A combined rapid microfluidics and design-of-experiment approach to optimize liposomal formulations

Nanomedicine has attracted enormous attention for the introduction of advantageous nanoscale materials. Among them, liposomes have devoted considerable concern owing to unique physicochemical and biological properties, as well as the tremendous potential for drug delivery applications. Over the past years, many liposome formulations comprised of various lipids have been approved by medical agencies (e.g., Doxil, DaunoXome, Myocet, Marqibo, or Onivyde) (Fig. 1a and 1b). However, the optimization of liposome characteristics during drug development is still challenging despite significant advances in formulation strategies. Thus, we suggested a combined Microfluidics and design-of-experiment (DoE) approach in which the DoE design was presented for nine different runs for each liposomal formulations and the effect of systemic variation in flow rate ratio (FRR from 1.5:1 to 5.5:1) and total flow rate (TFR 4 to 12 mL/min) was assessed on physicochemical characteristics of liposomes (size, PDI, and zeta potential). After the DoE-based specification of microfluidic settings, different lipid mixtures based on clinically approved compositions were prepared in Ethanol. Microfluidic flow settings were adjusted for each run, and liposomes were prepared by mixing ethanolic lipid solution with D-PBS.

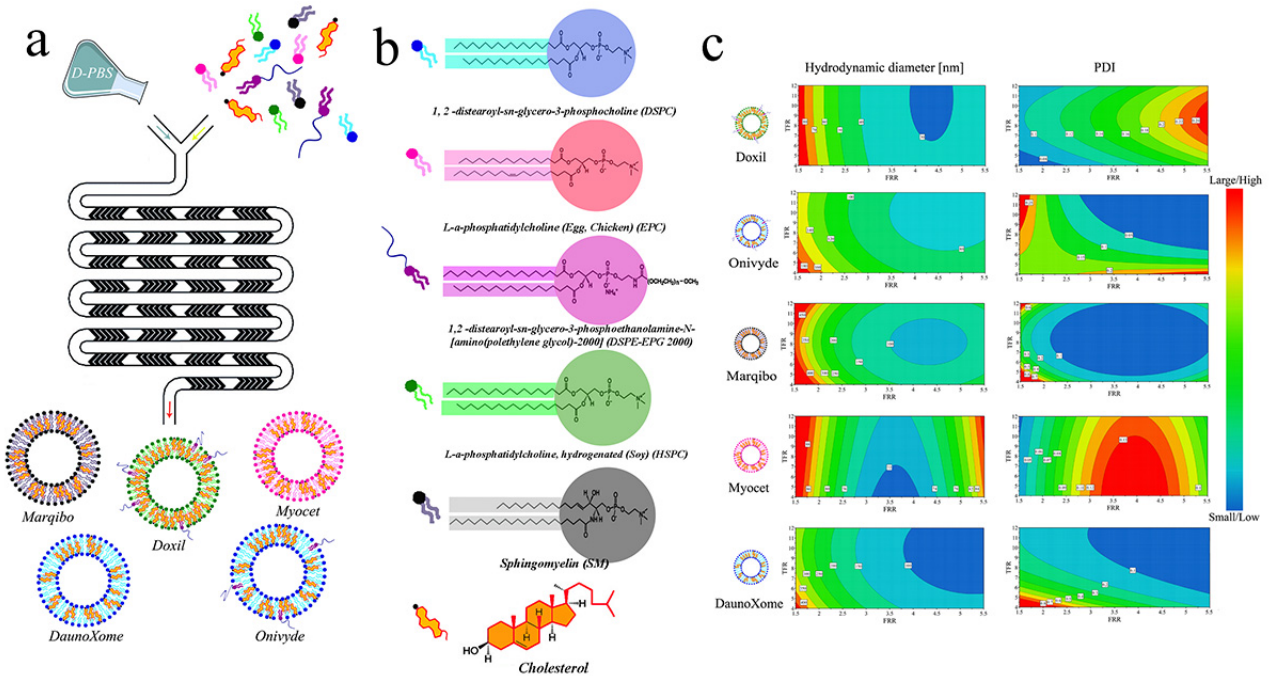

Fig. 1. Representation of rapid microfluidic-based liposome formulation development and the DoE approach results. (a) Liposomes based on clinically approved lipid compositions are formulated using a staggered herringbone micromixer. (b) Chemical structures of different lipids used in clinical liposome formulations. (c) The DoE based optimization of clinical liposome formulations using microfluidics.

Our results demonstrated no changes in zeta potential, indicating the maintenance of surface properties of different liposome compositions. For all formulations, the smallest hydrodynamic diameter was achieved at medium FRR (Fig. 1c). Notably, liposomes containing surface modifying lipids (i.e., Doxil, Onivyde) or lipids with a low transition temperature (i.e., Myocet) resulted in smaller liposomes at defined FRR as compared to other lipid compositions (i.e., Marqibo, DaunoXome). This result was also confirmed by resulting PDI values. These results revealed that FRR is a crucial parameter to control size and PDI of liposomes, while TFR did not significantly influence the size of resulting liposomes and size and, PDI for all liposomes remained constant for TFRs above 8 mL/min. This behavior of vesicle formation at increased TFR demonstrates the ability of microfluidics as a high-throughput formulation method, which is one of the key aspects for large scale manufacturing of liposomes.

