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Current potential and challenges in the advances of liquid crystalline nanoparticles as drug delivery systems

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Lyotropic nonlamellar liquid crystalline nanoparticles (NPs) (LCN), such as cubosomes and hexosomes, are useful tools for applications in drug delivery because of their unique structural properties. LCNs are highly versatile carriers that can be applied for use with topical, oral, and intravenous treatments. In recent years, significant research has focused on improving their preparation and characterization, including controlling drug release and enhancing the efficacy of loaded bioactive molecules. Nevertheless, the clinical translation of LCN-based carriers has been slow. In this review, we highlight recent advances and challenges in the development and application of LCN, providing examples of their topical, oral, and intravenous drug delivery applications, and discussing translational obstacles to LCN as a NP technology.

Introduction

Nanotechnology research has continued to focus on exploiting engineered nanosized particulate systems for the efficient delivery of bioactive molecules. In recent years, interest has increased on nanocarrier systems mimicking the curvature of cellular membranes [1–3]. This is because of significant advantages, including of higher membrane surface area:volume ratios, variations in membrane stress, and increased hydrophobic and membrane protein-loading capacities compared with planar structures [4]. Lipid-based systems are widely studied nano-self-assemblies with drug delivery, cosmetics, biosensing, and theranostic applications [5,6]. Among the various lipid self-assemblies, liquid crystalline (LC) phases have received particular research interest for use in drug delivery (Fig. 1).

LC phases are an intermediate form of matter between liquid and solid crystalline arrays, classifiable into structural types based on classes of order and orientation: (i) nematic; (ii) smectic; (iii) cholesteric; and (iv) columnar [7]. All materials demonstrating LC behavior are generally identified as being either: (i) lyotropic (i.e., a product of interactions between anisotropic aggregates of amphiphilic molecules); or (ii) thermotropic (i.e., the product of interactions among partially rigid anisotropic molecules that can change the order as a function of temperature) [8,9]. Physicochemical triggers, such as temperature, pressure, lipid/additives and water composition ratio, pH, and shearing, modulate the phase behavior and internal structure of self-assembled lyotropic LC mesophases, in which various forms have been characterized, including inverse bicontinuous cubic, discontinuous cubic, inverse hexagonal, inverse micellar, and sponge phases [10–13]. Within the bicontinuous cubic phase system, there are three phases [Im3m (Schwarz surface), Pn3m (diamond surface), and Ia3d (gyroid surface)] to describe the different geometries, all of which also show high drug delivery potential [14]. LCNs are formed by breaking down bulk LC phases to nanosized particles

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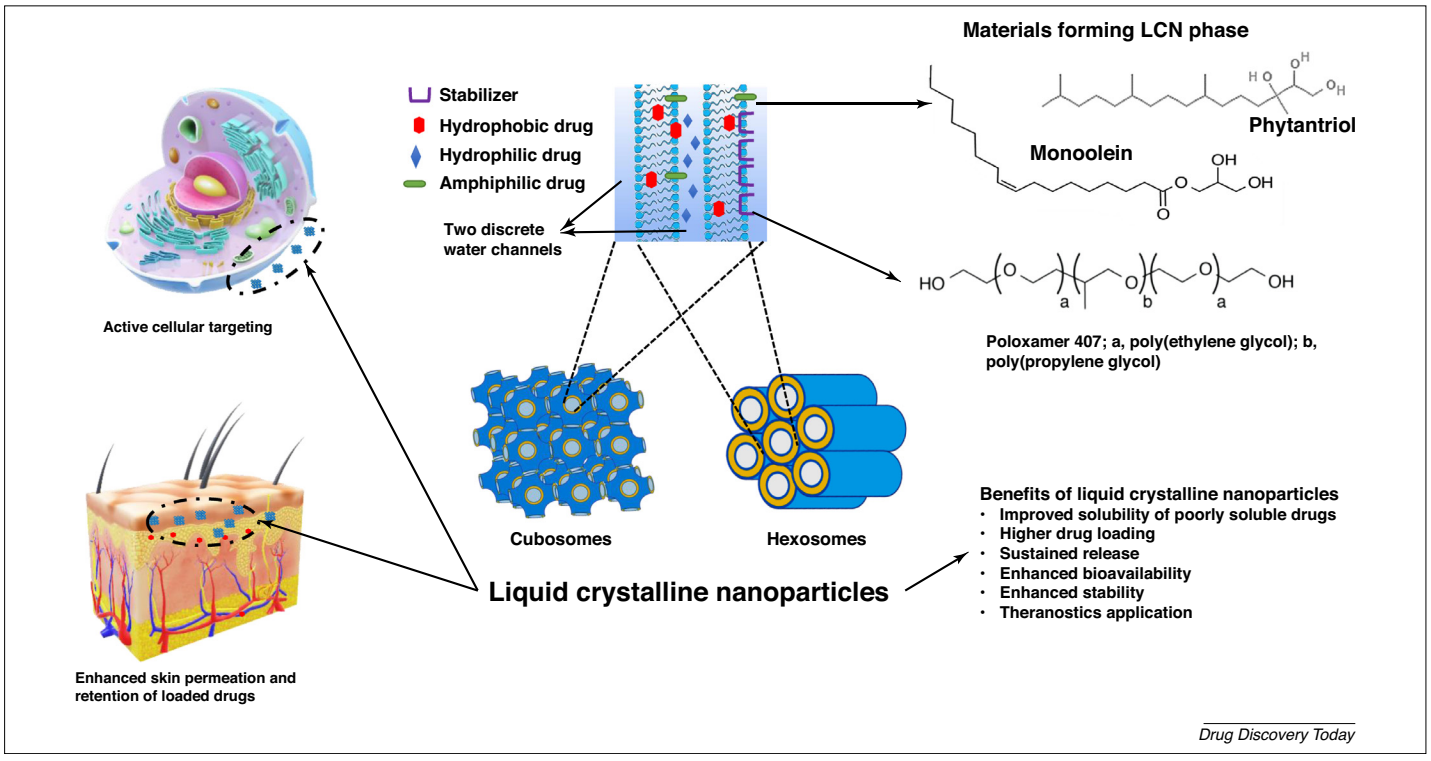

