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Lipid Nanoparticles for Drug Delivery

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Lipid nanoparticles have attracted significant interests in the last two decades, and have achieved tremendous clinical success since the first clinical approval of Doxil in 1995. At the same time, lipid nanoparticles have also demonstrated enormous potential in delivering nucleic acid drugs as evidenced by the approval of two RNA therapies and mRNA COVID-19 vaccines. In this review, an overview on different classes of lipid nanoparticles, including liposomes, solid lipid nanoparticles, and nanostructured lipid carriers, is first provided, followed by the introduction of their preparation methods. Then the characterizations of lipid nanoparticles are briefly reviewed and their applications in encapsulating and delivering hydrophobic drugs, hydrophilic drugs, and RNAs are highlighted. Finally, various applications of lipid nanoparticles for overcoming different delivery challenges, including crossing the blood–brain barrier, targeted delivery, and various routes of administration, are summarized. Lipid nanoparticles as drug delivery systems offer many attractive benefits such as great biocompatibility, ease of preparation, feasibility of scale-up, nontoxicity, and targeted delivery, while current challenges in drug delivery warrant future studies about structure–function correlations, large-scale production, and targeted delivery to realize the full potential of lipid nanoparticles for wider clinical and pharmaceutical applications in future.

1. Introduction

Numerous drug delivery systems have been developed for protecting active ingredients, improving drug efficacy and directing site-specific drug delivery.[1] Nanoparticles have been extensively investigated for drug delivery for decades.[1–3] Lipid-based nanoparticles such as liposomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs) have demonstrated tremendous clinical success in delivering both hydrophobic and hydrophilic therapeutics.[4] The first FDA-approved nanodrug, Doxil, is a doxorubicin (DOX)-loaded PEGylated liposome for treating breast cancer, ovarian cancer, and other solid tumors.[5,6] Compared to free DOX, the PEGylated liposomal doxorubicin Doxil offers several benefits including dramatic reduction of cardiotoxicity, prolonged retention time in human plasma, and passively targeted delivery to tumors by taking advantage of the enhanced permeability and retention (EPR) effect.[6] The clinical approval of Doxil in 1995 represents a big milestone for cancer nanomedicine and lipid-based drug delivery systems.

On the other hand, lipid nanoparticles (LNPs) have also been recognized as an ideal carrier for nucleic acids like DNA, mRNA, and siRNA due to their outstanding biocompatibility, biodegradability, and entrapment efficiency. ONPATTRO (Patisiran) is the first approved double-stranded small interfering RNA delivering LNP (2018).[7] Actually, LNPs containing cationic lipids or pH-responsive lipids have been employed for nucleic acids encapsulation and delivery since 1980s.[8–10] However, cationic lipids cause undesirable toxicity.[11] In contrast, ionizable cationic lipids, having positive charges at lower pH ($pH < 6.0$) but neutral at physiological pH, are favorable for formulating LNP systems. siRNA is entrapped inside LNPs consisting of ionizable cationic lipids (DLin-MC3-DMA), phospholipid (1,2-Distearoyl-*sn*-glycero-3-phosphocholine [DSPC]), cholesterol, and polyethylene glycol-modified lipids (PEG_{2000}-C-DMG). During systematic circulation, the PEG_{2000}-C-DMG coating is replaced by Apolipoprotein E (Apo E) recruited by cholesterol, which directs them to the liver and then be endocytosed by hepatocytes.[12] Upon entering the endosome, DLin-MC3-DMA in the LNPs becomes positively charged because of the acidic endosome condition disrupting the endosomal membranes, thus releasing the RNA cargo into the cytoplasm to achieve its function.[13] More recently mRNA COVID-19 vaccines developed by BioNTech/Pfizer and Moderna have been issued emergency use authorizations, and both of them use LNPs as mRNA carriers.[14] The LNP not only protects mRNA from degradation, but also enables their uptake by host cells and delivery of mRNA inside

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the cytosol, where the mRNA sequence is translated into the Spike protein.

The continuous success of these LNPs for various disease treatment has demonstrated their enormous potential as the next-generation drug delivery systems, evidenced by the exponential increase of publications from 1990s (**Figure 1**). Publication numbers increased dramatically from 23 publications in 1996 to more than 3000 in 2020. According to their nanostructure, LNPs can be classified as three systems, that is, liposomes, SLNs, and NLCs. In this review article, we will start with the definition of these three different LNPs. Several good review articles have been published about liposomes, so this review article will focus on other LNPs. Different methods of making such LNPs are reviewed including solvent-based emulsification method, nonsolvent emulsification method, bulk nanoprecipitation, microfluidic approaches, coacervation method, supercritical fluid (SCF) technology, and large-scale production. Their characterization is also briefly introduced. Then LNPs for encapsulating and delivering a wide variety of drugs are discussed including water insoluble drugs, water-soluble drugs, and RNAs. LNPs for addressing different delivery challenges are also reviewed including crossing the blood–brain barrier (BBB), targeted delivery, and different administration routes. Finally, we conclude this review with discussions about future challenges and opportunities of LNPs for various drug delivery applications.

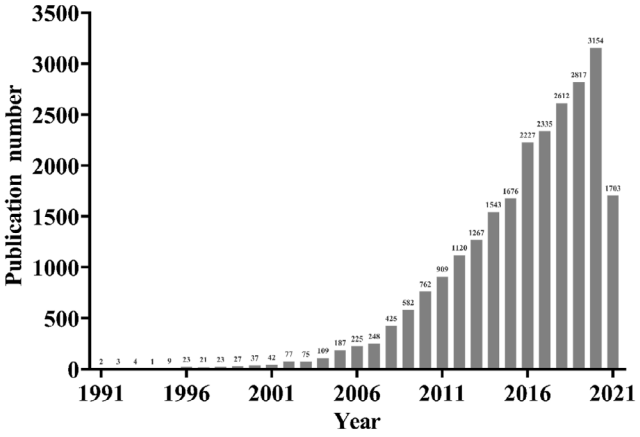

Figure 1. Publication numbers by searching "lipid nanoparticles" in Web of Science over the years since 1991.

2. Different Classes of Lipid-Based Nanoparticles

Among various classes of lipid-based nanoparticles for drug delivery, four typical classes include liposomes, SLNs, NLCs, and hybrid lipid-polymeric nanoparticles (**Figure 2**).

2.1. Liposomes

Liposomes discovered in 1965 are spherical structures typically consisting of an amphipathic phospholipid bilayer and an internal aqueous core.[15] The core–shell nanostructure of liposomes makes them suitable for loading both hydrophobic and hydrophilic molecules. Normally, hydrophobic drugs are encapsulated in the lipophilic bilayers of the shell,[16] and hydrophilic drugs are to be entrapped in the aqueous phase of the core.[17] For the liposomal doxorubicin Doxil, all the encapsulated DOX drugs are in the aqueous phase of the core, and they are in the form of aggregated or crystalline $(DOX)_2SO_4$ salt, which is loaded into the aqueous core driven by the transmembrane ammonium ion gradient.[6] Various methods have been developed for making liposomes including electroformation methods, microfluidic-based methods, thin-film hydration, etc.[18–20] As a hallmark of LNPs, liposomes have been intensely investigated and many good articles have reviewed liposomes.[21,22] Therefore, liposomes will be excluded from this review.

2.2. SLNs

SLNs consisting of fully crystallized lipid components are characterized by a drug-incorporated highly ordered crystalline structure with emulsifiers. In the middle of the 1990s, SLNs were first synthesized using lipids with melting points higher than both

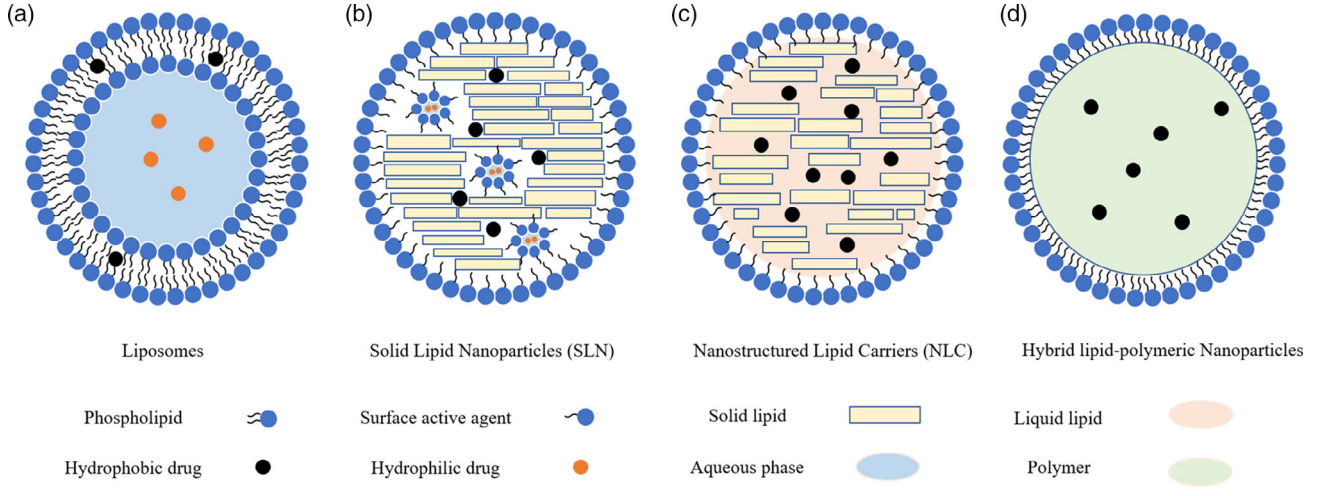

Figure 2. A schematic of three different classes of lipid-based nanoparticles: a) liposomes, b) SLNs, c) NLCs, and d) hybrid lipid-polymeric nanoparticles.

