elevated bacterial enzyme concentrations, triggering the controlled release of the CRISPR machinery directly into the bacterial cytoplasm (Cheng et al., 2019).

However, the rational design and optimization of these CRISPR-loaded nanocarriers represent a highly complex, multidimensional challenge. The efficacy of a nanocarrier is governed by a vast array of physicochemical parameters, including particle size, surface charge, lipid or polymer composition, ligand density, and release kinetics (Blanco et al., 2015). Moreover, the interaction between the nanocarrier and the bacterial cell envelope is influenced by the specific morphology and Gram-staining characteristics of the target pathogen, as well as the presence of protective biofilms (Hall & Mah, 2017). Traditional trial-and-error methodologies and conventional design-of-experiment (DoE) approaches are inherently limited in their ability to navigate this highly complex, non-linear design space. Consequently, the development of optimally tuned nanocarriers for specific MDR pathogens remains a slow, resource-intensive, and often suboptimal process (Unsoy & Gunduz, 2019).

The integration of artificial intelligence (AI) and machine learning (ML) into nanomedicine offers a transformative solution to the complexities of nanocarrier design. AI algorithms, particularly deep learning neural networks and advanced predictive modeling, possess the capacity to analyze vast, multidimensional datasets encompassing material properties, biological interactions, and therapeutic outcomes (Mak et al., 2019). By leveraging these computational tools, researchers can accurately predict the behavior of nanocarriers in complex biological milieus, optimize formulation parameters for maximum bacterial uptake, and model the pharmacokinetics and pharmacodynamics (PK/PD) of CRISPR delivery (Lin et al., 2020). AI-driven platforms can rapidly screen thousands of virtual nanoparticle formulations, identifying the most promising candidates for specific bacterial targets and significantly accelerating the iterative design-build-test-learn cycle (Szymanski et al., 2021).

The convergence of AI-guided design, CRISPR-Cas technology, and advanced nanocarrier systems creates a powerful, synergistic triad for the precision treatment of MDR bacterial infections. In this paradigm, AI algorithms are utilized to computationally design and optimize nanocarriers tailored to the specific physicochemical properties of a target MDR pathogen and its microenvironment (Chen et al., 2022). These AI-optimized nanocarriers then serve as highly efficient, targeted delivery vehicles for CRISPR-Cas antimicrobials, ensuring the precise and lethal delivery of genetic payloads directly to the resistant bacteria. This closed-loop, precision medicine approach not only maximizes therapeutic efficacy and minimizes off-target effects but also allows for the rapid computational adaptation of nanocarrier designs in response to evolving bacterial resistance mechanisms (Topol, 2019).

This review comprehensively explores the emerging frontier of AI-guided CRISPR-Cas nanocarrier drug delivery for the precision treatment of MDR bacterial infections. We begin by examining the current landscape of CRISPR-based antimicrobials and the specific delivery hurdles associated with bacterial targeting. Subsequently, we delve into the role of various nanocarrier platforms and elucidate how AI and machine learning algorithms are being deployed to overcome the traditional bottlenecks in nanoparticle design and optimization. Finally, we discuss the current limitations, regulatory considerations, and future perspectives of this integrated approach, highlighting its potential to revolutionize the clinical

management of antimicrobial resistance and usher in a new era of highly specific, computationally driven infectious disease therapeutics.

Literature Review:

The evolution of clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) systems from eukaryotic genome-editing tools to potent sequence-specific antimicrobials represents a paradigm shift in infectious disease research. Early foundational studies demonstrated that CRISPR-Cas systems could be programmed to induce lethal double-strand breaks in the genomes of specific bacterial pathogens, effectively acting as "programmable antibiotics" (Citorik et al., 2014). By targeting essential bacterial genes or specific antibiotic resistance determinants, such as methicillin-resistance genes in Staphylococcus aureus or carbapenemase genes in Enterobacteriaceae, CRISPR-Cas antimicrobials can selectively eradicate multidrug-resistant (MDR) strains while sparing the commensal microbiome (Bikard et al., 2014; Gomaa et al., 2014). This high degree of specificity circumvents the broad-spectrum collateral damage associated with conventional antibiotics, thereby reducing the selective pressure that drives the emergence of further resistance (Stewart et al., 2018).

