A Novel Ionizable Steryl Lipid (ISL) Based 3-Component Lipid Nanoparticles (3C-LNPs) for Selective Delivery of siRNA

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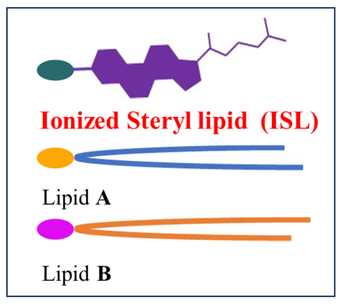
Abstract

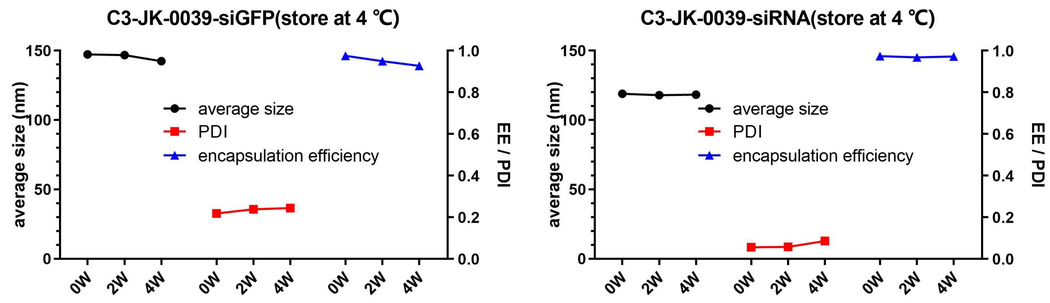
Currently, six siRNA drugs have been approved to treat from rare to common chronic disease, demonstrating the prospective application of RNAi therapy. siRNA are usually delivered by LNPs or GalNAc conjugates (for intrahepatic applications). However, existing LNP formulation lack the capability for precise siRNA delivery to specific extrahepatic organ. To overcome this limitation, here we have developed a three-components LNP (C3-LNPs) based on a novel ionizable steryl lipid and investigated whether the C3-LNPs could deliver siRNA in a cell-specific manner. In vitro assays indicated that C3-JK-0039 LNPs achieved targeted delivery specifically to keratinocytes, without affecting breast cancer cells. Additionally, the C3-LNPs demonstrated no cytotoxicity in CCK-8 assays and did not induce skin allergies in vivo. These results lay a preliminary foundation for the development of targeted siRNA delivery for specific tissues or organs, though further investigations are necessary to exactly understand their therapeutic potential in vivo.

Characterization and stability



LNP	Particle size(nm)	PDI	Zeta-potential	Encapsulation efficiency
C4-ALC-0315-siRNA	78.1	0.048	9.25	97.81%
C3-JK-0039-siGFP	147.25	0.218	10.93	97.50%
C3-JK-0039-siRNA	118.96	0.055	21.83	97.39%

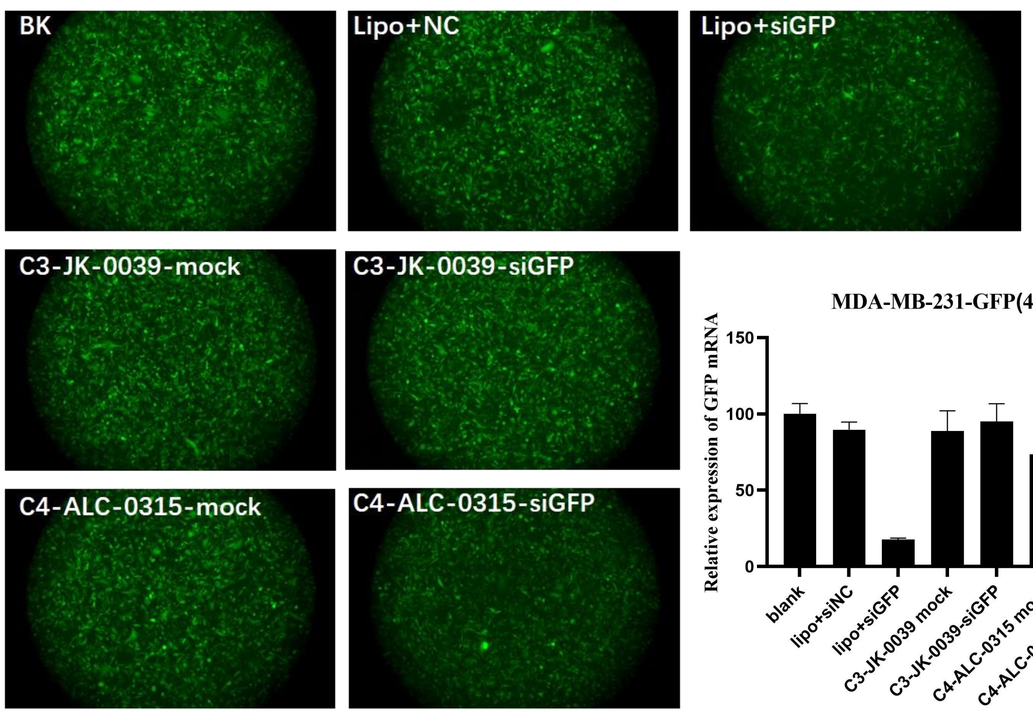
The physical and chemistry quality attributes of LNPs



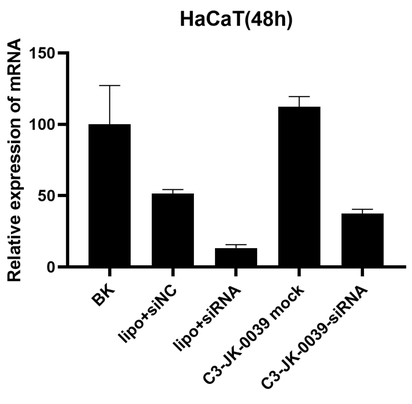
The stability of LNPs

1. LNP with different cationic lipids were prepared. The physical and chemistry quality attributes were characterized as above.
2. C3-JK-0039 LNPs were stable at 2-8℃ for at least 4 weeks.