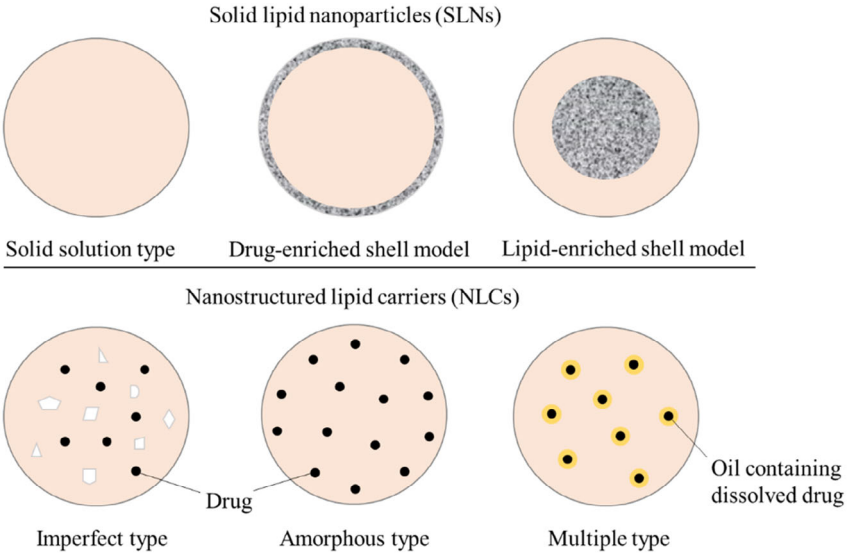

Figure 3. Schematic illustration of different types of SLNs and NLCs. SLNs: solid solution type, drug-enriched shell model, lipid-enriched shell model. NLCs: imperfect type, amorphous type, multiple type. Reproduced with permission.[26] Copyright 2016, Springer International.

body and ambient temperatures such as triglycerides, fatty acids, and waxes.[23,24] SLNs have many advantages such as improved nanoparticle stability, excellent drug protection, and controlled release, tunable properties by varying lipid components.[25] Based on the distribution of active ingredients in SLNs, three models can be used to describe SLNs including 1) solid solution model, 2) drug-enriched shell model, and 3) drug-enriched core model (**Figure 3**).[26,27] However, SLNs suffer from two major problems, that is, poor long-term drug retention and low drug loading capacity. During the storage, the lipid matrix underdoes polymorphic transition from high energy state to low energy state, leading to the formation of a more-organized crystalline lattice and the gradual expulsion of the encapsulated drugs. Thus, drug loading capacity is significantly limited by the polymorphism, especially for highly purified lipids.[27,28] Therefore, liquid lipid or solubilizers were introduced to improve the stability. Consequently, NLCs were developed with the solid lipid partially substituted with liquid lipids. Effectively, NLCs provide enhanced storage stability and drug loading capacity due to the impaired formation of crystallite.

2.3. NLCs

As the second-generation of SLNs system, NLCs were further developed by substituting fractional solid lipids components of SLNs with liquid lipids, resulting in a larger drug corporation space.[29] NLC is a prospective drug delivery system with improved drug retention and enhanced drug loading capacity. Three types of NLCs describe the structure of LNPs including imperfect crystal type, multiple type, and amorphous type (Figure 3).[26] Imperfect crystal type employs a highly disordered matrix by mixing spatially different lipids, leading to a high drug loading capacity. However, the encapsulation efficiency (EE) is relatively low, owning to the low solubility of drugs in solid lipids. Many NLCs utilize a higher content of oil mixing with a solid lipid to induce phase separation. The formation of drug-encapsulated oily nanocompartments increases drug solubility which was able to enhance EE. Amorphous NLCs contain a structureless solid matrix by adopting specific lipids like hydroxyl octacosanol, hydroxyl stearate, and isopropyl myristate, to avoid crystallization-induced drug leakage.[16,30] Different SLNs and NLCs have been illustrated **(**Figure 3**)**.[26]

2.4. Hybrid LNPs

Hybrid LNPs were introduced as an integrated system composed of at least two types of materials to achieve multifunctions or to address the limitations of single-component nanomaterials, combining the advantages of the two individual components.[31] Recently, a novel drug delivery system called hybrid lipid-polymeric nanoparticles has been reported with promising applications.[32–34] Basically, it consists of a therapeutic-containing polymeric core enveloped by an inner lipid layer and a PEGylated lipid outer layer (Figure 2).[35] Owing to the characteristics of both lipids and polymers, hybrid lipid-polymeric nanoparticles present great stability, sustained release, and high biocompatibility.[35–37] Lipid-polymer hybrid nanoparticles have been used for encapsulating a wide range of drugs including sorafenib (SFN), the clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (CRISPR/Cas9) plasmids, DOX, edelfosine, and raloxifene for improved efficacy, prolonged release, and higher bioavailability.[38–41] To fabricate hybrid lipid-polymeric nanoparticles for drug delivery, reproducibility and precise control of nanoparticle properties are essential. Therefore, microfluidics has been extensively used for preparing hybrid nanoparticles.[42,43]

In addition to lipid-polymer hybrid nanoparticles, hybrid lipid-metal nanoparticles such as hybrid lipid-coated silver nanoparticles (lipid-AgNPs), lipid-aluminum nanoparticles, and liposome gold nanoparticles (LiposAu NPs) have also been designed for various purposes.[44–48] Metal nanoparticles are widely used for probing and labeling in biomedical applications, but in vivo studies are limited by their low cellular uptake and poor colloidal

stability. Coating metal nanoparticles with lipids is ideal for improving their biocompatibility, nanoparticle stability, and endocytosis efficiency. A recent study demonstrated a significantly enhanced stability of phospholipid-functionalized gold (Au) nanoparticles.[49] Moreover, LiposAu hybrid nanoparticles exhibited photothermal effects against cancer cells, demonstrating a promising strategy to treat cancers.[47]

3. Synthesis of LNPs

Many different methods have been developed for the preparation of LNPs. Most methods are applicable for preparing both SLNs and NLCs. Different approaches commonly used for LNPs production are introduced in this section. Their advantages and disadvantages are discussed providing some guidelines for selecting the most appropriate method for a particular type of LNPs.

3.1. Solvent-Based Emulsification Method

The solvent-based emulsification method has been widely used to produce LNPs, including emulsion-solvent evaporation, solvent diffusion, solvent displacement, solvent injection, etc.[50,51] Briefly, solid lipids and hydrophobic drugs are dissolved in a water-immiscible organic solvent (e.g., cyclohexane, toluene, and chloroform), which is then dispersed in an aqueous solution to form oil-in-water emulsions. Then LNPs are generated as a result of the evaporation of organic solvent. This method is suitable to encapsulate temperature sensitive drugs. However, the complete removal of organic solvents could be difficult especially when the lipids used are not highly soluble in solvent, resulting in possible toxicity from residual solvents.[50,51]

3.2. Nonsolvent Emulsification Method

Instead of using solvents to dissolve lipids, nonsolvent emulsification methods, also called melting emulsification method, use melted lipids as the liquid phase to form oil-in-water emulsions.[28] Normally, solid lipids are melted into liquid at the temperature of 5–10 °C above their melting points. Then the melted lipids are mixed with an aqueous surfactant solution to produce nanoemulsions using high-pressure homogenization (HPH), microemulsions, high-speed stirring or ultrasonication, and membrane emulsification.[52–55] Dispersed SLNs can be obtained by cooling the nanoemulsions in an ice bath.[28]

