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Lipid/copolymer hybrid drug delivery systems: From design to performance

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ABSTRACT

In recent years, nanomedicine has become very popular in the pharmaceutical field as it raises opportunities for precise and more effective pharmacotherapy. Among others, hybrid lipid/polymer systems have gained a lot of interest as drug delivery nanoplatforms having several assets: combine the advantages of both lipid- and polymer- based nanoparticles, minimize the drawbacks, and exhibit new inherent properties. This review summarizes the most recent studies on hybrid nanostructures comprised of phospholipids and copolymers for drug and gene delivery. The general aspects of lipids and polymers alongside the formulation protocols, the characterization techniques, and the applications of hybrid systems are reported as well. From a critical point of view, the complexity of hybrids and their added value are considered, while the critical quality attributes as well as the still existing limitations are also highlighted. Embracing the quality by design-oriented formulation strategy, this work could, hopefully, enlighten the design and development of safe and efficient lipid/copolymer nanoparticles from a technological perspective having as the ultimate goal their clinical application.

Abbreviations: AFM, Atomic Force Microscopy; API, Active Pharmaceutical Ingredient; BMA, butyl methacrylate; Chol, cholesterol; CLSM, Confocal laser scanning microscopy; cryo-TEM, cryogenic transmission electron microscopy; CS, chitosan; DC-chol, 3β-[N-(N′, N′-dimethylaminoethane)-carbamoyl] cholesterol hydrochloride; DDAB, Dimethyldioctadecylammonium bromide; DEAEMA, Diethyl(aminoethyl) methacrylate; DIPAEMA, 2-(diisopropylamino) ethyl methacrylate; DLPC, Dilauroyl phosphatidylcholine; DLS, dynamic light scattering; DMAEMA, 2-(dimethylamino) ethyl methacrylate; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; DOPC, 1,2-Dioleoyl-sn-glycero-3-phosphocholine; DOPE, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; DOTAP, 1,2-Dioleoyl-3-trimethylammonium propane; DPPC, dipalmitoylphosphatidylcholine; DSC, differential scanning calorimetry; DSPC, 1,2-distearoyl-sn-glycero-3-phosphocholine; DSPE-mPEG, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methyl ether (polyethylene glycol)]; DSPE-PEG2K-MAL, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide (polyethylene glycol)-2000]; ELS, Electrophoretic light scattering; FESEM, Field Emission Scanning Electron Microscopy; FTIR, Fourier Transform Infrared Spectroscopy; GPC, Gel permeation chromatography; HPC, hydrogenated phosphatidylcholine; HPLC, High Performance Liquid Chromatography; HR-US, High Resolution Ultrasound Spectroscopy; HRTEM, High Resolution Transmission Electron Microscopy; HSPC, hydrogenated soybean phosphatidylcholine; ICP-MS, Inductively coupled plasma mass spectrometry; ITC, isothermal titration calorimetry; LC-MS, Liquid chromatography-mass spectrometry; LMA, lauryl methacrylate; LPHNs, Lipid/copolymer hybrid nanoparticles; LT, lauroxy tetraethylene glycol methacrylate; MAA, methacrylic acid; ManEMA, mannose conjugated ethyl methacrylate; MD, methoxy diethylene glycol methacrylate; mPEG, methoxypoly(ethylene glycol); MTS, 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetra-zolium; MTT, 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide; NMR, Nuclear Magnetic Resonance; OEGMA, oligo (ethylene glycol) methyl ether methacrylate; PBAE, disulfide-poly(β-amino esters); PBD, poly(butadiene); PC, phosphatidyl choline; PCL, poly(ε-caprolactone); PCMA, poly (cholesteryl methacrylate); PDL, poly(δ-decalactone); PDMS, poly(dimethylsiloxane); PEG, poly(ethylene glycol); PEG-P(8C-Chol), poly(ethylene glycol)-b-poly (cholesteryl-2-oxo-1,3,6-dioxazocane-6-carboxylate); PEO, poly(ethylene oxide); PLA, poly(D, L-lactide); PLGA, poly D,L-lactic-*co*-glycolic acid; PMMI, poly(monomethylitaconate); PMOXA, poly(2-methyl oxazoline)POEPC: 1-palmitoyl-2-oleoyl-sn-glycero-3- ethylphosphocholine; POPC, palmitoyl-oleoyl-sn-glycero-3-phosphocholine; POPS, palmitoyl-oleoyl phosphatidylserine; PPO, poly(propylene oxide); PTX, paclitaxel; PVA, polyvinyl alcohol; QCM-D, Quartz crystal microbalance with dissipation; QELS, Quasi-elastic light scattering; RFP, red fluorescent protein; SANS, Small angle neutron scattering; SAXS, Small angle X-ray scattering; SDS-PAGE, Sodium dodecyl-sulfate polyacrylamide gel electrophoresis; SEM, Scanning electron microscopy; siRNA, small interfering RNA; SLS, Static Light Scattering; TEM, Transmission Electron Microscopy; TGA, ThermoGravimetric Analysis; TK, thioketal; TPGS, D-α-tocopherol polyethylene glycol succinate; UPLC, Ultra-Performance Liquid Chromatography; XRD, X-ray Powder Diffraction.

