O3.5: Non-viral delivery of CRISPR/Cas9 DNA for gene editing via multivalent cationic liposome system

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Introduction

CRISPR/Cas9 gene editing technology has revolutionized medical research by opening new therapeutic possibilities for disease treatment such as cancer, cardiovascular, neuronal and immune disorders [1]. Even with the emergence of this technology, the lack of clinically viable delivery systems continues to hinder CRISPR therapeutic applications. Viral vectors have been sucessfully used for the delivery of CRISPR/Cas9, which whilst efficient, raise safety concerns regarding immunogenicity and insertional mutagenesis [2]. Non-viral vector such as cationic liposomes offer an attractive alternative to viruses given their low immunogenicity, high encapsulation capacity, easy synthesis and functionalization [3]. Their cationic charge mediates strong electrostatic interactions with the negative charges of nucleic acids, leading to the formation of complexes called "lipoplexes". However, cationic lipids efficiency is still not at the level of viruses as delivery vectors. In recent years, multivalent cationic lipids have been proposed as a promising strategy to effectively deliver nucleic acids into target cells [4]. In this work, we explored the potential of multivalent cationic liposomes to deliver plasmid-based CRISPR/Cas9 systems as well as to mediate gene-editing.

Materials and Methods

To study the *in vitro* suitability of multivalent cationic lipid-DNA complexes to deliver CRISPR/Cas9 DNA plasmids, a pentavalent cationic lipid (MVL5) was combined with three different helper lipids: 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), monoleein (GMO) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE). The lipoplexes were prepared with cationic-to-anionic charge ratios (CR (+/-)) of 3 and 10. To facilitate the detection of transfection in HEK 293T cells by flow cytometry and fluorescence microscopy, a plasmid containing both Cas9/sgRNA and reporter green fluorescence protein (GFP) expression cassettes was used. To evaluate CRISPR-mediated gene editing, a Cas9 expression plasmid containing a sgRNA to target the *GFP* gene was designed, and consequently transfected into HEK 293T cells stably expressing GFP. The depletion of fluorescence signal associated to GFP gene disruption was also assessed by flow cytometry and fluorescence microscopy. The cytotoxicity of MVL5-DNA complexes was assessed using a standard colorimetric cell viability assay.

Results and Discussion

We demonstrated that plasmids encoding Cas9 and sgRNA can be successfully transfected into HEK 293T cells via MVL5-based lipoplexes in a concentration-dependent manner for both CR (+/-). Neverthless, at CR (+/-) 10 lipoplexes showed a superior transfection ability, being comparable to the commonly used commercial transfection reagent Lipofectamine 3000. Regarding gene editing, MVL5-lipoplexes mediated a notable *GFP* gene disruption in HEK 293T-GFP cells, achieving a knockout superior to 50%. However, a non-specific gene knockout was also verified, probably resulting from the considerable cytotoxicity of these formulations. Altogether, these results show that multivalent lipid-based lipoplexes are promising CRISPR/Cas9 plasmid delivery systems, and by further optimization and functionalization could achieve an effective and safe delivery system.

Conclusions

In summary, we have demonstrated the potential of MVL5-based lipoplexes to deliver sgRNA/Cas9 plasmids for gene editing. These formulations exhibited both high transfection efficiency, as well as gene knockout ability, achieving results

comparable to Lipofectamine 3000® commercial reagent. Although MVL5-based formulations also showed considerable cytotoxicy and, probably consequent non-specific gene knockout, their versatility opens significant opportunities for further optimization, either by tuning the cationic-to-anionic CR (+/-) to lower cytotoxicity, or by including additional lipids or surface functionalization, which is important for *in vivo* applications and may constitute an alternative to viral-delivery methods.

Acknowledgments

This work was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte and the Project FCOMP-01–0124-FEDER-021053 (PTDC/SAU-BMA/121028/2010). This research was also supported by Microfluidic Layer-by-layer Assembly of Cationic Liposome - Nucleic Acid Nanoparticles for Gene Delivery project (032520) co-funded by FCT and the ERDF through COMPETE2020. Diana A. Sousa (D.A.S) and Celso J.O. Ferreira (C.J.O.F) acknowledge FCT for the grants PD/BD/139083/2018 and SFRH/BD/149199/2019, respectively.

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