Highly effective and safe non-viral ionizable lipid nanoparticle as universal transfection vectors

Ramon González-Rioja‡ [1, 4], Vivian A. Salazar‡ [1], Carla Franco [1], Helena Isla-Magrané [1], Hugo Calderon [2], Joan Joseph-Munne [1], Montse Martínez-Marcos [3], Xavier Martinez-Gomez [3], Anna Duarri [1], Maria Francesca Cortese [1], Manel Juan [2], Neus G. Bastús[4, 5] and Victor Puntes [1,4 5, 6**]

[1.] Vall d'Hebron Institut de Recerca, Barcelona, Spain.[2.] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain. [3.] Servei de Medicina Preventiva Vall d'Hebron University Hospital, Barcelona, Spain.[4.] Institut Català de Nanociència i Nanotecnologia (ICN2), Consejo Superior de Investigaciones. Científicas (CSIC), The Barcelona Institute of Science and Technology (BIST), Universitat Autònoma de Barcelona (UAB), Barcelona, Spain.[5.] Networking Research Centre for Bioengineering, Biomaterials, and Nanomedicine (CIBER-BBN), Instituto de Salud Carlos III, Madrid, Spain.[6.] Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.‡ equally contributed to the work,

rgonzalez@icn2.net

The use of oligonucleotide therapy offers significant potential for treating a wide range of diseases by delivering functional DNA or RNA into targeted cells or tissues and modulate gene expression. However, their clinical application has been hindered by the lack of safe and efficient oligonucleotide delivery systems. Normally, oligonucleotides have to be administered with a carrier; otherwise, they are rapidly degraded, can be immunogenic, and do not enter inside cells.

In the last decades, a high number of biocompatible materials has been engineered to form complexes, encapsulate, and deliver oligonucleotides with varying degrees of effectiveness [1]. Among the diverse formulations, lipid-based nanocarriers have been one of the most employed [2]. They present good biocompatibility and versatility, but their drug loading and cell penetration capacities are normally low, specifically for hydrophilic or polar molecules [3]. This led to the development of charged (cationic) nanocarriers to favor electrostatic oligonucleotide aggregation in the lipidic NP, dramatically increasing loading density, cell membrane interaction, and transfection efficacy. Unfortunately, the remaining positive charge in the nanocarrier also presented unacceptable levels of immunogenicity and toxicity at the necessary doses to produce therapeutically relevant effects [4]. In addition, they commonly presented hemodynamic toxicities, such as the activation of the complement system and an increase of blood coagulation time [5]. In this context, around the 2000s, Pieter Cullis at the University of British Columbia in Canada developed ionizable lipids as an answer to these problems, leading to the development of ionizable aminolipid NPs (iLNPs) [6], displaying cationic charge at acidic pH for high-density oligonucleotide encapsulation, small size and neutral charge at physiological pH for entering the cell by endocytosis (safer than by transcytosis and membrane fusion) [7], become protonated again during acidification of late endosomes recovering the cationic charge and disrupting the endosomal membrane, and delivering their cargo at the cytosol. Since then, effective ionizable cationic aminolipids have been identified in large-scale screening programs in several biotech companies [8]. The first approved for human use iLNP was Onpattro® (patisiran) containing the ionizable lipid MC3, $C_{43}H_{79}NO_2$, identified from a library of 56 ionizable lipids, with an apparent pKa of 6.44 [9]. Patisaran delivers small interfering RNA to treat polyneuropathies caused by transthyretin mediated amyloidosis in the peripheral nervous system. Followingly, BionTech and ModeRNA developed their own ionizable lipids for their vaccines, the ALC-0315 and SM-102, with pKa of 6.09 and 6.75 respectively. Initial studies showed a strong correlation between gene-silencing activity and the apparent pKa of iLNPs based on different ionizable lipid components [10]. The correlation followed a Gaussian distribution with an optimum pKa between 6.2 and 6.5. These type of lipidic NPs have been the preferred choice for COVID-19 vaccines, and are now the vehicle for next generation of mRNA vacines for cancer immunotherapy to reach the market. These lipids exhibit an inverted cone geometry which is said favor both, high curvature radii and for NP size, and high endosomal membrane disrupting capacities for similar geometrical reasons. It is interesting to note that these developments were industrialized very soon, therefore the description of formation degrees of liberty, intermediates, and characteristics of the structure activity relationship are not yet found in the scientific literature despite clinical penetration.