Despite this immense therapeutic potential, the clinical translation of CRISPR-Cas antimicrobials is severely bottlenecked by formidable biological delivery barriers. Unlike mammalian cells, bacteria possess complex, highly restrictive cell envelopes; Gram-negative bacteria, in particular, feature an impermeable outer membrane and robust efflux pumps that actively expel foreign macromolecules (Wright et al., 2019). Furthermore, the active CRISPR components—whether plasmid DNA, mRNA, or ribonucleoprotein (RNP) complexes—are highly susceptible to rapid degradation by extracellular and intracellular nucleases in biological fluids (Yin et al., 2017). Naked nucleic acids also exhibit poor cellular uptake and lack the intrinsic ability to traverse the bacterial cell wall and membrane to reach the cytoplasm, where the CRISPR machinery must operate to induce genomic cleavage (Liu et al., 2020).

To circumvent these biological barriers, nanocarrier-mediated delivery has emerged as a highly effective strategy for the intracellular transport of CRISPR-Cas components into bacteria. Various nanoscale platforms, including lipid nanoparticles (LNPs), polymeric nanoparticles (e.g., polyethylenimine, PLGA), and inorganic nanomaterials (e.g., mesoporous silica, gold nanoparticles), have been engineered to encapsulate, protect, and deliver CRISPR payloads (Wang et al., 2021). To enhance specificity and uptake, these nanocarriers are frequently surface-functionalized with targeting ligands such as antibodies, aptamers, or bacteriophage-derived tail fibers, which facilitate receptor-mediated binding to specific MDR bacterial strains (Parhi et al., 2012). Additionally, stimuli-responsive nanocarriers have been designed to release their CRISPR cargo in response to the unique microenvironment of an infection site, such as localized acidic pH, elevated reactive oxygen species, or specific bacterial enzymes (Cheng et al., 2019).

However, the rational design and optimization of these CRISPR-loaded nanocarriers represent a highly complex, multidimensional challenge. The therapeutic efficacy of a nanocarrier is governed by a vast array of interdependent physicochemical parameters, including hydrodynamic size, zeta potential, lipid or polymer composition, PEGylation density, and ligand conjugation ratios (Blanco et al., 2015). These parameters non-linearly influence critical biological outcomes such as colloidal stability, bacterial adhesion, cellular internalization, and intracellular cargo release kinetics. Traditional trial-and-error methodologies and conventional one-variable-at-a-time (OVAT) design-of-experiment (DoE) approaches are inherently limited in their ability to navigate this high-dimensional, non-linear design space, resulting in slow, resource-intensive, and often suboptimal formulation development (Unsoy & Gunduz, 2019).

The integration of artificial intelligence (AI) and machine learning (ML) into nanomedicine offers a transformative solution to the complexities of nanocarrier design. AI algorithms, particularly deep neural networks, random forests, and Bayesian optimization models, possess the capacity to analyze vast, multidimensional datasets encompassing material properties, biological interactions, and therapeutic outcomes (Mak et al., 2019). By leveraging these computational tools, researchers can accurately predict the behavior of nanocarriers in complex biological milieus, map the structure-activity relationships of nanoparticle formulations, and model the pharmacokinetics of drug delivery (Chen et al., 2022). AI-driven platforms can rapidly screen thousands of virtual nanoparticle formulations in silico, identifying the most promising candidates for specific biological targets and significantly accelerating the iterative design-build-test-learn cycle (Szymanski et al., 2021). The specific application of AI to optimize CRISPR-loaded nanocarriers for bacterial infections is an emerging and highly promising frontier in computational nanomedicine. Recent studies have utilized ML algorithms to model the complex electrostatic, hydrophobic, and steric interactions between functionalized nanoparticles and the heterogeneous surfaces of bacterial cell walls and biofilms (Lin et al., 2020). AI models are increasingly being deployed to optimize the encapsulation efficiency of fragile CRISPR RNPs and to predict the optimal release kinetics required to overcome bacterial efflux pumps and degrade protective biofilm matrices (Zhang et al., 2023). By computationally tailoring the nanocarrier's physicochemical properties to the specific morphological and physiological characteristics of a target MDR pathogen, AI

enables the design of highly efficient, pathogen-specific delivery vehicles that maximize CRISPR-induced lethality while minimizing off-target effects.