FIGURE 1

Summary of the benefits and applications of liquid crystalline nanoparticles (LCNs).

without altering the structural integrity of bulk phases. In addition, LCNs self-assembled from amphiphilic lipids in the presence of excess aqueous media can serve as novel carriers because they combine the advantages of liposomes and NPs by providing protection and enabling sustained release of compounds solubilized in the LCN matrix in a biocompatible manner [15].

In this review, we discuss recent advances in the development of LCNs towards potential therapeutic applications, with a focus on the lipid materials used in the formation of the LC phase, including basic characterization techniques. We also suggest areas to be explored for the translation of LCN-based delivery systems into promising strategies for the delivery of various therapeutically active molecules in the clinic.

Materials forming liquid crystalline phases

The key consideration in the formation of LC phases is the self-assembly of amphiphilic molecules, usually lipids that form various mesophases, including hexagonal, cubic, and micellar phases. Among the various phases, hexagonal and cubic phases are the most commonly occurring lyotropic phases and are widely studied as drug delivery carriers. The hexagonal phases comprise 2D hexagonal lattices comprising micellar cylinders formed by lipid self-assembly. Depending on the orientation of the hydrocarbon chain of the amphiphilic molecules, hexagonal phases are classified as either normal (H_1) or inverse (H_2). In H_1 phases, the hydrocarbon chains are orientated towards the inner core, whereas in H_2 phases, the chains have a polar head. The H_2 phase forms a channel of water inside the micellar cylinder and there is no contact with water between the inside and outside of the aqueous domain [16–18].

The bicontinuous cubic phase is of particular interest in drug delivery owing to its unique structural features. It is an intermediate phase between the flat lamellar phase and cylindrical hexagonal phases. The bicontinuous structure is regarded as a 3D periodic lipid structure and comprises two continuous but nonintersecting water channels separated by a curved bicontinuous lipid bilayer [19].

Monoolein (MO), with the chemical name 1-Monoolein [1-(cis-9-Octadecenoyl)-rac-glycerol], has wide applications in the pharmaceutical and food industries because of its biodegradable and biocompatible properties. MO forms various phases of amphiphilic systems, including lamellar, hexagonal, bicontinuous cubic, sponge, and discrete micellar phases [15]. These differences in the self-assembly of MO are influenced by various physicochemical factors that result in the formation of a range of phases. This includes lipid hydrocarbon chain length, saturation, temperature, and water content. In addition to MO, the other lipid extensively studied is phytantriol (PHY), which is an active ingredient used in various cosmetics and personal care products. Similar to MO, PHY forms various mesophases depending on the concentration of lipid, water, and temperature. PHY is a cosmetic ingredient that is generally considered as a safe amphiphilic lipid with biocompatibility and good mucoadhesive properties. The unique advantage of PHY over MO is its ability to retain a LC structure under physiological conditions, with less susceptibility to rapid cleavage by lipase because of the absence of ester linkages and presence of phytanyl functional groups [20–22]. Glyceryl monooleyl ether is an alternate to MO that forms a cubic phase with the addition of propylene glycol and is less prone to hydrolytic degradation [23]. Table 1 summarize the examples of materials used in the LCN preparation and its significance application.