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1. Introduction

Lipids and polymers are characterized as lifelong companions due to their principal roles and functionalities in the field of pharmaceutical technology. They are both used as excipients in several dosage forms, i.e., emulsions, suppositories, capsules, etc. Additionally, due to their physical properties, they can self-assemble into a variety of colloidal systems and nanosystems [1]. Namely, amphiphilic block copolymers exhibit different structures, which are strongly dependent on the architecture, the chemical properties, and the ratio of their blocks. Several lipid- and polymer-based nanosystems are in clinical trials for cancer treatment, including the most frequent types of cancer, i.e., prostate, breast, lung, and pancreatic tumors [2].

Only ten years ago, lipid-polymer hybrid nanoparticles were considered a new generation of drug delivery platforms. The main reason for their development was the combination of the physical stability of polymer nanoparticles with the biocompatibility of liposomes. On the other hand, different block polymers such as polylactic acid-*co*-glycolic acid (PLGA), poly(ε-caprolactone) (PCL), polylactic acid (PLA) and polyglycolic acid (PGA) are also characterized as safe for their use in pharmaceutical development due to their biocompatibility and biodegradability. The stealthiness and stability in human plasma, as well as in different biorelevant media, were the driving forces for the development of polymer modified liposomal carriers. Several technological properties, i.e., loading and encapsulation efficiencies, as well as the achievement of a sustained or stimulus-responsive drug release profile, are also very important features leading to the combination of lipid- and polymer-based materials as building blocks of a nanosystem. The chemical versatility of polymeric materials and their functionalization with stimulus responsive groups responding to the conditions of the human body make them ideal for increasing the therapeutic efficacy of lipid-based nanocarriers [3,4].

From a biophysical point of view, "polymer-lipid hybrid membrane" is a new functional material with unique properties that are strongly dependent on the outcome of the hybridization process, which can lead to homogenous membranes or phase-separated membranes. The last ones exhibited polymer- and lipid- rich domains. In most cases, the distribution of the polymer into the lipid bilayer governs the release profile of the hydrophobic drugs because the hybridization process alters the mechanical properties, the lateral diffusivity, and the permeability of the polymer-lipid hybrid membrane in comparison to pure liposomal or polymeric membranes [5].

The main category of these systems was lipid-polymer hybrid nanoparticles, which were core-shell nanoparticle structures made of polymer cores and lipid or lipid-PEG shells. Bangera et al., 2023, in their recent paper, categorized the polymer-lipid hybrid nanoparticles into different generations, including the older class of polymer-modified liposomes (which are already marketed as nanomedicines), solid lipid nanoparticles with polymeric core and polymer hybrid lipid nanoemulsions, and the more recent classes of cell membrane-biofunctionalized nanoparticles and cell-polymeric nanoparticle hybrid nanocarriers. Each class of lipid-polymer hybrid nanoparticles exhibits unique characteristics that make them ideal for drug delivery and targeting specific tissues and subcellular organelles. All the routes of administration have been described in the literature [6].

