Polymeric Nanoparticles as Cas9 Ribonucleoprotein Delivery Vehicles

Lucian Williams[1], Jessica Larsen[2]

[1] Department of Bioengineering, Clemson University, Clemson SC 29634

[2] Department of Chemical Engineering, Clemson University, Clemson SC 29634

March 10th, 2022, 3:30 PM Rhodes Annex 111

Clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein (Cas) have emerged as the leading gene-editing technology in research since its first successful use in mammalian cells in 2013[1]. CRISPR has emerged over other technologies due to its simplicity, ease of design, and preparation. CRISPR uses a guided-RNA system with a nuclease to make precise and accurate double-stranded cuts at the targeted location. The recent CRISPR revolution is stalling because there is no current efficient and effective way to deliver it into cells and tissues. The Cas9 complex induces a heavy immune response and requires a successful delivery vehicle for significant clinical translations[2].

CRISPR systems can be complexed into three different payloads. The first method involves using plasmid DNA containing the CRISPR sequences. This method is the simplest with high stability but is associated with the most off-targeting issues and delayed onset. Another technique involves encapsulating the CRISPR mRNA with the sgRNA. This method has more transient expression and allows for better dosing control but is vulnerable to RNase degradation within cells and limits delivery potential since separate delivery of the materials are required. The last method involves the direct use of the ribonucleoprotein (RNP), the Cas9 protein in complex with the targeting gRNA. This method is the most transient and has the lowest off-targeting issues but is challenging to package due to its large volume of about 191 kDa. The RNP method has a high potential for clinical translations and is therefore selected as the delivery payload for this study.

Current delivery vehicle research for CRISPR systems more commonly involves viral methods[3]. The viral methods include adenoviruses (AV), lentiviruses (LV), and adeno-associated viruses (AAV). Common obstacles for these methods are host integration, immune responses, and limited packing sizes. Researchers have transitioned to nonviral methods for their delivery vehicles to avoid and overcome these barriers. Nonviral methods include electroporation, cell-penetrating peptides (CPPs), gold nanoparticles, and lipid and polymeric nanoparticles. For our research design, we chose a cationic, biodegradable polymeric micelle system of poly-L-lysine (PLL) grafted to poly(lactic-o-glycolytic) acid (PLGA). Polymeric micelles offer a promising strategy compared to viral vectors due to their larger packing size and safer profile. We chose the cationic core-shell structure for its advantages of cellular entry, lysosomal escape, better protection, and high binding efficiency with the negatively charged RNP. **The objective** of this study is to create an effective and efficient nonviral delivery strategy for Cas9 for *in vivo* applications. Our study divides into **three aims** to explore our nanoparticle system's biophysical, biochemical, and biogenetic effects on Cas9 uptake. Our study provides the groundwork for using nonviral delivery systems to successfully target, release, and edit a desired gene in cells.

References

1. Yang, L.; Esvelt, K. M.; Aach, J.; Guell, M.; Dicarlo, J. E.; Norville, J. E.; Church, G. M. RNA-Guided Human Genome. 2013, No. February 823–827. **2**. Crudele, J. M.; Chamberlain, J. S. Cas9 Immunity Creates Challenges for CRISPR Gene Editing Therapies. Nat. Commun. 2018, 9 (1), 9–11. **3**. Liu, C.; Zhang, L.; Liu, H.; Cheng, K. Delivery Strategies of the CRISPR-Cas9 Gene-Editing System for Therapeutic Applications. J. Control. Release 2017, 266 (June), 17–26.