TABLE 1

Examples of LCN systems as drug delivery vehicles for various applications

Type of liquid crystalline phase/NP	Self-assembling material	Stabilizer	Application	Therapeutic/ diagnostic molecule	Advantages	Refs
Cubosomes and hexosomes	MO	P407	Topical	Celecoxib	Enhanced skin permeation and modulation of drug release	[50]
Cubic and lamellar	MO	–	Topical	Crocetin	Improved skin retention for cutaneous application	[54]
Cubic	MO	–	Transdermal	Metformin	Transdermal delivery of hydrophilic drugs	[56]
Cubosomes	MO	P407	Hepatocellular carcinoma	Sorafenib	High cellular uptake and greater apoptotic effects in HepG2 cells	[57]
Cubosomes	MO	P108	Theranostics	Codelivery of paclitaxel and a fluorescein-based lipid dye	Active delivery of paclitaxel to human adenocarcinoma cell line; enhanced cellular uptake via receptor-mediated endocytosis	[58]
Hexosomes	PHY	P407, Pluronic® (F-68 and F-87)	Oral delivery	Insulin	Demonstrated stability of NPs with higher Caco-2 cellular uptake and sustained glucose lowering profile	[60]
Hexosomes	MO and PHY	P407, Pluronic® (F-68 and F-87)	Oral delivery	CoQ10	Improved oral bioavailability	[61]
Hexosomes	MO	P108 conjugated to fluorescent (rhodamine) and cancer cells targeting (folate residues)	Theranostics	Docetaxel	Sustained release of drug with enhanced cytotoxic effect against HeLa cells	[63]
Hexosomes	MO	P407	Theranostics	Phytoestrogen and tetraphenylethene	Enhanced targeted oncotherapy and noninvasive detection of tumour	[69]

LCN preparation and characterization

LCNs can be prepared by various methods, including size reduction of bulk LC phases using homogenization, sonication, and shearing [24]. Briefly, the methods are classified as either top-down or bottom-up depending on the type of energy sources used to break the bulk phases. Top-down approaches involve the utilization of high-pressure homogenization and sonication, whereas bottom-up approaches reduce the energy inputs by incorporating hydrotopes. Both the methods use Pluronic F127(P407) as a stabilizing agent to form a homogenous dispersion. P407, known as triblock polymer, comprises a polyethylene oxide-polypropylene oxide-polyethylene oxide copolymer, which stabilizes the LCN by forming a steric barrier and preserving the LC internal structure of the particle [25]. However, other stabilizers, such as Myrj 59, Tween 80, PEGylated-phytanyl copolymers, and Cremophor, have been shown to be a better alternate to P407 [26–29].

The use of high-energy conditions results in the formation of stable and reproducible LCNs without the addition of hydrotopes, which can alter the phase behavior. In the bottom-up approach, the addition of a hydrotope reduces the energy input and achieves a stable NP formation [30,31]. The limitation to the use of hydrotopes is the formation of additional vesicular structures and their adverse effects in the body. The colloidal stability of LCN in aqueous dispersion is one of the major limitations of all the aforementioned preparation methods. To overcome this limitation, studies have aimed to develop powdered cubosomes by using spray-drying techniques. The involves the production of spray-dried LC phases forming a precursor that reforms to LCNs on hydration with excess water [32–34]. However, this results in the high polydispersity of particle size because of the utilization of bulk-scale mixing of ethanol and water.

To overcome the limitations associated with conventional methods, there is a demand for an appropriate technique to achieve narrow-size LCNs with a scalable process. Conventionally prepared LCNs display numerous features of a reliable nanocarrier system, including excellent stability, ability to load multiple drugs, with tailored flexibility of surface modifications for targeting ligands. However, the preparation step needs further investigation for scaling up to production with clinically proven qualities. The preparation process can have a vital role in improving the physicochemical attributes of LCNs. Laboratory-scale production by manual pipetting or vortex mixing introduces significant process variability, which can impact the quality and nanocarrier efficacy of LCNs. The difficulties in production of LCNs in a controlled, reproducible, and scalable manner also delay their clinical translation [35].