The aim of this review is to present the properties and applications of different lipid/copolymer hybrid drug delivery systems. The formulation protocols and techniques for the physicochemical characterization of these drug delivery platforms will be presented. An extensive literature review with special attention to the connection between the properties of the building blocks and the applications of the systems will be analyzed, giving emphasis to the advantages and limitations of each system. The critical quality attributes (CQAs) and the controllable formulation parameters are also discussed, in connection with the biological properties of these systems which may lead to fast clinical translation of these systems. To the best of the authors' knowledge, this is the first report in the literature where all the technological aspects, from the selection of the raw materials and formulation protocols to the characterization and applications of lipid/copolymer hybrid drug delivery systems, are presented.

2. Methodology for literature search

An extensive search and literature review were performed in the electronic databases PubMed, Scopus, and Google Scholar for English-language publications. The publications from the past ten years (2014–2024) served as a comprehensive guide for the systematic investigation of lipid/copolymer hybrid drug delivery systems. The authors performed a literature search excluding the studies with full text unavailable, publication language other than English, and conference abstracts. After the screening of titles and abstracts, lipid/copolymer hybrid drug delivery systems full-text studies were included in the examples from the recent literature on lipid/copolymer hybrid drug delivery systems.

3. General aspects for lipids

Lipids are amphiphilic molecules and thus when they are dispersed into aqueous environments, they have the ability to self-assemble, resulting into different mesophases as a function of both concentration and temperature, due to their lyotropism. The surfactant/amphiphilic character of the lipids is owned to their chemical structure that consists of a polar head group and a hydrophobic hydrocarbon tail. Upon their contact with water, the lipidic molecules position spontaneously themselves in such a way, in order to minimize the free energy of the system, by exposing their hydrophilic regions to the aqueous environment and simultaneously hiding their hydrophobic domains, forming bilayers, in order to minimize the interface with the existing solvent [7,8]. Due to the above ability, the resultant lipidic nanostructures are able to accommodate both hydrophobic and hydrophilic active agents, as well as amphiphilic ones, exhibiting great flexibility.

3.1. Self-assembly ability to form nanostructures

Depending on the lipid distinctive chemical characteristics, expressed by the critical packing parameter (CPP) ($CPP = v/(A^{*}l)$), different types of the self-assembled nanostructures/mesophases are formed. The CPP is a geometrical value consisting of the ratio between the volume of the hydrophobic lipid tail (v), the product of the cross-sectional lipid head area (A) and the lipid chain length (l). Depending on the changes of the CPP, the order – order transitions that are governed by changes in the curvature of the water – lipid interface can be predicted. Upon the lipid dispersion to the aqueous environment, the following types of liquid crystalline mesophases are formed, depending on lipid structural characteristics. Cylinder shaped lipidic molecules (CPP $\sim$ 1) tend to form planar membranes (fluid lamellar [$L\alpha$] phase), and conversely, cone-shaped (CPP <1) and wedge-shaped lipidic molecules (CPP >1) prefer the formation of convex (type 1/normal type, oil-in-water (O/W) phases) and concave (type 2 /inverted type, water-in-oil (W/O) phases) interfaces, respectively. Between type 1 and type 2, the nanostructures of inverted-type/type 2 liquid crystalline phases and micellar solutions are more stable and therefore are more suitable for drug delivery applications [7,9–11].

From a chemical point of view, lipids with double hydrophobic tails, like phospholipids tend to form lamellar structures, while monoglycerides with a single hydrophobic tail tend to form inverted-type/type 2 liquid crystalline structures. The most well-defined, thermodynamically stable lipidic mesophases are the following, lamellar one (L_α or L_C depending on whether the alkyl tail is amorphous or has been crystallized), transforming eventually to vesicular structures like liposomes, as well as the inverted liquid crystalline phases, such as hexagonal (H_{II}) and bicontinuous (Q_{II}) cubic phases, forming eventually

hexosomes and cubosomes, respectively [7,8,12,13]. All the aforementioned lipidic nanostructures can be utilized for drug delivery applications, while each category presents different advantages and disadvantages that could be either enhanced or decreased respectively, by their evolution to hybrid lipid-polymer nanostructures, as it is described in the following paragraphs.