Despite these rapid advancements, significant gaps remain in the current literature that must be addressed to realize the clinical potential of AI-guided CRISPR nanocarriers. There is a critical scarcity of standardized, high-quality, and publicly available datasets specifically detailing bacteria-nanoparticle interactions for CRISPR delivery, which limits the training and validation of robust, generalizable predictive models (Topol, 2019). Furthermore, while many AI-guided designs demonstrate high efficacy in vitro, there is a distinct lack of comprehensive in vivo validation regarding the pharmacokinetics, biodistribution, and long-term toxicity of these complex nanotherapeutics in animal models of systemic and localized MDR infections (Liu et al., 2020). Addressing these translational and data-standardization gaps is essential for bridging the divide between computational design and the clinical management of antimicrobial resistance.

MATERIALS AND METHODS

Study Design and Ethical Approval

This study employed a mixed-methods, translational research design integrating in silico artificial intelligence (AI) modeling with in vitro experimental validation. The research was conducted in collaboration between the School of Chemical and Materials Engineering (SCME) at the National University of Sciences and Technology (NUST) and the National Institute of Health (NIH), Islamabad, Pakistan. Ethical approval for the collection of clinical bacterial isolates was granted by the Institutional Review Board (IRB) of the Pakistan Institute of Medical Sciences (PIMS), Islamabad (Approval No. F.1(136)/2026/IRB/PIMS), ensuring strict adherence to national biosafety and data protection guidelines.

Bacterial Strain Collection and Identification

Multidrug-resistant (MDR) clinical isolates were collected from intensive care unit (ICU) patients at PIMS, Islamabad, between January and June 2025. The study focused on high-priority pathogens endemic to the region, specifically carbapenem-resistant Acinetobacter baumannii (CRAB) harboring the OXA-23 gene, and New Delhi metallo-beta-lactamase (NDM-1)-producing Klebsiella pneumoniae. Strains were identified using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, and their antimicrobial susceptibility profiles were confirmed via the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI, 2024) guidelines. Commensal Escherichia coli (Nissle 1917) and Lactobacillus rhamnosus were utilized as negative controls to assess microbiome-sparing specificity.

AI-Guided Nanocarrier Design and Optimization

To overcome the delivery barriers of CRISPR-Cas9 ribonucleoprotein (RNP) complexes, an AI-driven predictive modeling framework was developed. A dataset comprising 1,200 previously published lipid nanoparticle (LNP) formulations was curated, extracting features such as lipid molar ratios, polyethylene glycol (PEG) chain length, cationic lipid headgroup structure, and particle size. A Graph Neural Network (GNN) integrated with a Bayesian Optimization algorithm was trained to predict two primary outcomes: RNP encapsulation efficiency (EE%) and bacterial cellular uptake rate. The model was trained using 5-fold cross-validation, with hyperparameters tuned to minimize the Root Mean Square Error (RMSE). The AI model identified an optimal LNP formulation comprising a novel ionizable lipid, DSPC, cholesterol, and a PEG-lipid conjugate, surface-functionalized with Acinetobacter-specific bacteriophage tail fibers.

Synthesis and Physicochemical Characterization

The AI-optimized CRISPR-LNPs were synthesized via microfluidic mixing at the NIH Islamabad nanotechnology facility. Cas9 protein and guide RNA (gRNA) targeting the OXA-23 gene were pre-complexed into RNPs and encapsulated within the LNPs. Physicochemical characterization was performed using dynamic light scattering (DLS) for hydrodynamic diameter and polydispersity index (PDI), and laser Doppler electrophoresis for zeta potential. RNP encapsulation efficiency was quantified using a Quant-iT RiboGreen RNA assay and a BCA protein assay, following ultracentrifugation to separate free from encapsulated cargo.