Towards scaling up the formulation for mass production, a more sophisticated method is required. Vortex mixing with low heat production has been proven to generate homogenous LCNs. Alternatively, high-energy ultra-sonication can be used to produce

LCNs on a larger scale. LCNs can encapsulate large amounts of gene therapeutics, including small interfering (si)RNA compared with conventional liposomes. Significantly, the membranes that form cubosomes have intrinsic fusogenic properties that promote fast endosomal escape. Regardless of their potential, traditional routes of forming cubosomes lead to particle sizes too large to fulfill the state-of-the art requirements of delivery vectors. Recent microfluidic platform-based rapid-mixing protocols offer a unique advantage in generating small-sized LCNs. Recently, Kim *et al*. synthesized cubosomes and siRNA-loaded cubosomes using a microfluidic approach. The preparation methods involve the utilization of a microfluidic chip to form cuboplexes and characterized by cryogenic transmission electron microscopy (cryo-TEM) and small-angle X-ray scattering (SAXS). Time-resolved mechanisms were elucidated in which microfluidic devices allow the production of small cuboplexes (75 nm) that outperform commercially available delivery vectors, as well conventional liposomes [36]. When scaling up the formulation, there should be a trade-off between the production yield and the desired particle characteristics. This needs to be considered when tailoring large-scale production to meet the requirements of specific applications. Despite the prevalence of the microfluidics method in LCN fabrication, there are certain limitations that prevent it from being suitable for all applications [35,37].

SAXS is the most widely used technique to characterize the phase behavior of LC phases. The diffraction patterns obtained from SAXS analyses provide information on lattice parameters that are useful to characterize the LC mesophases. In addition, SAXS data are essential to investigate the effect of lipid composition, stabilizer, and temperature on the phase transition and to establish the phase boundaries [38–41]. To observe the morphological characteristic of LCN, cryo-TEM and cryo field emission scanning electron microscopy (cryo-FESEM) were used to visualize the internal structure of LC phases [42,43]. A dynamic light-scattering technique is used to measure the particle size, surface charge and stability of the dispersion. Other analytical tools, such as differential scanning calorimetry and nuclear magnetic resonance (NMR), are used to characterize the fundamental material properties [1]. Polarized light microscopy (PLM) is a well-known tool for visual inspection of LC phases. Using this technique, the hexagonal and cubic phase can be identified easily. Under polarized microscopy, a hexagonal phase shows birefringent properties because of its anisotropic properties, whereas a cubic phase shows no typical texture owing to its isotropic property [44]. A brief summary of the characterization techniques is provided in Table 2.

Applications of liquid crystalline nanoparticles

Enhancing oral delivery

LC phases are formed by the self-assembly of amphiphilic lipids in excess water to result in thermodynamically stable structures, such as bicontinuous cubic, hexagonal, and lamellar phases. LCNs are prepared by dispersing lyotropic LC phases in aqueous media with suitable surfactant. Owing to the unique structural properties of these nanostructures, they have various advantages, including loading of hydrophilic, hydrophobic, and amphiphilic bioactive molecules. In addition, LCNs have attracted special interest as drug carriers for enhancing the solubility of poorly soluble drugs. For example, Freag *et al*. loaded rapamycin, a poorly soluble anticancer drug, into surface-modified LCN,s to enhance its water solubility and anticancer activity. The study demonstrated a higher percentage of encapsulation efficiency with sustained drug release and enhanced cytotoxic effect against human breast cancer cell lines, such as MCF-7 and MDA-MB-231. An in vivo study showed a 3.35-fold increase in the bioavailability of rapamycin compared with free drug. Furthermore, the study demonstrated higher antitumor activity against an Ehrlich ascites tumor model with reduced nephrotoxic and hyperglycemic effects associated with free rapamycin [45]. In addition to synthetic anticancer drugs, clinical applications of certain natural compounds often have poor solubility and low bioavailability. Curcumin is a curcuminoid obtained from the turmeric plant (*Curcuma longa*) and is proven to inhibit various malignant cell types. The clinical application of curcumin is limited owing to its poor solubility. Baskaran *et al*. incorporated curcumin into LCNs and demonstrated its enhanced stability and cellular uptake in a human colonic cancer cell line [46].

