Cellular delivery of CRISPR/Cas9 plasmid using multivalent cationic liposomes

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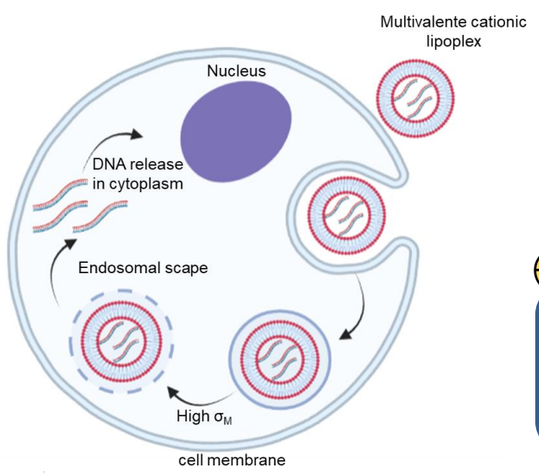
Introduction

Lack of clinically viable delivery systems for CRISPR/Cas9 applications caused by ineffective intracellular transfection

Viral Vectors
Immunogenicity
Low encapsulation capacity
insertional mutagenesis
Off-target effects
High transfection efficiency

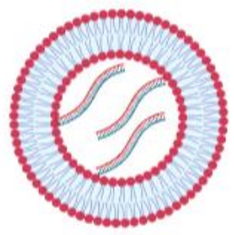
Nonviral Vectors
low immunogenicity
high encapsulation capacity
Easy synthesis and functionalization
Low transfection efficiency



Multivalent Cationic Liposomes
High membrane charge density (σ_M)
→ Endosomal scape
→ Boost Transfection efficiency

Multivalent cationic liposomes are a promising strategy to delivery CRISPR/Cas9 systems

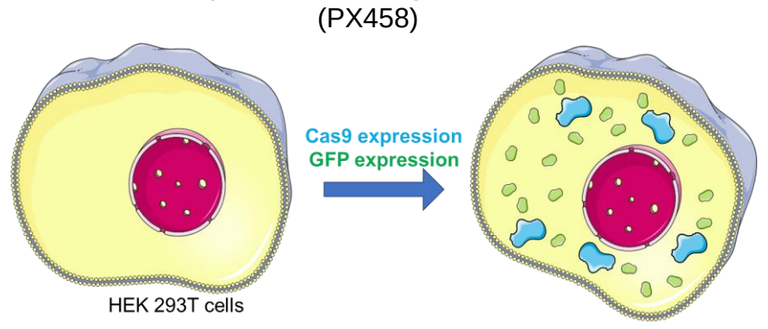
Methodology



Liposomes Composition
Multivalent Lipid (MVL5) + **Helper neutral Lipid (DOPC, DOPE or GMO**)
50:50 MVL5: DOPC
50:50 MVL5: DOPE
75:25 MVL5:DOPE
50:50 MVL5:GMO
Charge Ratio (CR(+/-)) of 3 and 10

Multivalent Cationic Lipoplexes characterization
Size and Charge characterization (DLS)
Cell viability (MTT)
Transfection efficiency (Flow Cytometry and Fluorescence Microscopy)

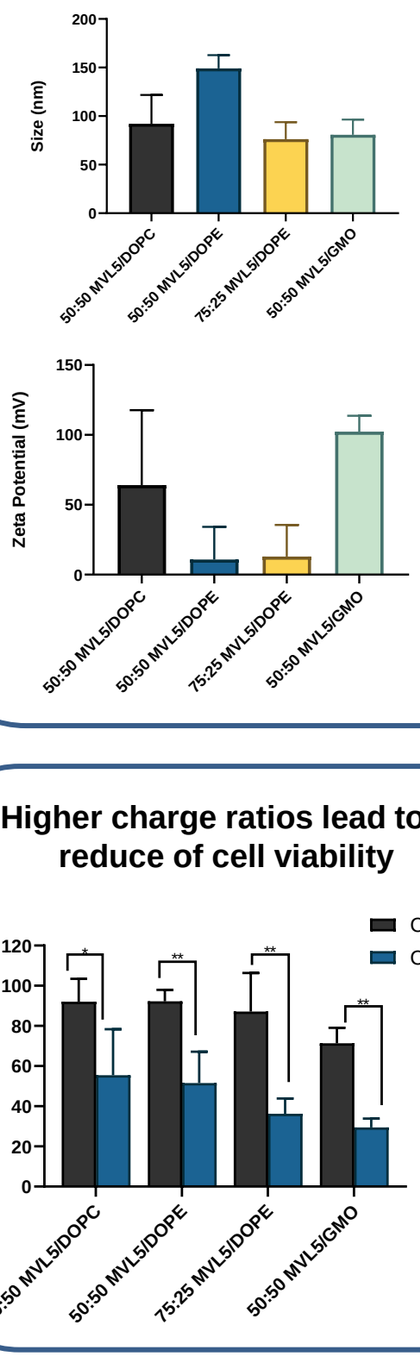
Indirect Cas9 expression detection
Transfection of a plasmid encoding Cas9 and GFP cassettes (PX458)