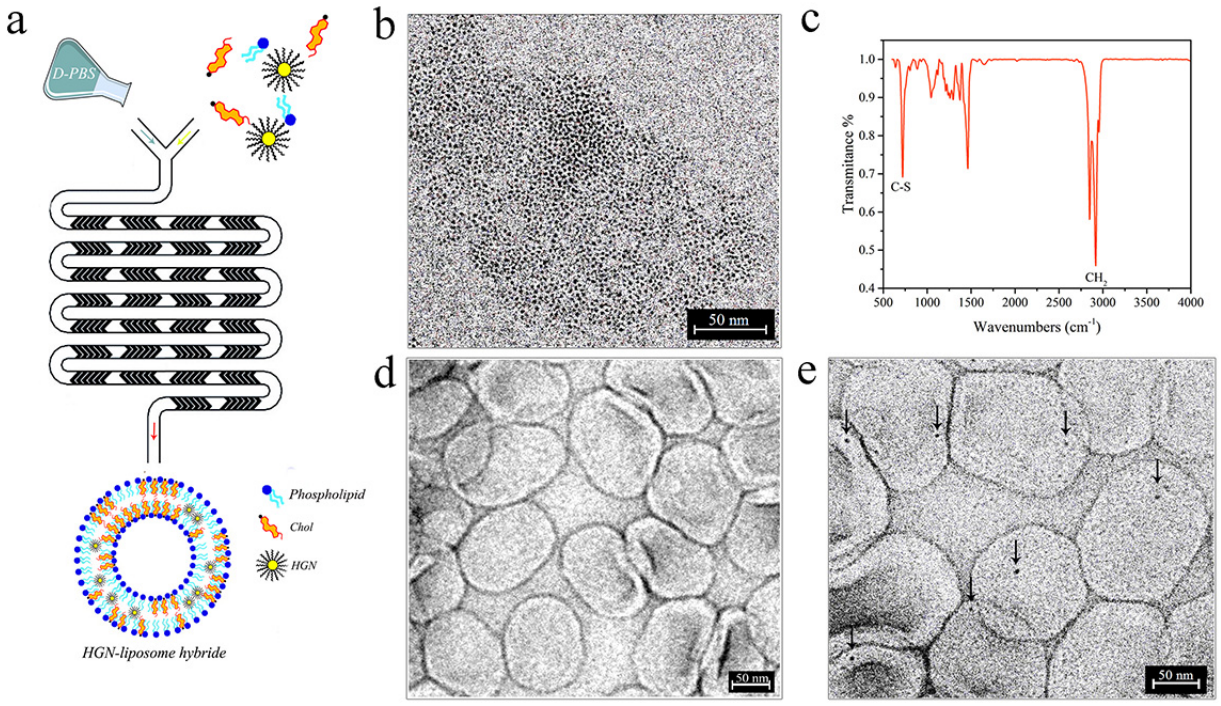

Fig. 2. Validation of the DoE prediction model. (a) Schematic representation of microfluidics-based encapsulation of hydrophobic gold nanoparticles (HGNs) into a clinical liposome formulation. (b) Representative transmission electron micrograph of HGNs with a defined size of 1.5-3 nm. (c) Fourier transform infrared (FTIR) spectrum of HGNs functionalized with dodecanethiol (DDT). Transmission electron microscopy (TEM) analysis of (d) empty liposomes and (e) HGN nanocomposites. Single HGNs are visualized within liposomes (black arrows).

To validate our DoE-based formulation strategy, we used the flow rate settings identified during the DoE to incorporate hydrophobic gold nanoparticles (HGNs) into liposomes (Fig. 2a) with a defined size of 1.5-3 nm (Fig. 2b). The FTIR analysis of HGNs (Fig. 2c) confirmed the successful synthesis of ideal HGNs for the incorporation into the liposomes. HGN-liposome composites were formulated by the rapid mixing of an ethanolic solution containing HGNs/lipids and an aqueous phase (D-PBS) at optimized FRR and TFR. Resulting HGN-liposome nanocomposites had a similar hydrodynamic diameter as compared to empty clinical liposomes with sizes of 111±17.3 nm and 109.3±15.3 nm, respectively (Fig. 2d and Fig. 2e). Electron microscopy analysis confirmed the incorporation of HGNs (dark spot) into liposomes without the formation of HGN clusters (Fig. 2e). It is tempting to speculate that HGNs associate with hydrophobic lipid tails in the lipid bilayer due to a strong thermodynamic driving force. As a result, flow rate settings selected based on DoE models resulted in predicted diameters demonstrating the potential of our approach to formulate liposomes with specific physicochemical characteristics. This strategy presents an exciting alternative to time-consuming traditional methods for the formulation of liposomes.

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