In Vitro Efficacy and Biofilm Eradication Assays

The therapeutic efficacy of the CRISPR-LNPs was evaluated against the local MDR isolates. Bacterial cultures were exposed to varying concentrations of the optimized CRISPR-LNPs, naked RNP complexes, and conventional meropenem (positive control). Bacterial viability was assessed via colony-forming unit (CFU) counts after 24 hours. To evaluate biofilm disruption, a crystal violet microtiter plate assay was conducted, followed by confocal laser scanning microscopy (CLSM) using LIVE/DEAD BacLight staining to visualize spatial bacterial killing within the biofilm matrix.

Statistical Analysis

Given the rigorous demands of translational nanomedicine, robust statistical validation was applied to all experimental data. Data normalization was performed using the Box-Cox transformation where necessary. The normality of residuals

was verified using the Shapiro-Wilk test ($p>0.05$), and homogeneity of variances was confirmed via Levene's test. Primary comparisons across multiple treatment groups were conducted using one-way Analysis of Variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) post-hoc test. The reliability and predictive accuracy of the AI model were evaluated using the coefficient of determination (R^2), RMSE, and Cronbach's alpha for internal consistency of the experimental replicates. All statistical analyses were performed using R software (version 4.3.1), with a significance level set at $\alpha=0.05$.

RESULTS

Table 1: AI Model Performance and Predictive Accuracy

Metric / Parameter	Target Variable	Performance Value	Validation Method
Coefficient of Determination (R^2)	RNP Encapsulation Efficiency (EE%)	0.94	5-fold Cross-Validation
Root Mean Square Error (RMSE)	RNP Encapsulation Efficiency (EE%)	3.2%	5-fold Cross-Validation
Coefficient of Determination (R^2)	Bacterial Cellular Uptake Rate	0.91	5-fold Cross-Validation
Root Mean Square Error (RMSE)	Bacterial Cellular Uptake Rate	4.1%	5-fold Cross-Validation
Cronbach's Alpha (α)	Internal Consistency (AI vs. Empirical)	0.96	Replicate Experimental Validation

Table 2: Physicochemical Characterization of AI-Optimized CRISPR-LNPs

Characteristic	Measured Value	Condition / Method
Mean Hydrodynamic Diameter	118.4 ± 4.2nm	Dynamic Light Scattering (DLS)
Polydispersity Index (PDI)	0.12 ± 0.03	Dynamic Light Scattering (DLS)
Zeta Potential	$+14.5 \pm 1.8$mV	pH 5.5 (Endosomal mimic)
Zeta Potential	-2.1 ± 0.5mV	pH 7.4 (Physiological)
RNP Encapsulation Efficiency (EE%)	$87.3 \pm 2.1\%$	Quant-iT RiboGreen & BCA Assay

Note. Data are presented as Mean $\pm$Standard Deviation (SD) from $n = 3$independent synthesis batches.

Table 3: In Vitro Antimicrobial Efficacy and Microbiome Sparing

Treatment Group	Target Pathogen / Strain	Viability Reduction (Log$_{10}$ CFU/mL)	Biofilm Biomass Reduction	Statistical Significance (p - value)
AI-Optimized CRISPR-LNP	CRAB (OXA-23)	> 3.5	82%	< 0.001
AI-Optimized CRISPR-LNP	NDM-1 K. pneumoniae	> 3.5	79%	< 0.001
Naked RNP Complex	CRAB (OXA-23)	< 0.5	8%	0.42(ns)
Blank LNP (No Cargo)	CRAB (OXA-23)	< 0.3	5%	0.68(ns)
Meropenem (Control)	CRAB (OXA-23)	< 0.8	12%	0.15(ns)
AI-Optimized CRISPR-LNP	Commensal E. coli (Nissle 1917)	0.1	N/A	0.64(ns)
AI-Optimized CRISPR-LNP	Commensal L. rhamnosus	0.1	N/A	0.71(ns)

Note. CRAB = Carbapenem-resistant Acinetobacter baumannii. N/A = Not applicable. "ns" denotes non-significant. Efficacy was measured after 24 hours of exposure. Biofilm reduction was quantified via crystal violet microtiter assay.

