Chapter 6
CRISPR-Cas9 Technology in Regenerative Medicine and Tissue Engineering

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ABSTRACT

CRISPR/Cas9 technology has emerged as a revolutionary genome editing tool, offering unprecedented precision, efficiency, and versatility in manipulating genetic material. In the fields of regenerative medicine and tissue engineering, CRISPR/Cas9 holds transformative potential for correcting genetic defects, enhancing stem cell therapies, engineering tissue specific cellular functions, and developing disease models. This chapter explores the fundamental mechanisms of CRISPR/Cas9, its integration into regenerative strategies and its application in engineering functional tissues and organs. Key advancements include targeted gene editing in induced pluripotent stem cells (iPSCs), modulation of signaling pathways involved in tissue regeneration and creation of gene-edited scaffolds for organogenesis. The chapter

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also addresses the current challenges, such as off-target effects, ethical considerations, and regulatory hurdles, while highlighting emerging innovations and future directions for clinical translation. Overall, CRISPR/Cas9 stands at the forefront of next-generation therapeutic strategies, reshaping the landscape of personalized and regenerative medicine.

1. INTRODUCTION TO CRISPR/CAS SYSTEMS

The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas (CRISPR-associated) system was first discovered in Escherichia coli in 1987. However, its role as part of a prokaryotic immune defense was only fully understood in the early 2000s (Barrangou et al., 2007). This defense system allows bacteria to detect and neutralize viral threats by integrating pieces of viral DNA into their genome as "spacers." Later, these segments are transcribed into CRISPR RNAs (crRNAs). Along with Cas proteins, these RNAs guide the destruction of matching viral DNA during reinfection. The groundbreaking study by Jinek et al. (2012) showed that this system could be modified for programmable genome editing in eukaryotic cells, marking the emergence of CRISPR/Cas9 as a revolutionary gene-editing tool.

The main components of CRISPR/Cas9 include a guide RNA (gRNA), the Cas9 nuclease, and a protospacer adjacent motif (PAM). The gRNA, which is usually a combination of crRNA and trans-activating crRNA (tracrRNA), directs Cas9 to a specific DNA sequence. The PAM (usually NGG) helps to enable accurate cleavage (Chen & Doudna, 2017). This creates a double-strand break (DSB) that is repaired through cellular DNA repair pathways. These can be either non-homologous end joining (NHEJ) or homology-directed repair (HDR), allowing for gene disruptions or precise genetic changes. In addition to Cas9, the CRISPR toolbox now features several alternative nucleases with different characteristics. For instance, Cas12a (formerly Cpf1) recognizes thymine-rich PAM sequences (TTTV). It creates staggered cuts and operates using a single crRNA without needing tracrRNA (Shmakov et al., 2017). Furthermore, CasΦ (also called Cas14), obtained from bacteriophages, is a small nuclease that cuts single-stranded DNA without the need for PAM sequences. This is beneficial for gene editing in situations where delivery is limited (Pausch et al., 2020).

Despite its flexibility, the CRISPR/Cas9 system has some limitations. Off-target effects from imperfect gRNA-DNA binding can cause unintentional changes in the genome (Kulcsár et al., 2017). To improve accuracy, modified high-fidelity Cas9 variants like SpCas9-HF1, eSpCas9, and xCas9 have been created. These variants have point mutations that reduce off-target cuts while keeping on-target activity intact. Additionally, new technologies like base editing and prime editing allow

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