3.2. Chemical classification & applications

Starting from the phospholipids, either natural or synthetic, they comprise a hydrophilic "head", carrying a phosphate group, and two hydrophobic "tails" derived from fatty acids and are conjugated together by an alcohol residue. Depending on the alcohol, they are further categorized as glycerophospholipids and phosphosphingolipids. The distinctive chemical structure of glycerophospholipids, which are extensively utilized for drug delivery nanocarriers, is further differentiated by the head group, the length and the saturation grade of hydrophobic chains, the bonding mode between the aliphatic moieties and the glycerol backbone. Some of the most typical head groups of glycerophospholipids are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidic acid (PA), phosphatidylinositol (PI), phosphatidylglycerol (PG) and cardiolipin (CL), while some of the most typical hydrocarbon tails are derived from lauric acid (12:0), myristic acid (14,0), palmitic acid (16,0) and stearic (18,0) acid. Regarding the unsaturated ones, a typical example utilized in drug delivery applications is oleic acid (18,1) (Fig. 1) [14–18]. In contrast to phospholipids, the monoglycerides that consist of a molecule of glycerol joined to a fatty acid via an ester bond, carry a single hydrophobic tail. The most representative monoglyceride, being used for lipidic nanoparticle formulations, is Glycerol Monooleate (GMO), due to its property to form non-lamellar mesophases, like cubic and hexagonal ones [19].

All these different head-tail possible combinations produce a large library of candidate lipids, with different characteristics and functionalities that are transmitted to the respective nanostructures. The chemical structure plays a key role in the lipid properties, like the main transition temperature (T_m) of the lipid state from the gel phase to the liquid crystalline one. Therefore, the chemical structure affects the respective behavior of a lipidic nanostructure, like the physicochemical characteristics, thermodynamic behavior, morphology, their charge or the drug loading capacity and release kinetics. For example, the unsaturation in the lipid tail, owed to the presence of C—C double bonds, provides more fluidic character to the lipid and facilitates fusion with the endosomal membrane. Shorter or unsaturated lipid chains tend to have lower T_m than longer and saturated chains, thus producing less well packed, less compact membranes with defects. At physiological temperature, the low T_m of shorter (e.g., 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) or unsaturated (e.g., 1,2-dioleoyl-sn-glycero-