A high number of investigations conclude that the iLNPs formed by nanoprecipitation, displayed low cargo encapsulation efficiency, poor reproducibility, and heterogeneous therapeutic efficacy. Consequently, the technology has been developed by microfluidics where reagent solutions mix in very small volumes continuously. This is ideal for large scale production but is hampering the availability of simple and accessible methodologies for preclinical research, a prerequisite for therapeutic development.

This reported failure of iLNPs made without microfluidic devices led us to explore which boundary conditions lead to efficient iLNPs synthesis. The objective of this study was to investigate the formation of ionizable lipid nanoparticles (iLNPs) using a straightforward benchtop mixing technique that yields reproducible highly functional iLNPs characterized by monomodal size distributions and high encapsulation efficiency

for various oligonucleotide types, including antisense, mRNA, ssDNA, and plasmids with high loading, reproducibility and size dispersions smaller than some commercial products. Additionally, we assessed the transfection capabilities of the synthesized iLNPs across different cell lines, namely HEK-293, Jurkat, RAW 264.7, HepG2-NTCP, and ARPE-19, as well as in-vivo retinal cells in C57BL/6J mice. The obtained results confirm the capacity of the synthesized iLNPs to encapsulate diverse oligonucleotides, ensuring cargo protection and efficient delivery to all tested cells.

References

[1]. Fus-Kujawa, A. et al. Front. Bioeng. Biotechnol. 9, 1–15 (2021).

[2]. Buck, J., Grossen, P., Cullis, P. R., Huwyler, J. & Witzigmann, D. ACS Nano 13, 3754–3782 (2019).

[3]. Khan, M. S. et al. Membranes (Basel). 13, 343 (2023).

[4]. Tam, Y. K., Madden, T. D. & Hope, M. J. Drug Target. 24, 774–779 (2016).

[5]. Plank, C., Mechtler, K., Szoka, F. C. & Wagner, E. Gene Ther. 7, 1437–1446 (1996).

[6]. Semple, S. C. et al. Biochim. Biophys. Acta - Biomembr. 1510, 152–166 (2001).

[7]. Gandek, T. B., van der Koog, L. & Nagelkerke, A. Advanced Healthcare Materials vol. 12 (2023).

[8]. Semple, S. C. et al. Nat. Biotechnol. 28, 172–176 (2010).

[9]. Kim, M. et al. ioeng Transl Med 8, (2023).

[10]. Whitehead, K. A. et al. Nat. Commun. 5, 1–10 (2014).

Figures

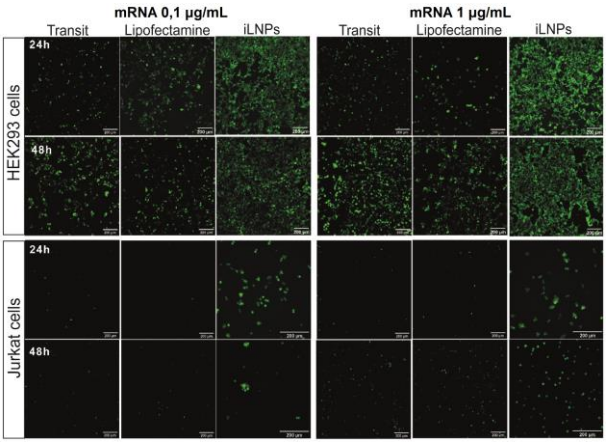

Figure 1. In vitro transfection experiments for EGFP mRNA encapsulated with in-house iLNP. Fluorescence microscopy images after 24 and 48 hours of transfection in both cell lines at different concentration of mRNA.