Several factors have been reported to influence NP formation and their properties, including homogenization time, sonication time, surfactant concentration, lipid concentration, drug concentration, lipid type, and surfactant type.[56] Among them, drug loading capacity was found to be significantly impacted by the solubility of a drug in lipids. Regarding the choice of surfactants, the optimal surfactant should have a hydrophilic–lipophilic balance (HLB) value within the range of 12–16, such as Chromophore EL (12–14), Tween 20 (≈16), and Tween 80 (≈15). Additionally, higher surfactant concentration and longer sonication time led to smaller particle size. Initial drug loading exerted no adverse effect on lipid particle size when it was below 0.75%, but a tremendous increase of particle size was observed when the drug loading was over 1%. Further increasing the drug loading to 2% led to the formation of drug crystals as a result of the crystallization of unencapsulated drugs.[57]

Compared to the solvent-based emulsification methods, nonsolvent-based methods avoid using toxic organic solvents, thus eliminating the potential toxicity from residual solvents in the final LNPs suspension. But drug loading could be limited by its solubility in the lipids, thus affecting drug loading capacity and EE. Low solubility of a drug in melted lipids results in poor drug loading. Additionally, the high melting temperature of lipids could also affect the chemical stability of drugs.[58]

3.3. Bulk Nanoprecipitation

Nanoprecipitation, also known as solvent displacement, was first developed and patented by Fessi et al. in 1989.[59] Briefly, a water-miscible solvent containing lipids and hydrophobic drugs, so-called the organic phase, mixes with an aqueous phase.[60] Rapid desolvation of the lipids and drugs leads to a rapid precipitation of LNPs and immediate drug encapsulation.[61] Typically, LNPs are produced by mixing an organic phase with an aqueous phase under magnetic stirring in bulk solution.[62,63] The nanoprecipitation process is influenced by the Marangoni effect, which is an intricate and accumulated phenomenon of interfacial turbulence due to flow, diffusion, and surface tension changes at the interface of two miscible solvents.[64] Using nanoprecipitation, Chaudhari et al. produced Amphotericin B-loaded glyceryl dilaurate-formulated nanoparticles which could be easily redispersed in water and remained stable for 3 months under refrigeration condition.[65] Polysorbate 80-coated kokum butter LNPs were prepared via nanoprecipitation for the target delivery of Nevirapine to the brain, and a sustained release of more than 24 h was observed after being administrated in Wistar rats.[66] Nanoprecipitation has also been applied for making LNPs for gene therapies. Huang et al. prepared siRNA-encapsulated LNPs for treating retinal diseases via ethanol injection.[67] The size of nanoparticles and drug EE can be tuned by adjusting parameters such as stirring rate, organic solvent/antisolvent ratio, and lipid/surfactant/drug concentration.[63] To narrow down nanoparticle size distribution, a homogeneous and supersaturated solution of lipids is favored for spontaneous nucleation.[68] Mixing time plays a critical role in controlling nanoprecipitation.[69] To get a smaller nanoparticle size, mixing time should be less than the characteristic time of precipitation, which means that mixing completes before precipitation occurs.[28] However, the main drawback of bulk nanoprecipitation is the limited control of fluidic dynamics, thus resulting in nanoparticles with a wider size distribution, especially for big mixing volumes and large-scale production. Furthermore, incomplete mixing could also lead to batch-to-batch variation in nanoparticle quality and property which makes it unsuitable for large-scale production.[70]

3.4. Microfluidic Approaches

Microfluidics have been widely used for making a variety of nanoparticles via nanoprecipitation, where fluids are manipulated in microchannels with the dimension on the order of tens of microns.[71,72] Compared to bulk nanoprecipitation, microfluidic

approaches for fabricating LNPs offer very attractive advantages, such as smaller particle size, narrow size distribution, scale-up feasibility, enhanced EE, and excellent reproducibility.[73] For the manufacture of LNPs, microfluidic devices can be generally divided into two types: 1) chip-based microfluidic devices and 2) capillary-based microfluidic devices (**Figure 4**).[74]

The formation of LNPs using microfluidic devices is typically based on a hydrodynamic flow focusing (HFF) design.[74] HFF employs two vertical shearing forces squeezing the central channel to obtain a narrow focused stream with an extremely small width, resulting in rapid mixing through diffusion.[75] A comparative study was conducted for siRNA-LNPs produced using a HFF microfluidic chip and vortex mixing.[76] siRNA-LNPs with an average size of 38 nm were yielded using the microfluidic method, generating nanoparticles with a narrower size distribution and a 20% increase in EE compared to those nanoparticles prepared via vortex mixing.

The mixing driven by diffusion force in HFF could be further enhanced, so different micromixer structures were developed to improve mixing efficiency.[74,77] A Tesla-structured HFF microfluidic device was developed containing tortuous microchannels that enable the fluid to be split and merged repeatedly for rapid mixing.[78] Lipid-polymeric hybrid nanoparticles were synthesized using the Tesla-structured HFF microfluidic device (**Figure 5**). Complete mixing could be achieved within the fourth turn of the mixer on a time scale of 10 ms at a flow rate of 50 μL min^{-1}. Different nanoparticles with well-controlled size distribution were prepared using this device, including polymer nanoparticles with a size of 40–50 nm, LNPs with a size of about 250 nm, and polymer nanoparticles with a lipid coating of 40 nm.[78] The main drawback of microfluidic HFF is its relatively low concentration of the final nanoparticles, which might need further processes to concentrate.[73]

A microfluidic device with staggered herringbone micromixers (SHMs) structure was also developed for producing LNPs in millisecond with high throughput (**Figure 6**).[79] An ethanol solution containing lipids was mixed with an aqueous solution in the SHM microfluidic device. Increased aqueous/ethanol flow rate ratio (FRR) resulted in reduced LNP size regardless of the SHM cycle numbers. A minimum SHM cycle numbers of ten was required to produce small LNPs. The SHM device has also been reported for producing siRNA-encapsulated LNPs with

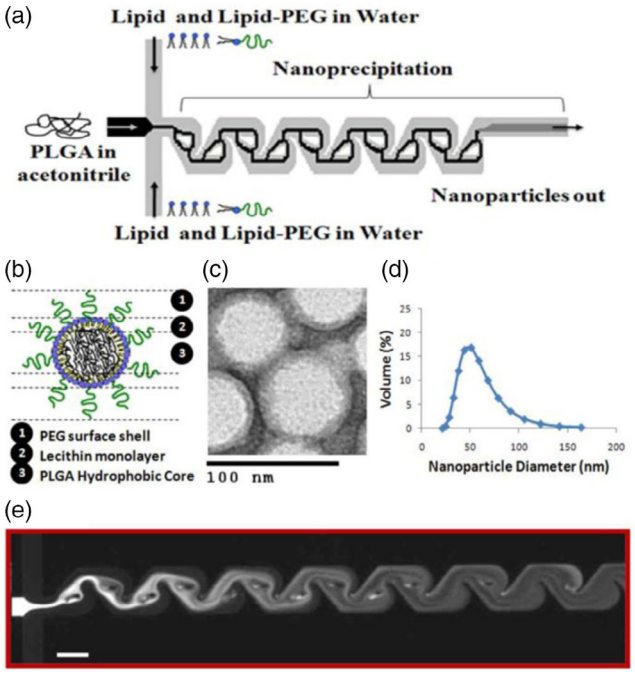

Figure 5. Tesla structure mixer used for the fabrication of hybrid lipid-polymeric nanoparticles. Reproduced with permission.[78] Copyright 2010, American Chemical Society.

almost 95% EE, which is 1.36-fold higher than a T-tube mixer device.[80] Riewe et al. compared three different micromixers: a segmented-flow micromixer, a high-pressure micromixer, and a SHM, for making LNPs.[69] They showed that the presence of surfactants can decrease particle size for all the three devices. Moreover, for both high-pressure micromixers and SHMs, a higher FRR produced smaller particles. Comparatively, segmented-flow micromixers generated a relatively larger particle size. Importantly, for the SHMs structured microfluidic device, the choice of solvents should be compatible with the device materials.[73]

Chip-based microfluidic devices can be manufactured by micromilling, microcmaching, lithography, and mold replication.[81] Specific geometry of microchannels can be designed having the dimension of tens of microns, thus allowing rapid mixing for

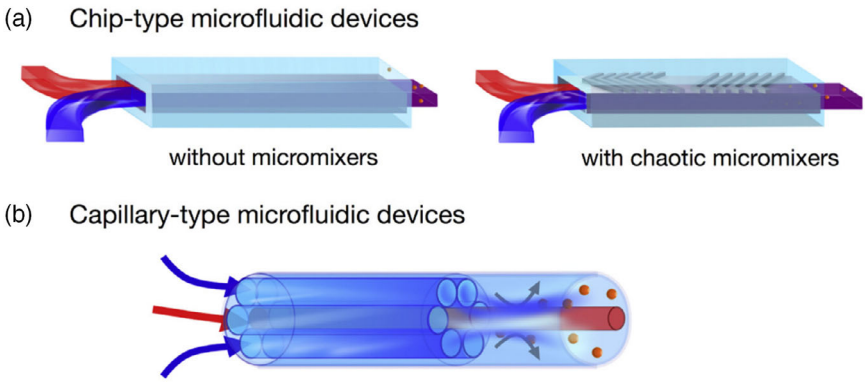

Figure 4. Microfluidic devices for the formation of LNPs. a) Chip-type microfluidic devices. b) Capillary-type microfluidic devices. Reproduced with permission.[74] Copyright 2018, Elsevier B.V.