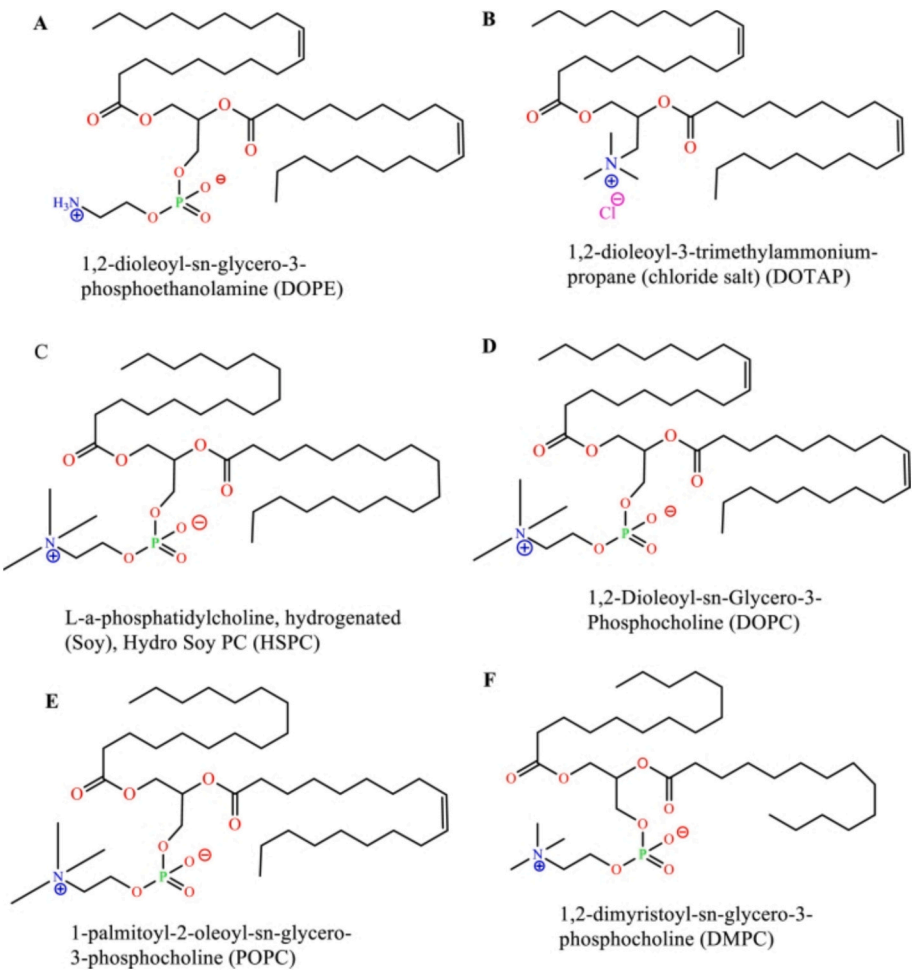

Fig. 1. Examples of different types of lipids, including A) 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), representing phosphoethanolamines B) 1,2-dioleoyl-3-trimethylammonium-propane (chloride salt) (DOTAP), representing cationic lipids, C) L-a-phosphatidylcholine (HSPC), D) 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine (DOPC), E) 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), and F) 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC). C) and F) represent examples of saturated phosphatidylcholines, while D) and E) represent examples of unsaturated phosphatidylcholines of different carbon atoms in alkyl chain. Adapted from [15].

3-phosphocholine (DOPC)) lipid chains impart a more fluidic character with loose, less condense packing than of a lipid with longer carbon tail, like the 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) one [20]. The packing mode further plays a key role in the cargo release kinetic profile. By selecting the proper lipid/lipid combination, an optimization of the formulation can be taken place, and as a result, the release kinetics can be controlled, for example either to sustained or to burst release, depending on the therapeutic requirements.

For example, DSPC which is a neutral saturated phosphatidylcholine, exhibits phase transition temperature of approximately 54 °C and a cylindrical conformation that allows lamellar phase formation, providing stabilization of lipid nanoparticles and avoiding premature content release at lower temperatures [21–23]. Thus, DSPC is being already used in market approved nanomedicines. On the other hand, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), which is a phosphoethanolamine with two unsaturated tails, exhibits a phase transition temperature of about 30 °C and a conical geometry, promoting the formation of an inverted hexagonal H(II) phase, therefore being an ideal lipid to be used to destabilize endosomal membranes and facilitate endosomal escape, allowing subcellular targeting, localised pharmacological action and thus increased drug bioavailability [21,24,25]. The phase transition from lamellar to hexagonal one is controlled by the intrinsic curvature of the lipids. The increase of tail unsaturation from 0 to 2 *cis* double bonds leads to a transition from a bilayer to a non-bilayer structure, causing membrane disruption and drug release [26]. The intrinsic curvature is explained by the shape of lipids, for example, the more cylindrical-shaped lipids like phosphatidylcholine (PC) tend to form lamellar structures, while conical helper lipids, such as phosphatidylamine (PE), verge to form hexagonal structures [27].

The careful selection of the lipids is strictly correlated with not only nanoparticle properties, such as particle stability, but also with their interaction mode with biological systems like delivery efficacy, tolerability, targeting, absorption, distribution, metabolism, and excretion–toxicity (ADMET) profile and thus the safety and efficacy of the final product. Therefore, lipid selection is a key parameter during any formulation process of such nanoparticles, either pure lipidic or hybrid ones with polymers. For example, formulations of lipid nanoparticles may contain many different lipid components, including phospholipids (sometimes both of phosphatidylcholine and phosphatidylethanolamine type), ionizable lipids, cationic lipids, cholesterol and polyethylene glycol (PEG)-functionalized lipids (PEG-lipids), with each category covering a special scope.