Topical and/or transdermal delivery

MO- and PHY-based LC mesophases have been extensively investigated as carriers for topical administration to enhance the permeation of bioactive molecules through the skin and to sustain drug release on the superficial layer of the skin [29,47,48]. The

TABLE 2

Summary of analytical tools used in the characterization of LCNs

Characterization tool	Parameters measured	Observations
PLM	Macroscopic structure; preliminary phase identification	Cubic phase, a dark background without birefringence; hexagonal phase, a fan-like structure; lamellar phase, a birefringence structure
SAXS	Molecular dimensions; phase identification; impact of guest molecules on liquid crystalline structure	Bragg's peak: cubic phase; Pn3m, $\sqrt{2}$, $\sqrt{3}$, $\sqrt{4}$, $\sqrt{6}$, $\sqrt{8}$, etc.; Im3m, $\sqrt{2}$, $\sqrt{4}$, $\sqrt{6}$, $\sqrt{8}$, $\sqrt{10}$, etc.; Ia3d, $\sqrt{6}$, $\sqrt{8}$, $\sqrt{14}$, $\sqrt{16}$, $\sqrt{20}$, etc.; hexagonal phase, $\sqrt{1}$, $\sqrt{3}$, $\sqrt{4}$, $\sqrt{7}$, $\sqrt{9}$, etc.; lamellar phase: a broad peak Lattice parameter and water channel radius: cubic phase, $\alpha = d(h^2 + k^2 + l^2)^{1/2}$, $r = 0.391\alpha - 1$, $r = 0.305\alpha - 1$; hexagonal phase - $\alpha = 4d/3(h^2 + k^2)^{1/2}$, $r = (\alpha - 2l)/2$
DSC	Phase transition evaluation; effect of guest molecules on liquid crystalline structure	Rise in enthalpy; transition of endothermic peak; temperature impact on system
NMR	Inner structure; diffusion pattern of molecule components	Interactions between mesophase components and loaded molecules
Cryo-TEM and cryo-FESEM	Morphological features	Visualization of inner structures

successful delivery of bioactive molecules through the skin using LC mesophases results from their unique structural resemblance to skin microstructures, which favors the easy diffusion of mesophases through the stratum corneum. In particular, the cubic structure of mesophases can be matched with the in vivo cubic organization of the epidermal barrier of the skin surface [49], increasing the partitioning of liquid crystalline phases and lipid lamellae of the skin.

Celecoxib is an orally administered anti-inflammatory and chemopreventive agent. To overcome the systemic toxicity associated with its oral administration, a LCN system with the addition of excipients such as propylene glycol and oleic acid was developed as a topical carrier. The authors concluded that the addition of oleic acid sustained celecoxib release and anti-inflammatory effects, whereas the oleic acid–propylene glycol combination increased the drug release rate [50]. Similar results were reported in studies in which the addition of excipients significantly altered the release of drug with lipophilic properties by influencing the structure of the LC phase [51–53].

In a recent study, Esposito *et al.* developed MO-based LC bulk phases to enhance the cutaneous permeation of crocetin, a poorly soluble compound. An in vivo study was carried out in healthy human volunteers to measure the amount of drug in the superficial layer via the tape-stripping method. Interestingly, this study showed a higher concentration of crocetin in the stratum corneum and decreased concentration in the deeper layers of the skin. This finding revealed that, for a lipophilic drug, the LC phase allows the targeting of the upper skin layers, achieving prolonged action on the skin and reducing the systemic exposure of the drug [54]. A similar result was obtained for 5α-reductase inhibitors with lipophilic properties with LCN-based formulations, where increased retention of 5α-reductase inhibitors in the upper layers of skin was observed [48].

Hydrophilic molecules have poor percutaneous permeability because of the lipophilic nature of the skin membrane [55]. Given the 3D bicontinuous structure of mesophases, with two intersecting water channels, limitation associated with the loading and delivery of hydrophilic molecules can be circumvented. For example, Yu *et al.* studied the mechanism of transdermal delivery of MO-based cubic phases containing metformin hydrochloride, an essential antidiabetic drug with hydrophilic properties. The authors extensively evaluated drug permeation through mouse skin in in vitro experiments combined with molecular docking techniques. The authors concluded that MO enhanced drug permeation by increasing the fluidity of skin lipids through interactions with skin components [56].