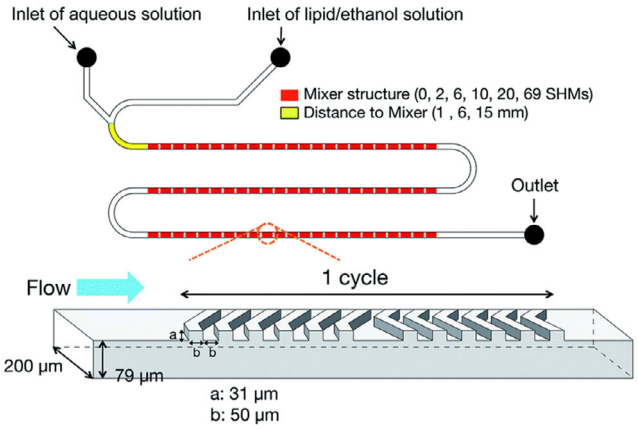

Figure 6. Schematic diagram of a microfluidic device with staggered herringbone micromixers. Reproduced with permission.[79] Copyright 2015, Royal Society of Chemistry.

homogenous LNPs production. However, the manufacture of microfluidic chips is costly and time-consuming. Besides, the adopted material for the fabrication of microfluidic chips is also a limiting factor. Also, chemical compatibility and surface hydrophobicity or hydrophilicity should be considered. Moreover, fouling is another drawback of chip-based microfluidic devices due to the relatively small dimension of microchannels and the direct contact of fluids with the inner wall of the microfluidic chips.[69,82]

Capillary-based microfluidic devices have been employed for producing cetyl palmitate-based LNPs to encapsulate SFN and paclitaxel (PTX).[83] Two capillaries were aligned coaxially with the inner capillary having a fine tip, which allows the fluids run in the same direction.[84,85] The resulted PTX-loaded SLNs achieved an EE of 54% and a drug loading (DL) of 1.4% with a PTX concentration of 0.75 mg mL^{-1}, while SFN-loaded SLNs had an EE of 79% and a DL of 1.04% with a SFN concentration of 0.5 mg mL^{-1}. Importantly, the synthesis temperature for SLNs should be maintained at around 60 °C to keep the LNPs as soft liquid nanoparticles, preventing their precipitation or adhesion onto the channel.[83] Hood et al. also reported a glass multicapillary array device for the fabrication of LNPs.[86] Seven identical small glass capillaries were assembled into a big capillary tube; the inner capillary was coaxially aligned with the big capillary for the lipid stream injection. Homogenous LNPs were produced with an average size of 53 nm and a PDI of 0.044. The influence of FRR and capillary dimension on the LNP size was also examined, concluding that smaller capillary and high FRR contributed to smaller LNP size with narrower size distribution.

Capillary-based microfluidic devices are relatively cost-effective and easier to fabricate.[81,82] These devices are normally made of glasses which make them compatible with various solvents and harsh chemicals. One drawback of the capillary devices is the difficulty in device assembly and alignment of the inner and outer capillaries.[82]

3.5. Coacervation Method

In order to overcome the disadvantages associated with those methods mentioned above, such as toxic organic solvent and sophisticated equipment, a new and solvent-free technology, called the coacervation method, was first reported for the formation of LNPs by Battaglia et al. in 2009.[87] Basically, a micellular solution of fatty acid alkaline salts precipitates as pH is lowered, due to the acidification-induced proton exchange between acid solution and alkaline salts.[87] LNPs of fatty acids can be produced with the gradual addition of a coacervating solution which decreases the pH to a certain point. Also, before the addition of the coacervating solution, the mixture of well-dispersed lipids and surfactants should be heated above the Krafft point of the salt of fatty acid and stirred constantly to obtain a clear solution.[3] An optimal formulation of baicalin-loaded SLN was produced using the coacervation method with 0.69% w/v lipid and 26.64% w/w drug/lipid ratio. The formulated SLN exhibited an EE of 88.29%, a particle size of 347.3 nm, and a polydispersity index (PDI) of 0.169.[88] Briefly, the aqueous solution containing stearate sodium and 1% hydroxypropylmethyl cellulose (HPMC) was stirred and heated over the Krafft point of stearate sodium (47.5 °C) until a clear solution was observed. Then baicalin was added as a model drug when the temperature of the solution was increased to around 60 °C and fully dissolved. HCl solution was added dropwise until the pH reached 4.0. Then the suspension was suddenly cooled to 15 °C and stirred to get the final nanoparticle product by incubating in an ice water bath. Spherical nanoparticles were acquired and the encapsulated drug in the nanoparticles was crystalline as confirmed by the differential scanning calorimetry (DSC). This method is simple, but the application of coacervation method is limited to those lipids capable of forming alkaline salts, such as fatty acids.[89]

3.6. SCF Technology

SCF technology represents a promising method for the fabrication of nanoparticles with advantages such as superior nanoparticle size control, uniform size distribution, complete solvents removal, and environmentally friendly.[90,91] SCF technology employs a material that has a supercritical form with tunable solvent power by altering pressure and temperature.[92] Carbon dioxide is the most frequently used SCF due to its excellent safety and low price. The production of LNPs using SCF is generally realized by changing the ambient pressure of supercritical CO_2 ($scCO_2$).[91] Briefly, $scCO_2$ is used as a solvent, and the solubilities of solid lipids and drugs in $scCO_2$ are increased when pumped into the high-pressure vessel. Then, through the depressurization process, the solid lipids and drugs are suddenly supersaturated and precipitated out, forming drug-loaded LNPs.[93–95] Based on this SCF method, other strategies have also been developed for the fabrication of SLNs, such as supercritical fluid extraction of emulsions (SFEE),[96] particles from gas-saturated solutions (PGSS),[97,98] and gas antisolvent (GAS)/supercritical antisolvent (SAS).[99]

PGSS is a solvent-free process that enables direct production of LNP powders.[91,100] Briefly, a gas-saturated solution of melted lipids and drugs in $scCO_2$ was formed, and then was sprayed through a nozzle into a depressurized chamber to produce atomized dry LNP powders. Curcumin-loaded LNPs were produced using the PGSS.[98] Helium was also used for the atomization of SLNs as it can maintain the chemical solubility of curcumin.

The presence of helium affected the size and drug loading of LNPs. Caffeine, glutathione, and ketoprofen loaded LNPs with the coating of silanized TiO_2 were produced successfully via PGSS.[97] As a result, hydrophobic ketoprofen exhibited much higher EE than hydrophilic caffeine and glutathione. SFEE is similar to GAS/SAS. It employs $scCO_2$ as an antisolvent for the extraction of organic solvents, but with different feedstock.[91,100] Although the solubility of drugs can be improved in SCF, most drugs still have limited solubility in $scCO_2$. Therefore, the SAS technique was further developed to overcome the solubility limitations.[91,92] LNPs were fabricated using SAS to encapsulate hesperidin (HES) for the improvement of cardioprotective effects.[99] Briefly, stearic acid as the solid lipid was dissolved in DMSO with HES and Tween 80, and $scCO_2$ was used as the antisolvent. The organic solution was pumped into the $scCO_2$ vessel with a controlled flow rate. HES-loaded SLNs were precipitated due to the saturation of solutes by depressurization. The residual solvent was removed with the flow of $scCO_2$ for 30 min. Tunable particle size and EE can be obtained under different operating temperature, pressure, and solution flow rates.

Although SCF technologies have advantages for the production of LNPs, the equipment for SCF technologies are costly, and precise computational modeling of the SCF manufacture process could be used to improve the technologies.[91,100] Comparative studies could be carried out in future for comparing SCF technologies with other methods like microfluidic-based methods and bulk nanoprecipitation.