PEG-lipids represent a special category of lipids with various applications in already market approved nanotechnological products, due to their protecting role. They are the basic component of nanoparticles also known as stealth ones, providing stability under physiological conditions by decreasing particle aggregation, due to steric phenomena. They result in prolonged circulation time, due to prevention of plasma proteins binding, like of albumin and avoidance of premature excretion owned to the kidneys and the mononuclear phagocyte system (MPS). Moreover, PEG-lipids are useful as conjugates to specific ligands for targeting purposes, for example the accumulation to specific tissues, such as tumors, due to exploitation of the enhanced permeation and retention effect (EPR) [28]. The extent and mode of these effects depend on the proportions and properties of the PEG-lipids. The specific characteristics of the PEG-lipid, like the structure and length/molecular mass of the PEG chain and the length of lipid tail are crucial parameters that differentiate the physicochemical characteristics of the resultant nanosystems, controlling also the nanoparticle excretion mode and immune response [29–34]. In a recent study, it was found that lipid nanocarriers with 1.5% PEGylated lipids exhibited approximately 80% transfection efficiency and the highest hepatocyte uptake, being compared with other candidates with PEGylated lipids ranging from 1 to 3% [35]. The PEG-lipids significantly outcome on nanoparticle formulation and size [36]. As found in a recent study, particle size decreases upon PEG lipid content increase [30].

Cholesterol is often used as helper/modulator in combination with lipids, in order to modulate the characteristics of the lipid bilayer, like membrane integrity, fluidity and rigidity, being able to act as a particle stability promoter. Cholesterol derivatives with various structures and molecular geometries are also used to adjust the functionality of the lipid nanoparticles [37,38]. Cholesterol acting as a filler to the spaces within the bilayer, can be a stabilizing factor, preventing leakage of the content, as well as preventing opsonization [34,39].

Cationic lipids are synthetic lipids that possess a head group with permanent positive charge. They are comprised of a hydrophobic part with two alkyl chains or a cholesterol moiety, a positively charged polar head group, and a linker connecting the polar group with the hydrophobic moiety. Due to their positive charge, cationic lipids are excellent for complexation with nucleic acids and thus for such delivery applications. Some representative cationic lipids are 1,2-di-*O*-octadecenyl-3-trimethylammonium-propane (DOTMA) and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) (Fig. 2), which are quaternary ammonium lipids and are formulated either alone or combined with other materials for mRNA delivery for vaccine applications, including infections, but also cancer [40,41]. However, there are some critical issues that should be taken into account during the utilization of cationic lipids, like toxicity issues. For example, DOTAP was found to increase liver enzyme release, suggesting possible hepatoxicity and toll-like receptor-mediated inflammatory response in some cases [42,43]. Dimethyldioctadecylammonium bromide (DDAB), another cationic quaternary ammonium lipid, is suitable to form complexes with nucleic acids, with a parallel role as vaccine adjuvant, due to its ability to stimulate innate immune responses [44–47].

Ionizable lipids is a special category of cationic lipids, with a pH-sensitive character, with a non-permanent, tunable positive charge, being protonated only at low pH (in pH conditions below the acid-dissociation constant (pK_a) of the lipid) and remaining neutral at physiological pH, allowing less interactions with anionic membranes of blood cells and thus showing decreasing toxicity. When they are trapped in endosomes, where acidic pH values prevail compared to cytoplasm, the ionizable lipids are protonated and due to the proton sponge effect, they promote membrane destabilization and facilitate endosomal escape. 1,2-dioleyloxy-3-dimethylaminopropane (DODMA) is considered as a typical example [40,48–52]. Moreover, the lipid tail of the ionizable lipids exhibits also a great effect on the carrier stability and can regulate the fusion mode of the lipid carrier with the endosomal membrane, the release place, for example to be in the cytoplasm and the packaging mode of the bilayer. The head group also influences the nanoparticle internalization. For example, lipid nanoparticles with a piperazine headgroup accumulated more in the spleen and liver, compared to lipids with a tertiary amine headgroup, which accumulated more in the liver [20].

