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Novel lipoplexes for efficient microRNA delivery to human cardiac fibroblasts

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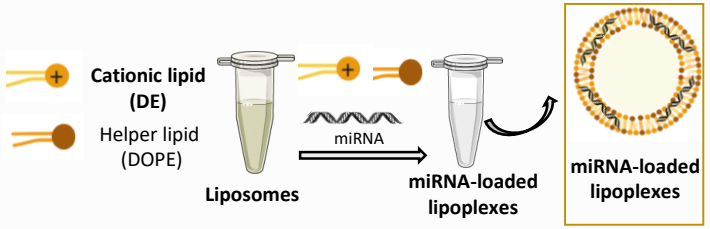
Purpose

Design of miRNAs-loaded lipoplexes composed of a mixture of cationic lipid [2-(2,3-didodecyloxypropyl)hydroxyethyl]ammonium bromide (DE) and dioleoylphosphatidylethanolamine (DOPE) showing:

- ✓ High encapsulation efficiency of miRNAs
- ✓ Cytocompatibility
- ✓ Efficient uptake by adult human cardiac fibroblasts (AHCF)
- ✓ Efficient delivery of miRNAs to human cardiac fibroblasts in the perspective of their direct reprogramming

Methods

miRNAs-loaded lipoplexes were prepared by mixing miRNA (negmiR or miR-1, 5 µM) and DE-DOPE (1 mg/mL, 6 µg) at varying molar ratios of protonated amino groups in DE to phosphate groups in miRNA, defined as N/P ratio (N/P: 3.0; 1.75; 0.70; 0.35).



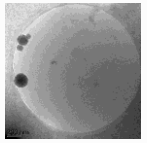
Results

Physicochemical characterization miRNA-loaded lipoplexes

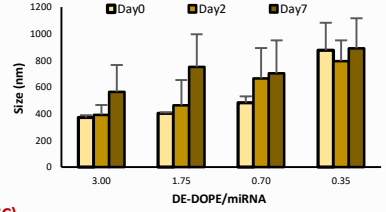
Lipoplexes containing negmiR or miR-1 showed a hydrodynamic diameter ranging from 372 nm to 876 nm and average zeta potential ranging from +40 mV to -26 mV depending on N/P ratio. DE-DOPE lipoplexes showed 99% encapsulation efficiency.

N/P	Size (nm)	PDI	Z-potential (mV)
3	372 ± 18	0.296	40 ± 12
1.75	403 ± 8	0.277	28 ± 17
0.70	483 ± 47	0.284	-18 ± 6
0.35	876 ± 200	0.590	-26 ± 6

CryoTEM N/P 3



Stability study over 7 days (4°C)

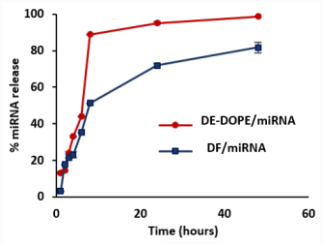


µl DharmaFECT µl DE-DOPE (N/P 3)	µl miRNA (5 µM)
6	10

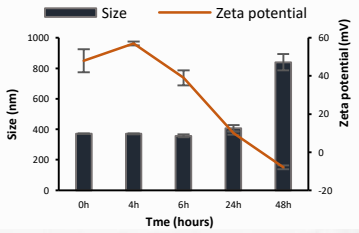
Encapsulation efficiency by Qubit analysis

Sample supernatant	Free miRNA concentration (µg/mL)	EE%
DE/DOPE miRNA	Too low	99%
DF/miRNA	0.255	64%

miRNA release study in PBS, N/P 3 (37°C)

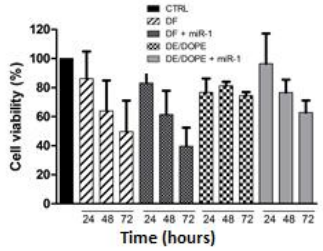


Stability study, N/P 3 (37°C)



In vitro validation studies

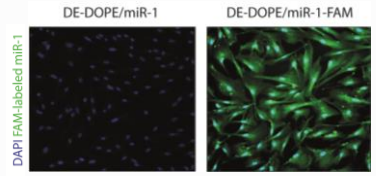
Cell viability assay using AHCFs

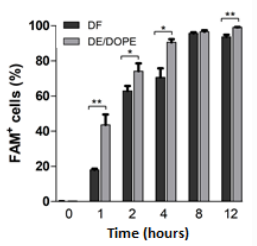


At 24h and 48h culture time (i.e. immediately after and 24h post transfection) both DE-DOPE and DF lipoplexes showed cytocompatibility.
At 72h, DE-DOPE showed superior cytocompatibility.

MiR-1 uptake efficiency of AHCFs mediated by DF and DE/DOPE

DE-DOPE and DF lipoplexes were efficiently internalized by AHCFs as suggested by trials using FAM-labelled miR-1.





Conclusion

New lipoplexes were developed showing efficient encapsulation and delivery of miR-1 to human adult cardiac fibroblasts, for future use in direct reprogramming. Future work will involve the encapsulation of miRcombo (miR-1, 133, 208, 499) to validate the newly developed lipoplexes as efficient vectors for direct cardiac reprogramming compared to commercial agents.

Acknowledgments

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