3.7. Large-Scale Production

Development of large-scale production approaches for making LNPs is essential to realize their full potential in pharmaceutical applications. Two strategies are available for large-scale production of LNPs, namely, solvent-based and nonsolvent-based technology. HPH and microemulsion methods are the two most commonly used nonsolvent-based techniques. HPH is a well-established scaling-up method for nanoparticle production and has been used since 1950s to produce parenteral emulsions.[101] Both drug-free and drug-loaded LNPs have been successfully produced in pilot scale by HPH.[102] Recently, a systematic study was reported for making Stavudine-loaded LNPs using the HPH method from lab scale up to industrial scale. Stavudine was dissolved in the melted lipid Dynasan at 2% by mass (0.15% drug + 1.85% lipid) with the addition of surfactants at 80 °C (Solutol 1%, Tween 80 1%, Plurol Oleique 1%, w/w). The hot solution was then dispersed in an aqueous solution containing 0.5% w/w Poloxamer 188 at 80 °C. Coarse microdroplets were first formed and then homogenized using different homogenizers. To obtain very small LNPs, the lipid content was kept very low (2%) and the surfactant to lipid ratio was high (35:10), resulting in a size of 53.1 nm with a PDI of 0.213 using just one homogenization cycle for the lab scale. For different types of homogenizers, no obvious changes of long-term stability were observed for similar-sized systems.[103] Gasco et al. used a microemulsion method to prepare LNPs. In industry, microemulsion was prepared in a thermal-controlled tank, subsequently pumped to a cold-water tank for precipitation.[104] However, the introduced drug could be degraded due to the heating process which is necessary for both methods mentioned above. Moreover, intensive energy is also required for the HPH method, and surfactants are involved in the microemulsion method.[28]

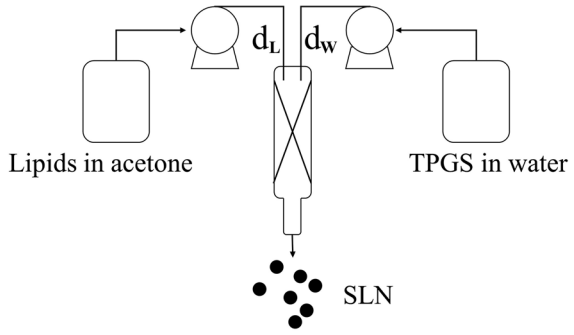

Figure 7. Schematic illustration of a device with the static mixer for large-scale production of LNP. Reproduced with permission.[28] Copyright 2012, Elsevier B.V.

On the other hand, bulk nanoprecipitation is suitable for making LNPs in small scale, but when scaling it up to big volume, perfect mixing with short mixing time is difficult to achieve. Therefore, mixing devices like confined impinging jet mixers, microfluidics, and T-mixer have been developed for large-scale production. Microchannel mixers have been developed for the synthesis of LNPs using continuous nanoprecipitation, but with low productivity.[28] The solution is to scale out by using as many as possible devices in parallel to increase the throughput.

For continuous and large-scale production of LNPs, static mixers have been developed with the components of tubes, columns, or reactors assembled by many identical and static elements with tortuous structures (**Figure 7**). Due to the tortuous structure, rapid and homogeneous mixing can be achieved with the introduced fluids be twisted and recombined repeatedly in the static mixers. Consequently, a throughput of 37.5–150 g h^{-1} of LNPs was achieved with a size below 200 nm (for 25 mg mL^{-1} lipid solution at a flow rate of 25–100 mL min^{-1}). The lipid concentration was also found to have a significant impact on the size of LNPs. Specifically, particle size can be increased by increasing the lipid concentration.[28]

4. Characterization of LNPs

Proper characterization of LNPs is critical not only for investigating the synthesis of LNPs but also for controlling their quality to meet the requirement of various applications. A few important properties of LNPs need to be carefully characterized including particle size and PDI, zeta potential (ZP), surface morphology, EE, drug release, crystallinity, and nanoparticle stability.[105]

4.1. Particle Size, ZP, and Surface Morphology

Particle size is a critical parameter for nanoparticle drug delivery applications. The average particle size of LNPs is normally between 100 and 400 nm, and LNPs with particle sizes ranging from 10 to about 150 nm are desirable for systematic drug

delivery via intravenous (IV) injection.[3,37] In addition, PDI indicates the extent of particle size distribution with a range of 0−1, and a PDI of less than 0.2 is often considered as narrow size distribution whereas most of studies set PDI value less than 0.3 as the upper limit. Normally, particle size and PDI can be determined by dynamic light scattering (DLS) which measures the intensity differences of fluctuated light due to the motion of particles.

ZP refers to the surface charge of particles measured by a ZP analyzer. It can be used to indicate the stability of formulated colloidal dispersions by determining the degree of repulsion force. High repulsion force prevents particles from aggregation. Normally ZP higher than +30 mV or less than −30 mV are considered strong enough to repel each other and remain electrostatically stable. It should be noted that LNP formulations containing nonionic surfactants such as polyhydroxy surfactants tend to have lower ZP values.[106] Meanwhile, it has been reported that an increase of oil content increases the ZP value of LNPs.[107] But for systematic drug delivery, near-neutral charge is preferable. So strong charges need to be screened off by either PEGylation or some other surface modification such as covering the LNPs with nonionic surfactants like Tween 80.[106]

Scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) are often be employed to determine particle size, as well as to observe particle surface morphology. SEM and TEM provide the morphological information via electrons transmitted from the surface of the particles and the inner structure of the particles, respectively. In contrast, AFM allows for a 3D profile of LNPs.

Fourier transform infrared spectroscopy (FTIR) is a technique to examine the IR radiation absorbed or transmitted by the tested samples, and the signal is converted into an infrared spectrum. FTIR is mainly used for verifying the successful encapsulation of drugs within LNPs and characterizing the chemical structure of LNPs via comparing the FTIR spectra of drug-loaded LNPs, pure LNPs and pure drugs.[108,109]

4.2. Drug EE

Drug EE is an important indicator for evaluating the preparation method for drug encapsulation. It is defined using the following formula

$$\%\mathrm{EE} = \frac{\text{Encapsulated drug amount by nanoparticles}}{\text{Total drug amount}} \times 100\% \quad (1)$$

To determine the amount of drug encapsulated in nanoparticles, UV–vis spectrometry or high-performance liquid chromatography (HPLC) is normally used.[110,111] Normally, a dialysis membrane is used to remove the unencapsulated drugs. Another choice is separating the entrapped drugs and free drugs via ultracentrifuging. For example, 35 000 × *g* for 40 min was used for centrifuging down the drug-encapsulated nanoparticles, and then the supernatant was collected to determine the drugs left in the solution.[112] There are two main methods to measure the EE including direct method and indirect method. The direct method calculates the entrapped drug amount in nanoparticles directly, whereas the indirect method measures the amount of unencapsulated drugs in the supernatant and then calculates the EE. Usually, the direct method is suitable for measuring the EE of lipophilic drugs while the latter is proper for hydrophilic drugs.[113]

EE is vital for understanding the percentage of drugs successfully entrapped in carriers. A higher EE is desirable for drug delivery applications.[114] Several key factors affect EE including types, composition and crystallinity of the lipid materials, and drug solubility in organic phase and aqueous phase.[115] Normally, the EE of drugs in LNPs is expected to be higher than 70%.[3] Compared to SLNs, NLCs usually have higher EE due to the presence of liquid lipids. The imperfect core formed during preparation offers a larger space for drug accommodation thus higher drug EE.[29] However, hydrophilic drugs usually have lower EE compared to hydrophobic drugs due to its high solubility in the external aqueous phase.[116]

4.3. Drug Release Studies

Normally, drug release from the LNPs is mainly controlled by two processes, namely, biodegradation and diffusion. In vitro studies are very useful to predict the in vivo behaviors of drug loaded LNPs. In vitro drug release studies simulating in vivo drug release are often conducted in phosphate-buffered saline (PBS) or simulated body fluids using side-by-side diffusion cells with biological or artificial membrane-like reverse dialysis sacs, ultracentrifugation, dialysis bags, centrifugal ultrafiltration, and ultrafiltration. Then UV spectrophotometer or HPLC is used to analyze the drug release profile. Many factors influence the in vitro release profiles of LNPs, including drug loading, drug location, particle size, size distribution, degree of crystallinity, lipids contents, types of release medium, morphologies, surfactants applied, and preparation techniques.[3]

4.4. Crystallinity

It is very important to determine the crystallinity of LNP components because the lipids and the drugs encapsulated may undergo polymorphic transformation during storage, leading to drug expulsion and instability.[117] Lipid crystallinity also strongly affects drug incorporation and drug release. DSC and X-ray diffractometry (XRD) are often used for the investigation of structure, content, and size of lipid crystalline.[118] DSC measures heat capacity changes of the tested samples during the warming up and cooling down process, and peaks are observed at different phase transition temperatures. DSC is a simple, quick, and intuitive way to establish the degree of LNPs crystallinity through enthalpy change. Nevertheless, the main drawback of DSC is that it is a destructive method because heat is applied. The nondestructive XRD indicates the crystallographic structure by measuring the intensity and angle of X-ray scattered through the tested samples.[119] However, the utilization of XRD has limitation because it measures powder samples, which means the LNP-containing suspension needs to go through drying, and polymorphic transitions may occur during the process. Usually, DSC and XRD can be combined to analyze the atom-to-atom distance of LNPs, thereby understanding the composition of LNPs.[3] The presence of both solid and liquid lipid

components is beneficial for promoting more therapeutical molecules accommodation and less drug leakage over the polymorphism transitions. It has been reported that an increase of liquid lipid content could alleviate the degree of crystallinity in the case of NLCs while slow drug release was observed in the case of SLNs because of the relatively high crystallinity of SLNs.[29]