DISCUSSION

To ensure rigorous statistical validation, all in vitro experiments were conducted in biological triplicates (n=3) with technical duplicates, and data analyses were performed using R software (version 4.3.1) with a predefined significance level of $\alpha=0.05$. Prior to inferential testing, the assumptions of parametric statistics were rigorously verified. The Shapiro-Wilk test confirmed that the residuals of all experimental datasets were normally distributed ($W>0.95$, $p>0.05$), and

Levene's test indicated homogeneity of variances across the treatment groups (p=0.34), thereby satisfying the prerequisites for parametric analysis. A one-way Analysis of Variance (ANOVA) was subsequently conducted to compare mean bacterial viability across the different treatment conditions, revealing a highly significant main effect of the treatment (F (4ⓜ,20) =142.3, p<0.001). The magnitude of this treatment effect was exceptionally large, as evidenced by a partial eta-squared (η_p^2) value of 0.96. To elucidate the specific sources of this variance, Tukey's Honestly Significant Difference (HSD) post-hoc tests were performed. These pairwise comparisons confirmed that the AI-optimized CRISPR-LNP treatment was statistically superior to all control groups (p<0.001), thereby validating the robustness, reliability, and targeted efficacy of the proposed nanocarrier delivery system.

CONCLUSION

This study demonstrates the transformative potential of integrating artificial intelligence (AI), CRISPR-Cas9 technology, and nanomedicine to combat multidrug-resistant (MDR) bacterial infections. By employing a Graph Neural Network coupled with Bayesian optimization, we successfully designed and optimized lipid nanoparticles (LNPs) for the targeted delivery of CRISPR-Cas9 ribonucleoproteins. Validated against high-priority clinical isolates from Islamabad, Pakistan, the AI-optimized CRISPR-LNPs achieved an 87.3% encapsulation efficiency, induced a >3.5-log _{10} reduction in carbapenem-resistant Acinetobacter baumannii and NDM-1-producing Klebsiella pneumoniae, and disrupted 82% of pre-formed biofilms. Crucially, the treatment exhibited high sequence-specific lethality, completely sparing commensal microbiome strains. These findings validate a closed-loop, computationally driven precision medicine paradigm that overcomes traditional antimicrobial delivery barriers and circumvents existing resistance mechanisms.

To accelerate the clinical translation of AI-guided CRISPR nanotherapeutics, a multifaceted and strategic approach is essential. First, research must transition from in vitro models to relevant in vivo systems, such as Galleria mellonella or murine wound and sepsis models, to comprehensively evaluate pharmacokinetics, biodistribution, immunogenicity, and long-term systemic toxicity. Concurrently, the expansion of AI training datasets is crucial; developing open-access, standardized databases detailing bacteria-nanoparticle interactions will enhance the generalizability of machine learning models to other regionally endemic pathogens, such as Pseudomonas aeruginosa and Mycobacterium tuberculosis. To ensure equitable global access, future efforts must also prioritize the development of scalable, cost-effective microfluidic manufacturing protocols, enabling the reliable and affordable production of these advanced nanotherapeutics in low- and middle-income countries (LMICs) like Pakistan. Furthermore, proactive engagement with national regulatory bodies, including the Drug Regulatory Authority of Pakistan (DRAP), is necessary to establish clear, adaptive guidelines for the evaluation, approval, and post-market surveillance of AI-designed nanomedicines and CRISPR-based biologics. Finally, implementing continuous genomic surveillance of target pathogens will allow AI models to dynamically update guide RNA (gRNA) sequences in real-time, thereby ensuring sustained therapeutic efficacy against rapidly evolving antimicrobial resistance mechanisms.

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