In vitro mRNA inhibition assay

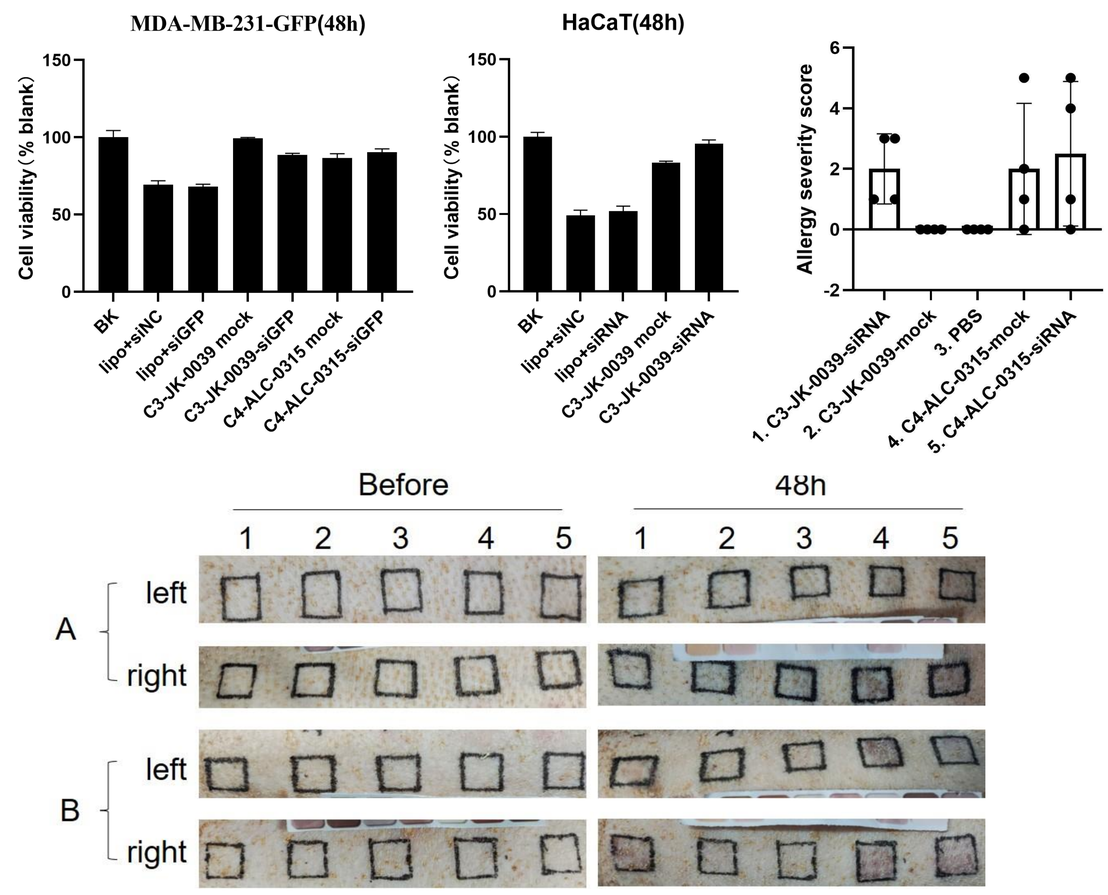


Three components- (C3-JK-0039-siRNA) and four components-LNP (C4-ALC-0315) were prepared separately and transfected in to MDA-MB-231-GFP cell. After 48h, relative mRNA expression level and average fluorescence intensity were assayed. It indicated C3-JK-0039 did not deliver siRNA into breast cancer (MDA-MB-231-GFP) cells.



Three components- (C3-JK-0039-siRNA) was transfected into HaCaT cell. The relative mRNA expression level was assayed by qPCR. It showed C3-JK-0039 could deliver siRNA into keratinocytes (HaCaT) and downregrulated gene expreesion. Therefore, LNP has potential for applications in cosmetic medicine.

Safety



1. The *in vitro* cytotoxicity study was analyzed by CCK-8 assay. C3-JK-0039 LNP showed no toxicity to both MDA-MB-231-GFP and HaCaT cells at 48h after transfection.
2. LNPs contained different ionizable lipid were administrated to the back of Bama xiang pig by subcutaneous injection. After 48h, no obvious allergic response was observed at the site of PBS and C3-JK-0039 LNP mock while C4-ALC-0315 based LNP did. Although C3-JK-0039-siRNA administration caused similar adverse effect to that of C4-ALC-0035-mock and C4-ALC-0035-siRNA, it was ascribed to the immunogenicity of siRNA. Further investigation will be carried out to explore the sensitization mechanism.

Conclusion

1. C3-LNP based on a novel ionizable steryl lipid (JK-0039) were successfully developed which showed high efficiency of siRNA encapsulation and delivery.
2. C3-JK-0039 LNP delivery behaved as cell selectivity for keratinocytes which suggested potential application in cosmetic medicine.
3. C3-JK-0039-LNP showed low toxicity *in vitro* and had little sensitizing effect on Bama Xiang pig skin.
4. C3-JK-0039-LNP was stable at 2-8℃ for at least 4 weeks.