4.5. LNPs Stability

The development of stable LNPs is still at an early stage of development and presents a series of challenges for the development of commercial products containing LNPs. The potential of LNPs in the next generation of therapeutic RNA vaccines and therapeutics will drive development of stable aqueous preparations. Experience from formulating therapeutic proteins, including the monoclonal antibodies,[120] aids in the prediction of the issues that may arise in LNP preparations, including chemical stability of the LNP components, physical stability of the LNP (disintegration, aggregation, and adsorption on surfaces), and stability of the RNA within the LNP. Successful therapeutic proteins products are invariably stable as a liquid preparation for over 18 months at $5 \pm 2\,^{\circ}C$. The stability tests to demonstrate ruggedness of the product are also well developed, and include accelerated stability (e.g., 40 °C for 4 weeks), freeze-thaw cycles (three or four cycles), and vibrational testing. These mimic the events that commonly occur in the cold chain that can cause product failure. Studies with synthetic nanoparticles in aqueous solutions have shown that many of the interactions between nanoparticles and cosolutes are predictable with the salts influencing nanoparticle solubility following the series established by Franz Hofmeister in 1888, displaying different behavior depending on the hydrophobicity of the nanoparticle.[121,122] Published stability studies with LNPs have successfully used DLS to study the relationship between mean particle size, PDI, and ZP, and storage time of LNPs in solution.[123,124]

5. LNPs with Different Drugs Encapsulated

5.1. Water-Insoluble Drugs

About 40% of approved drugs and 90% of drugs under development are hydrophobic, so developing delivery vehicles for hydrophobic drugs has attracted significant interests.[125] LNPs have been widely used for hydrophobic drug delivery. A great number of hydrophobic drugs have been successfully encapsulated in LNPs with improved bioavailability and controlled release. For instance, Docetaxel (DTX) is a very potent antineoplastic and antiangiogenic agent. However, its clinical application is limited by its poor water solubility and high cytotoxicity. To address these issues, DTX-loaded LNPs were prepared using Compritol 888 ATO as the lipid material, Pluronic F127, and Span 80 as the stabilizers.[126] The LNPs had a particle size of 128 nm with a PDI of 0.2, and achieved 86% EE of DTX, 2% DL, and a controlled release profile was observed. Importantly, the final DTX-loaded LNPs remained stable for 120 days.[127] Similarly, Das et al. prepared tretinoin-loaded LNPs with >75% EE using Precirol ATO5 and Compritol 888 ATO using an emulsification–ultrasonication method. The tretinoin-loaded LNPs remained stable for 3 months at 4 °C.[56] Moreover, IR-780 iodide-loaded c(RGDyK)-conjugated LNPs were designed for near-infrared (NIR)-imaging-guided photothermal therapy, using a solvent-diffusion method. A high EE (85.34%) was achieved, and high cytotoxicity and low adverse effect were observed in mice experiments.[128] Furthermore, LNPs have been fabricated to encapsulate drugs which are neither soluble in water nor soluble in oil, such as Cisplatin (CDDP). Poor solubility of such drugs poses challenges for the design and development of drug delivery systems. Gup et al. successfully synthesized lipid-coated CDDP nanoparticles with high EE up to 80.8%.[129,130]

5.2. Water-Soluble Drugs

Encapsulation of hydrophilic drugs and precise control of their release are challenging because of their high water-solubility, so drug leakage toward aqueous phase is difficult to avoid. LNPs have been employed as a potential vehicle for entrapping and delivering hydrophilic drugs.[131] Microemulsion and double emulsions have been widely used for hydrophilic drug encapsulation. A microemulsion approach was developed to encapsulate paromomycin, a broad-spectrum antimicrobial for treating parasitic infections. It is a highly polar organic molecule with a very high aqueous solubility of 79.9 mg mL^{-1}. Using the microemulsion method, a maximal EE (41.65%) of paromomycin in stearic acid nanoparticles was achieved with a drug-to-lipid ratio of 4.[132] Basically, an aqueous phase containing paromomycin at 85 °C was added to a transparent mixture of stearic acid and surfactant under stirring. The formed microemulsion was then homogenized at 18 000 rpm for 20 min to generate nanoemulsions. Then the stearic acid nanoparticles containing paromomycin were obtained after cooled down in double-distilled water (2–5 °C).

Double emulsions are also commonly used for encapsulating hydrophobic drugs. The unique structure and property of W/O/W double emulsions enables the formation of hydrophilic drug-loaded LNPs.[133] For example, a chitosan-coated insulin-encapsulated LNP was developed for oral administration of insulin. The insulin-containing aqueous phase was added to Witepsol 85E in an organic phase and then homogenized to form the primary W/O emulsion which was then poured into 2% Tween 80 solution and homogenized again to form the secondary W/O/W double emulsion. Lastly, the double emulsion was poured in to a 0.5% w/v chitosan solution containing 2% Tween 80 under magnetic stirring until the solvent was fully removed to form chitosan-coated insulin-encapsulated LNP.[134] But the EE of LNPs produced with double emulsion method is normally not very high because of the immature release of hydrophilic molecules to external aqueous phases.[89]

LNPs have also been developed for making peptide-based cancer vaccines. Melittin is a cationic hydrophilic peptide derived from European wee venom. It shows a wide range of immunomodulatory and antitumor effects, but its pharmaceutical application is limited by its positive charges, resulting in cytotoxicity, rapid clearance, and nonspecific biodistribution. Compared to free melittin, Yu et al. designed an alpha-melittin-LNP. The melittin-loaded LNP achieved a 3.6-fold increase in immune

response intensity but less cytotoxicity due to the successful shielding of its positive charges.[135] LNPs have also been fabricated to encapsulate a hydrophilic antibiotic for oral delivery. LNPs containing streptomycin sulphate (STRS) were prepared using a cold high-pressure homogenization method, and achieved 30% DL and $51.17 \pm 0.95\%$ EE.[136] The synthesized STRS-loaded LNPs were able to overcome the gastric barriers with a significant intracellular uptake increase and a controlled release. Furthermore, an enhanced bioavailability (1.6–7 times higher) was achieved in in vivo studies when comparing to free drugs.

5.3. RNA

Over the past two decades, various gene therapies have been developed to treat a wide range of diseases including cancers, the acquired immunodeficiency syndrome (AIDs), and Parkinson.[137–139] Since the recent approval of the first two RNA drugs: Patisiran (2018) and Givosiran (2019), significant interest has been drawn in developing clinical RNA therapeutics. Patisiran is a LNP formulation of siRNA for the treatment of hereditary transthyretin-mediated amyloidosis.[140,141] RNA therapeutics face many challenges upon administration. RNA molecules are normally unstable and are prone to be degraded by nucleases and rapidly cleared by the immune system. Also, the negatively charges of RNA molecules make them unable to passively cross cell membrane. In addition, it remains a big challenge to unleash the potential of RNA molecules to target cells or organs in a controlled manner. Thus, delivery vehicles for RNA molecules are essential for facilitating their entry into cytoplasm. Both viral-based and nonviral carriers have been developed for RNA delivery. Viral-based vectors mainly include retroviral vectors, lentiviral vectors, adenoviral vectors, and poxviral vectors.[142] Generally, viral-based vectors invade cells via virus infection pathways, so they have advantages such as high gene transduction efficiency, site-targeted gene delivery, and enhanced immune response, but they also have some disadvantages including their mutagenic and carcinogenic concerns, high production cost, and low packaging capacity.[143] Compared to viral vectors, nonviral vehicles such as cationic polymers, lipids, and lipid-based materials have demonstrated higher flexibility and better safety profile.[144,145]

The development of ionizable cationic lipids makes it possible to achieve RNA cytoplasmic delivery. Ionizable cationic lipids are positively charged in an acidic environment but remain neutral at physiological pH.[146] Patisiran formulation (Onpattro, Alnylam Pharmaceuticals, Cambridge, MA, USA) approved in 2018 was the first representative ionizable cationic lipid formulated RNA drug. It contains a synthesized ionizable cationic lipid (DLin-MC3-DMA). Compared to permanently cationic lipids, ionizable cationic lipids are beneficial for not only electrostatically interacting with the negatively charged RNA at acidic pH, but also for inducing endosomal escaping and reducing cytotoxicity.[147] In other words, ionizable LNPs are less prone to be recognized and then removed by the reticuloendothelial system.[148] Upon entering endosome, the ionizable LNPs become positively charged in the acidic environment of the endosome (pH 5−6) and spontaneously fuse with anionic endosomal lipids, thus releasing the RNA cargo into the cytoplasm. Optimal efficiency can be achieved for targeting the liver using RNA-loaded LNPs via IV administration when the pKa value of ionizable lipids is between 6.2 and 6.5.[149] Another RNA-encapsulated LNP has been designed to deliver CRISPR-Cas9 gene, which was verified to be capable of permanently destroying tumor genes. A NanoAssemblr microfluid mixing device was used to prepare the CRISPER-LNPs using ionizable lipid, DSPC, cholesterol DMG-PEG, and DSPE-PEG with the molar ratio of 50:10.5:38:1.4:0.1, which achieved 70% gene editing efficacy in vivo, 50% growth inhibition of cancer cells, and a large survival rate increase.[150]