Other categories of lipids, usually classified as solid lipids, are used in cases of solid and nanostructured lipid nanoparticles along with some stabilizing agents, usually surfactants, like Tween and Span and other coating materials. In these nanosystem categories, it is required that the matrix maintains its solid state at room temperature, and therefore polymorphism, crystallinity, miscibility and physicochemical properties of the lipid should be taken into account. Such lipid categories include fatty acids (like stearic acid and palmitic acid), fatty alcohols, glycerides, triglycerides (like trimyristin, tripalmitin), steroids, paraffin and waxes (like cetyl palmitate, beeswax) etc. [53,54].

3.3. Lipid-based nanocarriers categorization

There are different categories of lipid nanocarriers depending on the lipids used, the presence of components other than lipids like surfactants, the external and internal morphology, the internal conformation, the particle size, charge and the number of lamellas/bilayers. Some of the most representative ones are liposomes, solid lipid nanoparticles, nanostructured lipid nanoparticles, cubosomes, hexosomes, cationic

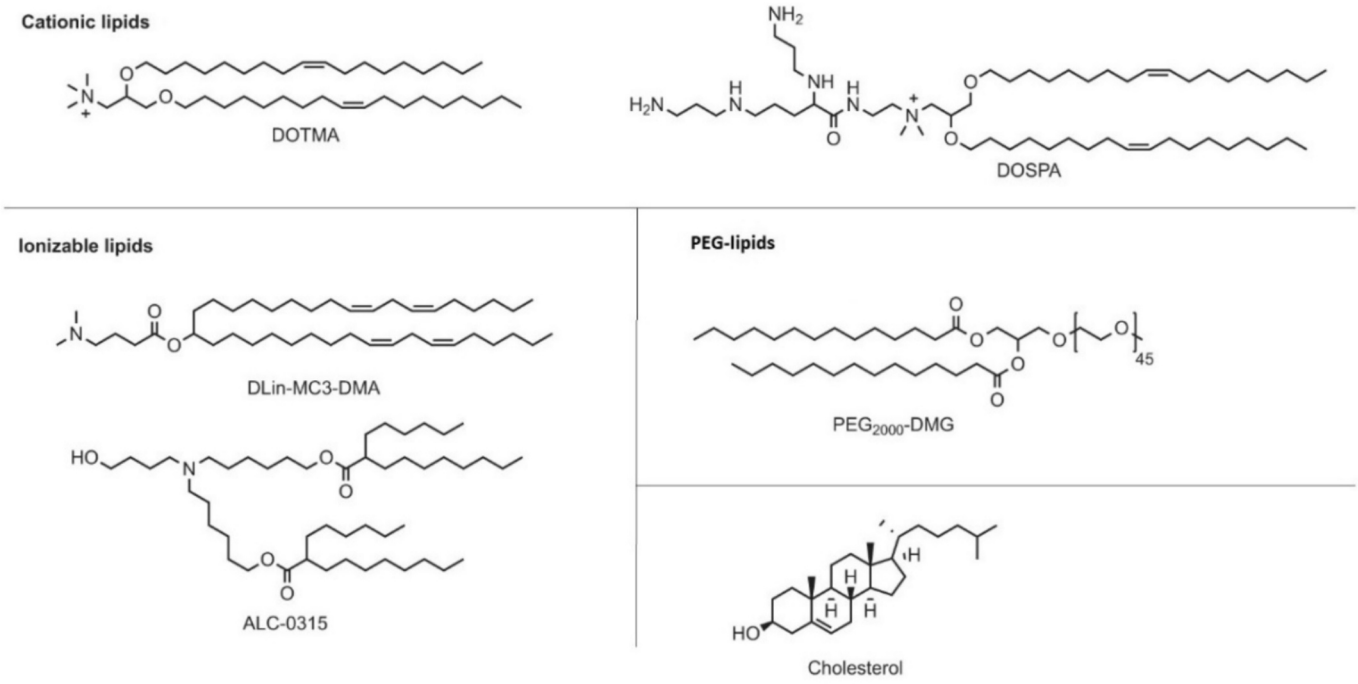

Fig. 2. Typical examples of lipid categories chemical structures, including cationic lipids, ionizable lipids, pegylated lipids, and cholesterol. Adapted from [40].

lipid−nucleic acid complexes, transferosomes, ethosomes, niosomes and nanoemulsions [53,55].