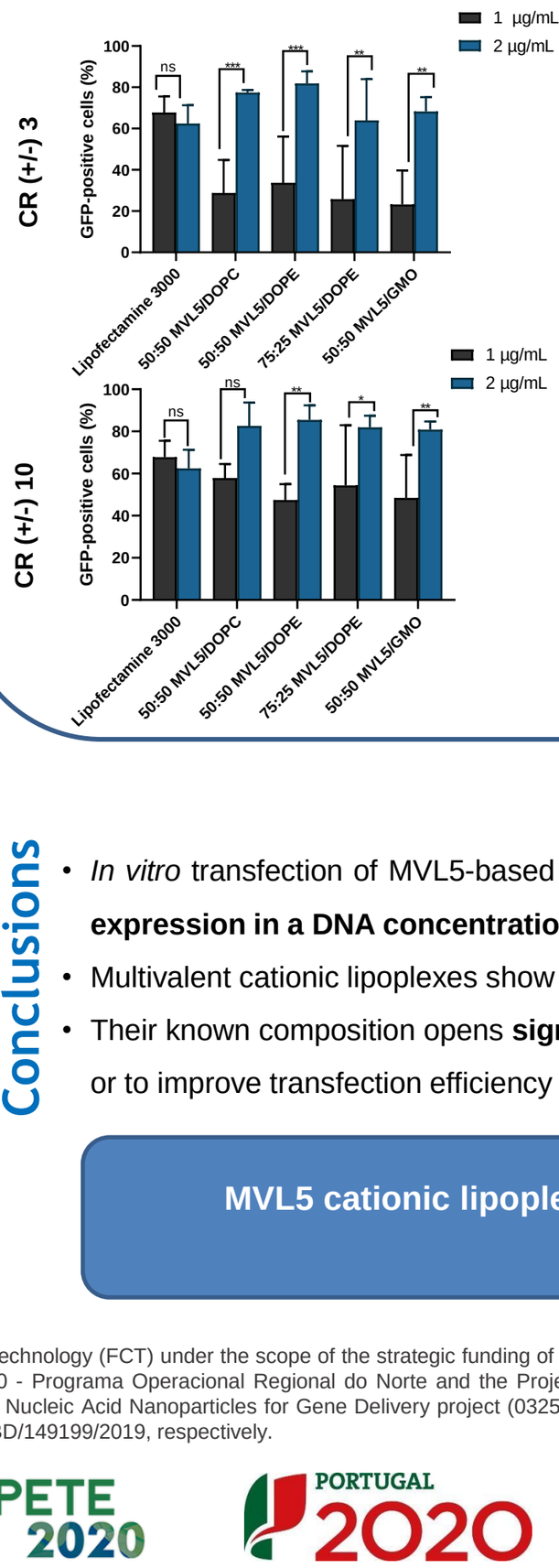
Proof-of-concept cell line
Easy to transfect