In addition, the two COVID-19 vaccines (BioNTech/Pfizer and Moderna) are also LNP formulations of mRNA. Both Pfizer and Moderna vaccines consist of ionizable cationic lipid, PEGylated lipid, neutral lipid, and cholesterol but relatively different molar lipid ratios. Pfizer vaccine is composed of [(4-hydroxybutyl) azanediyl]bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315), 2-[(polyethylene glycol)-2000]-*N*,*N*-ditetradecylacetamide (ALC-0159), DSPC, and cholesterol with a molar lipid ratio (%) of 46.3:9.4:42.7:1.6. Moderna vaccine contains heptadecan-9-yl 8-{(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino}octanoate (SM-102), 1-mono methoxy polyethyleneglycol-2,3-dimyristylglycerol with polyethylene glycol of average molecular weight 2000 (PEG_{2000}-DMG), DSPC, and cholesterol at a molar ratio of 50:10:38.5:1.5.[14]

Generally, lipid-based RNA delivery systems consist of four main ingredients, including ionizable cationic lipid, structural lipid, cholesterol, and polyethylene glycolipid. The ionizable cationic lipids are mainly positioned at the inner core of the LNPs, whereas the structural helper lipids form the outer layer of the LNPs.[151] The common way to produce such LNPs is to rapidly mix an ethanolic-lipid solution with an acidic aqueous solution containing negatively charged RNAs followed by dialysis in a neutral buffer. Many factors affect the formation of LNPs, including the ratio of lipid compositions, the ratio of the ethanol solution to the aqueous solution, and the mixing conditions. Staggered herringbone micromixers were used to improve mixing. Total flow rate (TFR) and FRR are two significant parameters affecting the mixing, thus the formation of LNPs. Regardless the type of cationic lipid, small mixing rates form larger particles, and higher flow rates generate smaller particles. Buffer and lipid content are two other important factors that affect the size and stability of LNPs.[148] In order to better understand the formation of LNPs, a recent study investigated the formation mechanism of siRNA loaded LNPs and found a fusion process occurring at pH neutralization. When LNPs synthesized at acidic conditions, they were small but fused into larger electron-dense particles during dialysis at pH 7.4 (**Figure 8**).[152]

6. LNPs for Addressing Challenges in Drug Delivery

Drug-loaded LNPs have been explored for treating a wide variety of diseases including brain diseases, cancer, and infection.[153] Herein, we are not aiming to provide a comprehensive review about LNPs for various diseases, but to select a few representative examples (**Table 1**) to highlight the recent development of

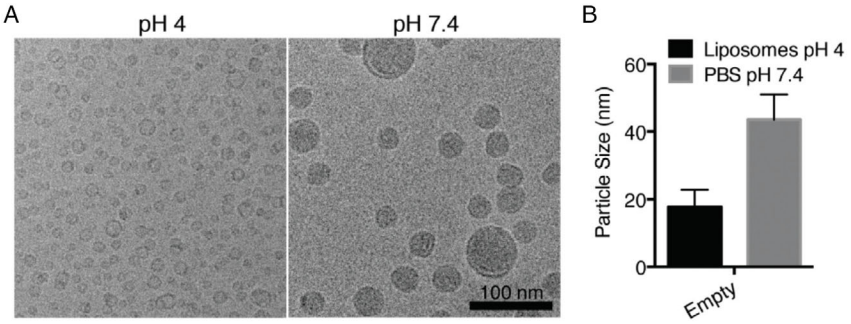

Figure 8. Morphological changes of LNPs by fusion during pH neutralization. A) LNP were generated at pH 4 and dialyzed into pH 4 buffer (left) or dialysed into PBS pH 7.4 (right). B) Particle size of LNPs at pH 4 and pH 7.4. Reproduced with permission.[152] Copyright 2019, Royal Society of Chemistry.

applying LNPs to address key drug delivery challenges, for example, crossing the BBB, targeted delivery, and oral delivery.

6.1. BBB

The BBB is a highly selective and semipermeable endothelial cell line that protects the brain from being invaded by foreign pathogens and unwanted substances in circulation.[154] Moreover, some efflux transporters like P-glycoprotein on the BBB surface mediate the active transport of a broad range of substances including nutrients and drugs, from the central nervous system back to the circulation.[155] This characteristic of BBB contributes to the difficulty for conventional brain drugs to be absorbed leading to low bioavailability.[154] To address this challenge, a wide variety of drug delivery systems have been designed to cross the BBB. LNPs have been extensively explored for noninvasive brain drug delivery because of their excellent safety, good stability, and potential improved therapeutic efficacy.[156] For instance, as a low-density lipoprotein (LDL), Apo E has been demonstrated to enhance nanoparticle penetration through the BBB, thus achieving the targeted brain drug delivery.[157] Consequently, various Apo E-coated LNPs were developed for treating brain cancer. Campthecin-loaded LNPs were synthesized with polysorbate 80 coating and then Apo E would preferentially be adsorbed onto the LNPs facilitating the targeted delivery across the BBB.[158] A similar strategy was adopted for the delivery of donepezil, an anticholinergic drug for the treatment of Alzheimer's disease. Donepezil and a fluorescent dye called Rhodamine B were co-encapsulated within the LNPs. Instead of taking advantage of Apo E present in blood, LNPs were directly modified with Apo E. The Apo E-modified Donepezil-loaded LNPs showed enhanced central nervous system targeting.[159] Similarly, Dal Magro et al. used Apo E-derived peptide-functionalized LNPs to target BBB.[160]

6.2. Targeted Delivery

Targeted delivery is a strategy for selective delivery of drugs to specific site of action without affecting or damaging normal cells and tissues. Targeted delivery utilizes LNPs to overcome the limitations of conventional drug delivery systems including nonspecific action, uncontrolled release, via ligand modification, composition alteration, pH-responsive, or other ways.

One common strategy for designing targeted delivery systems is to modify nanoparticles with targeting ligands which bind specifically to those overexpressed receptors on malignant cells.[161,162] For example, some carbohydrates can preferentially bind to the lectin receptors on some tumor cells, so carbohydrates functionalized LNPs have the potential for tumor-targeted drug delivery. Jain et al. produced mannosylated LNPs to carry an anticancer drug DOX. In vitro results suggested a more efficient cellular uptake in A549 lung epithelial cancer cell lines; meanwhile, a prolonged retention in circulation and a narrow biodistribution in tumor sites has been observed in tumor-bearing mice.[158] Transferrin (Tf) is another widely used targeting ligand. Tf-modified LNPs with curcumin loaded demonstrated enhanced breast cancer cell uptake.[163] Also, a LNP tumor-targeted drug delivery system was developed to selectively release the cargo at desired sites by mimicking high-density lipoprotein (HDL). A sub-30 nm HDL-mimicking LNP was prepared by conjugating epidermal growth factor (EGF) to LNPs to achieve enhanced accumulation in tumor cells.[164] Zhai et al. functionalized PTX-loaded LNPs with an EGF antibody for targeting at aggressive ovarian cancer.[165]

Alternatively, lipid compositions of LNP can be designed for targeted delivery.[166] LNPs formulated with a helper lipid 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) tend to accumulate in the liver, whereas LNPs containing helper lipid DSPC preferred to distribute in the spleen in vivo. When the nucleic acids (Cy3-siRNA or mRNA) encoding for firefly luciferase were encapsulated within LNPs containing a helper lipid DOPE, they increased the internalization of nucleic acids to the liver twofold leading to threefold increase of liver transfection efficiency. Meanwhile, the DSPC-containing LNPs increased twofold the delivery of siRNA to the spleen and improved fivefold the delivery of mRNA.[167] Another case proved the effects of lipid compositions on targeted delivery by systematically screening lipids and altering the different segments of lipids to test the impacts on drug cellular uptake in cancer cells. The order of cellular uptake from strong to weak, receptor-targeted has the greatest cell uptake, then is cationic head group, followed by zwitterionic head group and negatively charged head group. Besides, the length of the acyl chain in the lipid tail also affects the magnitude of cellular uptake. The longer the acyl chain is, the stronger is the cell uptake (18:0 > 16:0 > 14:0). When the tail length is the same, unsaturated fat is more absorbable than

Table 1. Different LNPs and their applications.

