Multi-dimensional Characterization of siRNA Delivery Nanoparticles: Heterogeneity Assessment through Nanoparticle Fractionation

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The development of lipid nanoparticle (LNP) based small interfering RNA (siRNA) therapeutics presents unique pharmaceutical and regulatory challenges. One of the key issues is the presence of heterogeneity in the nanoparticles. Here we present size-based separation and fractionation methods that permit the study of size, composition, and bioperformance polydispersity in LNPs. An analytical size-exclusion chromatographic (SEC) system coupled with multiple in-line detection systems including UV diode array, multi-angle light scattering (MALS), and refractive index detectors was first developed to permit real time separation and on-line characterization of the sizes, molecular weight, and siRNA cargo loading of LNPs. Semi-preparative SEC methods of LNPs were also developed and fractions were analyzed for sizes, chemical compositions as well as in vitro silencing activity and cytotoxicity. LNPs with similar bulk properties were evaluated in-depth using the above methods and profound differences in batch polydispersity were observed between them. Despite the similarity in the particle assembly process, it was found that one LNP (A) possessed a narrow size and molecular weight distribution while the other (B) was polydisperse. The majority of LNP A fractions after the semi-preparative SEC separation showed similar chemical compositions, in vitro silencing activities, and cytotoxicity. Interestingly, it was found that LNP B SEC fractions exhibited profound size and compositional variation along with 40 fold differences in the in vitro silencing activities. The impact of LNP size and formulation composition on in vitro activities is also discussed. The present results suggest that LNP drug products are highly complex and diverse in nature and care should be taken in examining and understanding them to ensure quality and consistency. The method developed here can not only serve as a method for understanding LNP product property, permitting control on product quality, but also could serve as a potential manufacturing method for product purification. Understandings obtained in this work can help to facilitate the development of LNPs as a well-defined pharmaceutical product.