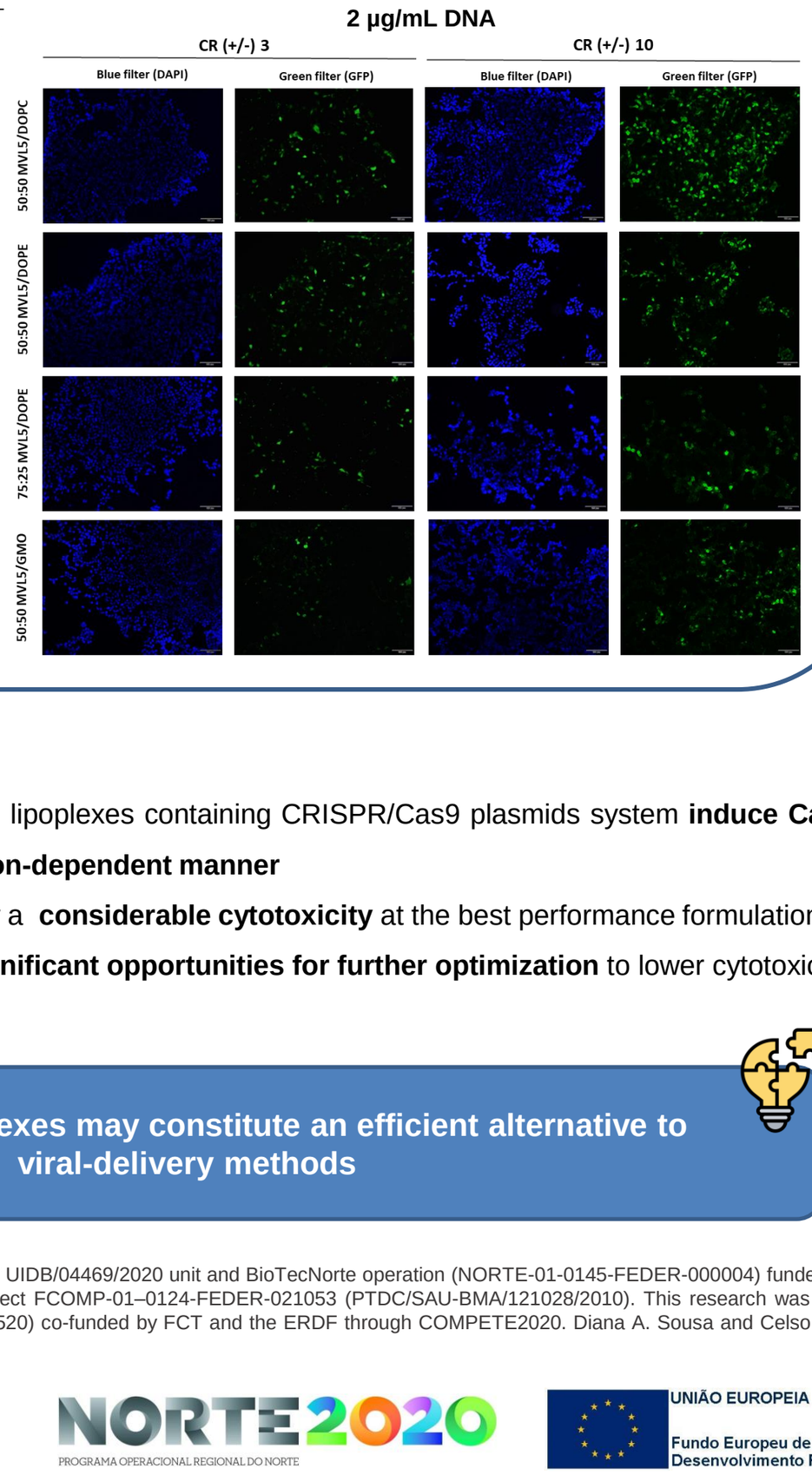
Results

Formation of positively charged lipoplexes with a size range from 85 – 120 nm



Cellular delivery of CRISPR/Cas9 plasmid systems via MVL5-based lipoplexes induce Cas9 expression





Higher charge ratios lead to a reduce of cell viability

Conclusions

- *In vitro* transfection of MVL5-based lipoplexes containing CRISPR/Cas9 plasmids system **induce Cas9 expression in a DNA concentration-dependent manner**
- Multivalent cationic lipoplexes show a **considerable cytotoxicity** at the best performance formulations
- Their known composition opens **significant opportunities for further optimization** to lower cytotoxicity, or to improve transfection efficiency

MVL5 cationic lipoplexes may constitute an efficient alternative to viral-delivery methods

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