Chapter 11
Nano-Delivery for CRISPR-Cas9 Technology in Precision Medicine

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ABSTRACT

The integration of CRISPR/Cas9 genome editing and nanotechnology is transforming precision medicine through the targeted, efficient, and safe delivery of gene-editing agents. With its great promise in the treatment of genetic disorders, the clinical application of CRISPR/Cas9 is hampered by delivery issues such as degradation, immunogenicity, and off-target effects. This chapter discusses the evolution and use of nano-delivery systems like lipid nanoparticles, polymeric carriers, dendrimers, gold nanoparticles, and exosomes to deliver CRISPR/Cas9 elements across biological barriers in a highly specific manner. Focus is given to nanoformulation strategies that maximize cellular uptake, endosomal escape, and

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nuclear localization with minimal systemic toxicity. Case studies of preclinical and clinical progress in nano-delivered CRISPR therapies for cancer, genetic diseases, and viral diseases are also presented. The chapter is wrapped up with perspectives on regulatory affairs and future directions on combining nanocarriers with genome editing for personalized medicine.

1. INTRODUCTION

1.1. Overview of CRISPR/Cas9 and its significance in genome editing.

CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9) is an innovative genome editing tool. Originally identified as part of the immune system of bacteria and archaea, it is now widely used by scientists to detect and eliminate viral infections by capturing viral DNA fragments and integrating them into their genomes. This natural defense has been transformed into a highly versatile and programmable gene editing system capable of precise modifications across almost all organisms (T. Li et al., 2023). The CRISPR/Cas9 system functions through a straightforward yet effective mechanism. A synthetic single-guide RNA (sgRNA), designed to match a specific DNA sequence, guides the Cas9 nuclease to the target location within the genome. Upon arrival, Cas9 introduces a double-stranded break (DSB) in the DNA. The cell then activates its repair processes, primarily utilizing two pathways: non-homologous end joining (NHEJ), which often results in insertions or deletions (indels) that can interfere with gene function; and homology-directed repair (HDR), allowing accurate insertion or correction of DNA sequences with the help of a homologous template (Redman et al., 2016).

CRISPR/Cas9 is notable for its accuracy, simplicity, and adaptability. Unlike earlier genome-editing tools like zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), which require complex protein engineering for each target, CRISPR/Cas9 uses a flexible RNA molecule, making it much easier and cheaper to use. This accessibility has democratized gene editing technology and sped up progress in various scientific fields (Redman et al., 2016). CRISPR/Cas9 has a broad and influential range of applications. In biomedical research, it is frequently employed to generate gene knockouts, study disease mechanisms, and create animal models of human conditions. Clinically, CRISPR-based treatments are under development for several genetic disorders, such as sickle cell anemia, β-thalassemia, and hereditary blindness. The agriculture industry also leverages CRISPR to engineer crops with enhanced resistance to pests, greater environmental