Cancer targeting

Thapa *et al.* developed an efficient layer-by-layer polymer-assembled LCN based on MO and stabilized by P407 to deliver sorafenib, a poorly soluble drug used in the treatment of advanced hepatocellular carcinoma. The developed sorafenib-loaded LCN was coated with six layers of poly-L-lysine and polyethylene glycol-b-polyaspartic acid. The coating of NPs was proposed to overcome the limitations with the intravenous administration of LCNs, including bioadhesivity, rapid removal from the blood circulation, and LCN-induced hemolysis. Furthermore, it provided controlled drug release, targeted delivery, and improved therapeutic indices of anticancer activities. This study demonstrated that multilayered LCNs promote high cellular uptake with superior apoptotic effects. In addition to single anticancer drug delivery, authors recently reported the possibility for combination chemotherapy using LCN, wherein drugs with different solubility profiles were loaded into a LCN system. In vivo studies showed enhanced antitumor activity because of higher cytotoxicity and improved expression of apoptotic markers with minimal adverse effects. This study provides useful information on the viability of LCNs as dual drug delivery systems for the effective treatment of metastatic breast cancer [57]. Similarly, Aleandri *et al.* developed cubosomes stabilized by modified PF108 for paclitaxel, a poorly soluble anticancer drug. In this study, PF108 was conjugated with biotin to actively target overexpressed biotin receptors in HeLa cells. The results showed increased cellular uptake of paclitaxel-loaded biotinylated cubosomes in HeLa cells via biotin receptor-mediated endocytosis [58].

He and coworkers developed LCN nanotransformers with satisfactory siRNA-loading efficiency and low cytotoxicity in the intracellular acidic environment for efficient siRNA delivery for cancer treatment. The desirable safety along with high gene transfection efficiency provided a broad application potential of LCN nanotransformers, even as a cancer treatment [59]. Agrawal *et al.* successfully developed LCN for the treatment of diabetes mellitus. In this study, attempts were made to improve the stability and therapeutic efficacy of insulin following oral administration. In line with the results of higher Caco-2 cell uptake, LCNs demonstrated more than double the cumulative hypoglycemia compared with a subcutaneously administered standard insulin solution [60].

Swarnakar and coworkers investigated the implications of the lipase digestibility of LCNs on the oral bioavailability of coenzyme Q10, their in vivo antioxidant potentials, and the in vitro–in vivo relationship of coenzyme Q10-loaded LCNs prepared using monoolein and phytantriol. The authors demonstrated the potential of this formulation strategy to improve the oral bioavailability of coenzyme Q10, which a difficult-to-deliver drug [61].

Hong and a team of researchers focused on understanding the mechanism of formation of cubosomes using an enzymatic approach and extending this approach to lipid types other than phytantriol. The authors used an alternative nondigestible lipid to phytantriol, selachyl alcohol, which was found to be a route to enzymatic-induced production of LCNs. Incorporation of triglycerides with greater chain lengths yielded a more efficient means of disrupting phase structure. It was also determined that most of the fatty acids produced during digestion remained in the particles and did not need to diffuse into the aqueous bulk for structure reformation to occur [62].

Meli *et al.* developed a novel monoolein-based cubosome formulation engineered for possible theranostic applications in oncology. The researchers explored and demonstrated that the NP surface of cubosomes loaded with an anticancer drug, docetaxel, can be decorated with both a cancer cell-targeting ligand and an imaging probe. Docetaxel was successfully loaded within cubosomes, and the cubosome surface was simultaneously decorated with targeting and imaging moieties, with significant cytotoxic effects (more than one order of magnitude larger than the molecularly dispersed drug) of the docetaxel-loaded LCN against HeLa cells [63].

Josephine *et al*. studied the use of PEG as a steric stabilizer for cubosomes and to establish structure–property relationships, developing several successfully sterically stabilized cubic lyotropic LC nanostructured particles. Application of these amphiphilic PEGylated-lipid copolymers in other lipid-based self-assembly systems might be possible. The amphiphilic copolymer PEG-PHYT series synthesized in this study illustrates the potential of using customized steric stabilizers [28].

Guan *et al*. investigated alternatives to the low-throughput approaches for the preparation of polymeric NPs containing LC mesogens with tunable anisotropic morphologies. They developed an efficient route to prepare anisotropic morphologies of azobenzene-containing block copolymers (BCPs) with a high solid content via polymerization-induced hierarchical self-assembly in ethanol. Their work also significantly expanded the scope of accessible morphologies in PISA and suggested an impactful role for the underexplored LC BCPs in the polymerization-induced self-assembly field [64].