Nanoparticles	Lipid	Surfactant	Drug	Method	Disease	Properties	References
LNPs	Wax cetyl palmitate, DMPC	Polysorbate 60 or 80	Camptothecin	Nonsolvent emulsification	Glioblastoma	130.2 ± 7.3–159.7 ± 8.0 nm −19.8 ± 1.6 to −21.8 ± 2.0 mV 91.3 ± 2.1–92.9 ± 3.0% EE	[158]
Apo E-targeting LNPs	Dynasan 116	Tween 80	Donepezil	Homogenization-sonication	Alzheimer's disease	≥140 nm, 86% EE, –9.6 ± 0.5 mV	[159]
Fas ligand antibody conjugated PEGylated LNPs	Medium chain triglyceriade, Amino-terminated polyethylene glycol monostearate	Polysorbate 80	3-*n*-Butylphthalide	Solvent diffusion method	Brain ischaemic stroke	61.0 ± 8 nm, 93.1 ± 0.4% EE, 15.51% DL	[173]
Lactoferin-modified LNPs	Glyceryl monostearate, stearic acid	Tween 80, Soy-lecithin	Docetaxel	Emulsification and solvent evaporation	Brain tumour	121.0 ± 5.7 nm, 91.0% EE, 22.8% DL, −21.5 ± 1.2 mV	[174]
Mannosylated LNP	Tristearin, stearyl amine	Soya-lecithin	Doxorubicin	Solvent injection	Lung cancer	359.9 ± 0.5 nm, 70.3 ± 0.9% EE, 4.1 ± 0.2 mV	[175]
Transferrin-mediated-LNPs	Hydrogenated soya phosphatidylcholine, DSPE, Cholesterol, Triolein	Poloxamer 188	Curcumin	High-speed homogenization – high-pressure homogenization	Breast cancer	206.0 ± 3.2 nm, 77.3 ± 2.3% EE, 8.2 ± 0.9 mV	[163]
Self-assembled LNPs	Monoolein	Pluronic F127 triblock copolymers, Tween 80, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-PEG M_w=3400-maleimide	Paclitaxel	Solvent evaporation	Aggressive ovarian cancer	170–250 nm, 0.15 PDI	[165]
Magnetic LNPs	Cetyl palmitate	Tween 80	Sorafenib, superparamagnetic iron oxide nanoparticles	Microemulsion-solvent evaporation	Hepatocellular Carcinoma	248 ± 113 nm, 90% EE, -23.0 ± 5.3 mV	[176]
pH-responsive LNPs	Trilaurin, sodium laurate	PEG	Doxorubicin	Microemulsion-evaporation	Tumor	105 nm, 3.3 ± 0.3 mV, 90.1–91.5% EE	[168]
Chitosan-coated LNPs	Cetyl palmitate	Tween 80	Rifampicin	Microemulsion	Tuberculosis	524 ± 39 nm, 90.2 ± 1.3% EE, 4.5 ± 0.1% DL, 30.85 ± 2.7 mV, Mucoadhesive	[169]
LNPs	Stearic acid, lecithin	Myrj 52	Curcumin	Emulsion solvent evaporation	Asthma	190 nm, −20.7 mV, 75% EE	[171]
LNPs	Compritol 888 ATO	Polysorbate 80, soy lecithin	Isoniazid	Nonsolvent emulsification	Tuberculosis, oral delivery	48.4 nm, 69.0 ± 0.7% EE, slow release (60%, in 24 h)	[170]

saturated fat (18:1 > 18:0). In terms of the application of lipids for cancer therapy, those LNPs are composed of phospholipids with shorter acyl chains (12:0 and 14:0), due to the destabilization of cell membranes, hence reduce cells growth. Therefore, LNPs containing phospholipids with longer carbon tails such as dipalmitoylphosphatidylcholine (DPPC) should be avoided for anticancer therapy because they can enhance the internalization of exogenous lipids, thus support cancer cells proliferation. It should be noted that when cholesterol is formulated into anticancer LNP systems to rigidify the particles, the cellular uptake of short lipids like 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC) is optimized whereas resulting in less efficient endocytosis for that long-chain lipids.[166]

PH-responsive LNPs have also been designed for targeted drug delivery especially in tissues or organs have different pH values compared with normal tissues such as cancerous tissues. Chen et al reported a type of pH-responsive cholesterol-PEG adduct coated, DOX-encapsulated LNP which had a higher drug unloading percentage (63.4%) observed at pH 4.7 compared to pH 7.4 (25.2%) due to the weakened electrostatic force among the negatively charged laurate and the positively charged DOX. In vivo results indicated that pH-responsive DOX-loading LNPs preferentially accumulated in tumor organs in contrast to free DOX with no adverse effects observed, and efficiently inhibited the tumor growth in Balb/c nude mice.[168]

6.3. LNPs Applications in Other Diseases

Drug-loaded LNPs have been used for other drug delivery applications. A mucoadhesive chitosan-incorporated, rifampicin-loaded LNPs system was developed to treat tuberculosis, one of the leading causes of death worldwide. The chitosan-incorporated LNPs demonstrated a stronger mucoadhesive strength with mucin and greater cellular uptake, representing a promising delivery system for pulmonary-targeted drug delivery.[169] Another study prepared isoniazid-loaded LNPs using a microemulsion technique for antitubercular applications. The isoniazid-loaded LNPs demonstrated an improved oral bioavailability and prolonged retention, low hepatotoxicity, and neurotoxicity.[170] Wang et al. developed curcumin-loaded LNPs to treat asthma with a significantly higher bioavailability and efficacy.[171] Lopinavir-loaded LNPs were fabricated for intestinal lymphatics targeting delivery. Compared to the conventional drug formulations, lopinavir-loaded glyceryl behenate-based LNPs exhibited fivefold higher lymphatic accumulation and 2.13-fold higher oral bioavailability with longer shelf life.[172]

7. Conclusion

LNPs have been widely used for drug delivery applications not only in preclinical studies but also in clinical contexts. Many LNPs have been approved for clinical uses, demonstrating their unique advantages compared to other drug delivery systems. This review highlights LNP as a drug delivery system with a focus on three lipid-based systems, that is, SLNs, NLCs, and hybrid lipid-polymeric nanoparticles. Various approaches have been developed for synthesizing these LNPs, including traditional methods (solvent-based emulsification, nonsolvent-based emulsification, bulk nanoprecipitation) and more advanced technologies (SCF technology, coacervation method) or microfluidics (chip-based microfluidics, capillary-based microfluidics) and mixers (static mixers). Meanwhile, a wide range of drugs including water-insoluble drugs (DTX, tretinoin, IR-780 iodide), water-soluble drugs (paromomycin, insulin, melittin, STRS), and RNAs (CRISPER-Cas 9, mRNA COVID-19) have been successfully incorporated into LNPs. Encapsulation of hydrophobic drugs in LNPs is easier than encapsulating hydrophilic drugs because of the compatible hydrophobicity of hydrophobic drugs and hydrophobic lipids. But new strategies have been developed to address the challenge of encapsulating hydrophilic drugs. For example, the development of ionizable lipids allows the encapsulation of negatively charged nucleic acids in LNPs, laying foundation for the success of LNPs for RNA delivery. Stability of LNPs, retention of drugs in the particles, and their controlled release are essential for drug delivery applications, but remain challenging. Designing new lipids and improving the formulation of LNPs will provide new opportunities for their drug delivery applications.

Another significant challenge is targeted drug delivery. Although continuous efforts have been directed to develop new targeted delivery systems, it is far away from clinical reality. Some promising strategies have been developed for LNPs targeted delivery. For example, Apo E-functionalized LNPs have been demonstrated capable of overcoming the obstacles of the BBB. Also, ligand-modified LNPs (carbohydrates-functionalized LNPs, Tf-modified LNPs, EGF-conjugated LNPs), and stimuli-responsive LNPs (pH-responsive LNPs) haven been designed for targeted delivery. In addition, lipid components have impact on the biodistribution of drugs. DOPE-formulated LNPs tend to accumulate in the liver, whereas DSPC-formulated LNPs prefer the spleen. Better understanding of the lipid chemistry/structure–function relationships will definitely improve future design of more effective LNPs for drug delivery. Also, new technologies such as machine learning or meta-data analysis on studies in the literature will also provide powerful tools for future LNP design.

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Conflict of Interest

The authors declare no conflict of interest.

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drug delivery, large-scale production, lipid nanoparticles, microfluidics, nanostructured lipid carriers, RNA delivery, solid lipid nanoparticles

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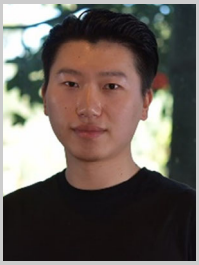

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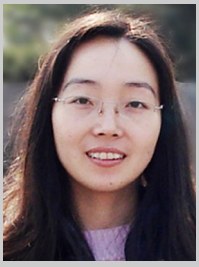

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