Zhai *et al*. investigated chemical reactive stabilizers for enhanced functionalization and specificity in therapeutic delivery applications. They successfully detailed DSPE-PEG5000-stabilized cubosomes that were less toxic than DSPE-PEG3400 or Pluronic F127-stabilized dispersions, attributed to the increased surface coverage of PEG chains on the particle surface, implicating the role of PEGylation in future in vivo investigations in these systems. They also showed that the PEG chain length has an important effect on the resulting nanostructure within the NPs, including the internal water channel size [65].

Kim and coworkers developed an industrially accessible method producing LC lipid NPs with various internal structures based on phytantriol, Pluronic F127, and vitamin E acetate. The authors were successful in economically producing LC lipid NPs in large quantities with well-defined internal structures with hexagonal lattices (mostly inverted cubic), lined/coiled patterns (inverted hexagonal), and disordered forms (inverse microemulsion) depending on the compositions [66]. Tran and colleagues reported the controlled manipulation of mesophase structures of monoolein and phytantriol NPs by adding unsaturated fatty acids (FAs). The researchers pointed out the substantial differences between the phase behavior of NPs with *trans*-FA, *cis*-FAs with one double bond, and *cis*-FAs with multiple double bonds. Their research has aided the selection and development of NP-based drug delivery systems with the desired mesophase [67].

Theranostic applications

The aim of theranostics is the clinical development of an agent or carrier for therapeutics and diagnostics to achieve improved prognoses of diseases such as cancer. LCNs have many unique features, such as ease of surface modification with functionalized targeting and imaging moieties. In addition, the viscosity of dispersion of cubosomes and hexosomes is identical to water, which is an essential characteristic for intravenous administration and the possibility of dual loading with drugs and imaging agents, with surface decoration of cancer-specific targeting moieties. For example, monoolein-based hexosomes were loaded with docetaxel, a poorly soluble anticancer drug stabilized by Pluronic conjugated with rhodamine and folate, by preserving the internal structure of the LC phase [68]. In a related study, the same authors developed a novel monoolein-based cubosome formulation loaded with docetaxel and explored its potential as a theranostic nanomedicine to treat cancer. The engineered NPs were stabilized with a mixture of Pluronic (PF108), folate-conjugated PF108, and rhodamine-conjugated PF108 to exert multifunctional effects, such as targeting, therapeutic, and imaging abilities. The cytotoxic effect was studied in a human adenocarcinoma (HeLa) cell line, and results showed that docetaxel-loaded cubosomes significantly induced cell toxicity. Furthermore, the authors also estimated the surface area and hydrophobic volume of cubosomes compared with multilamellar liposomes. Interestingly, the hydrophobic volume of the cubosomal nanostructure was three times larger than that of single-bilayer liposomes despite the fact that 60% of the cubosomal surface is exposed to water compared with liposomes and equal that of liposomes when the bilayer increases (i.e., multilamellar liposomes). These results suggest using cubic bicontinuous LCNs to deliver hydrophobic drugs for therapeutic/diagnostic purposes [63]. Another possible use of the LC phase in theranostic was demonstrated by Urandur *et al*. The authors developed MO-based inverse hexagonal LCNs stabilized by anisamide-grafted Pluronic F127 to deliver formononetin, an anticancer agent, and tetraphenylethene, an optical beacon with aggregation-induced emission signature properties. The authors demonstrated that this LCN-based theranostic carrier significantly inhibited tumor growth in a 4T1-induced breast cancer xenograft model and provides a platform for noninvasive detecting and imaging of cancer [69]. Meli *et al*. engineered a novel monoolein-based cubosome formulation for possible theranostic applications in oncology. The researchers demonstrated that cubosomes loaded with docetaxel could be formulated by decorating their NP surface with both a cancer cell-targeting ligand and an imaging probe. Docetaxel was successfully loaded within cubosomes, the surface of which was simultaneously decorated with targeting and imaging moieties; the authors reported significant cytotoxic effects (more than one order of magnitude higher than the molecularly dispersed drug) of the docetaxel-loaded LCN against HeLa cells [63].

The common approach for active targeting is the surface modification of NPs with antibodies or ligands for a specific cell surface receptor. However, there are many potential drawbacks associated with surface modification of NPs, such as poor stability, lack of specificity if the target receptor is shared with normal cells, and a high cost [70]. A recent approach to overcome these limitations was to target the chemical reaction so that it does not interfere with normal biochemical processes at the cellular level. To achieve this goal, Alcaraz *et al*. developed cubosomes using a copper-free click chemistry method to provide an antibody-free targeting option for these emerging drug delivery and imaging systems [71].

Concluding remarks and outlook

LCNs have been widely investigated at the preclinical level and are gaining increasing attention in clinical trials because of their attractive properties suitable for efficient drug delivery. However, several important challenges and hurdles need to be addressed before the successful translation of LCNs into the clinic.

Key advances include tailoring of pore sizes in the lipid cubic phase, the library of stabilizers available to target different cancer cells of interest, structural studies to understand access to the internal lipid membrane, and the design of systems for controlled release.

Key outstanding challenges remain that would further enhance the applications of LCNs, including a deeper understanding of the stabilizer–membrane interaction, demonstration of pore size tuning, analogous to the bulk phase work, further cytotoxicity studies, including mechanism of interaction with cancer cells and intracellular trafficking, and demonstration of therapeutics release.

An interesting future development would also be to exploit LCNs comprising cell-derived lipids (CDLs) [72,73]. CDL-coated NPs have demonstrated promising results in preclinical studies because of their enhanced biocompatibility and robust targeting of the target tissues [74].

Here, we have briefly reviewed LCNs, their structure, constituents, characterization, preparation techniques, and application advantages. LCNs as a drug delivery system also have some limitations, such as the high amount of water in the system, which makes it difficult for to load hydrophilic drugs in the bilayer mesophase. In addition, their small size and resulting short diffusion pathways make it difficult to control the rate of hydrophilic drug release. In addition, methods to characterize LCNs are expensive and difficult, and require complex procedures. The dynamic nature of the crystals makes their characterization particularly difficult. Furthermore, the dynamic nature itself poses problems for LCN production. A slight change in a parameter such as temperature or pressure during the process can result in the formation of a product with a different crystal structure. Therefore, appropriate optimization methods for the process are required. These process optimizations are costly, especially when considering industrial scale-up. Additionally, reverse bicontinuous cubic phases, as well as hexagonal phases, are highly viscous and this mechanical stiffness renders them unfit to be used as parenteral dosage forms. Exposure to higher volumes of fluid also causes phase transitions, which leads to undesired drug release rates.

In the attempt to translate formulations into clinical applications, LCNs need to have long-term stability and sterility. To extend the shelf life of LCNs, freeze drying methods can be used. However, these can cause changes to internal structure of LCNs, especially upon reconstitution. Hence, further characterization and formulation developments need to be carried out to generate a suitable LCN formulation that can expand its shelf life for clinical use without disrupting its structure and stability.

Despite these disadvantages, LCNs are still in use because of their multifunctionality, their ability to protect drug stability for longer durations compared with other dosage forms, their ability to incorporate all types of drug, their easier handling compared with other lipid-based dosage forms, reduced toxicity because of their component parts, and their enhanced permeation ability. The theranostics applications of LCNs have rendered them a favored dosage form among researchers, particularly for improving current drug delivery systems. Our improved understanding of the lyotropic LC phase has resulted in the development of low-energy, but efficient preparation techniques, with the aim of improving formulation procedures. There have been few examples of the use of LCNs as therapeutics delivery systems for the treatment of brain diseases. Cubosomes have shown great potential as permeation enhancers and are able to cross the blood–brain barrier when administered nasally. In addition to the potential effect of LCNs as drug delivery systems, further studies should also focus on other important issues such as their biological safety and metabolism, new structure-forming materials and larger-scale production methods. Such developments could improve LCNs and their nanodispersed forms as drug delivery systems.

Fully understanding the factors that affect the structure of lyotropic liquid crystals is conducive to controlling the preparation conditions and obtaining a stable LC phase. Furthermore, we can use these factors to design intelligent LCN carriers. Additionally, studying the relationship between drug release and structures can provide a reference for encapsulating diverse cancer therapeutics and imaging agents. However, many challenges need to be overcome for the clinical application of LCNs, such as the biocompatibility and safety of lipid material. For example, MO causes hemolysis, especially when administered intravenously, although PEGylation can be used to improve the hemocompatibility of MO-based systems. Nevertheless, LCNs remain suitable candidates for drug delivery, and further research is expected to reveal additional applications for them as drug carriers.

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