Lipid nanocarriers are versatile nanostructures with tunable characteristics, as well as biocompatible and biodegradable nanosystems, since lipids are also endogenous components of cell membranes. They exhibit the typical advantages of nanocarriers, like enhancing drug bioavailability, solubilization, penetration and reducing toxicity. However, there are still great challenges and opportunities to the roadmap of market approval of such products, as well as problems to be solved, like stability issues, more specific targeting, lowering immunogenic side effects, etc. In this direction, there is still an emerging need for evolution, fine tuning and more precise control of their characteristics and behavior. Thus, their upgrade to more advanced hybrid polymer-lipid ones could be the solution to the aforementioned restrictions.

4. General aspects for copolymers

Controlled radical polymerization (CRP) or controlled/living radical polymerization, has transformed the field of polymer science and industry by serving as a valuable method for crafting a diverse array of polymers. This technique empowers the synthesis of polymers with precise regulation over molecular weight, narrow dispersity, and the ability to create various architectures such as block, random, graft copolymers, star or hyperbranched polymers, and other functionally specialized polymers [3,56–58]. The mechanistic transformation reaction opens significant possibilities for adjusting the chemical and physical attributes of copolymers. In an ideal living polymerization process, all chains commence at the initiation stage and experience uniform growth rates, thereby preserving the fidelity of their chain ends. Well-defined polymers with different macromolecular architecture such as block, graft, star or hyperbranched copolymers can be synthesized by living/controlled polymerization methods. CRP, commonly referred to as reversible-deactivation radical polymerization (RDRP), encompasses various fundamental techniques, including nitroxide-mediated polymerization (NMP), atom-transfer radical polymerization (ATRP), reversible addition–fragmentation chain transfer (RAFT) polymerization. The copolymers synthesized by the above polymerization methodologies are capable of self-assembling and forming various polymer formations in aqueous solutions with small sizes, narrow size distribution and controllable structural characteristics. These polymeric characteristics make them potential candidates for drug or gene delivery carriers.

Block copolymers (BCPs) are a unique type of copolymers characterized by distinct monomer units organized into separate blocks along the polymer chain. As shown in Fig. 3, BCPs can adopt various architectures, including linear and branched such as graft and star macromolecular structures [3]. Advances in polymer synthesis, such as controlled polymerization methods and efficient post-polymerization functionalization, have enabled the precise control of molecular weights and macromolecular architectures for BCPs. This remarkable structural and compositional flexibility has driven significant innovation, leading to synthetic approaches capable of achieving unprecedented levels of complexity in man-made polymer architectures. Significant advancements have been made in the past decade in developing nanoscale systems through the self-assembly of BCPs, particularly for applications in drug delivery. Amphiphilic block copolymer micelles are highly appealing for drug delivery applications due to several key advantages. Firstly, they can encapsulate hydrophobic drugs within their core, enabling transport at concentrations far exceeding the drugs' inherent water solubility. Secondly, hydrophilic blocks, often made of poly(ethylene oxide) (PEO), create a stabilizing shell around the micellar core by forming hydrogen bonds with the surrounding aqueous environment. Additionally, the PEO corona minimizes protein adsorption and cellular adhesion, safeguarding the hydrophobic drug from hydrolysis and enzymatic degradation [59–61].

In recent years, hyperbranched polymers have garnered significant attention from polymer scientists due to their exceptional structural properties. These macromolecules, which possess a high degree of polymerization, feature tree-like topologies similar to dendrimers. They are distinguished by an extensive network of internal and external functional groups, making them versatile for various applications. The hyperbranched structure endows polymers with unique characteristics, including low viscosity and compact conformations in both solutions and melts, compared to their linear counterparts. Also, hyperbranched polymers possess an incomplete or irregular branching pattern along with a high density of reactive or functional groups on both their surface

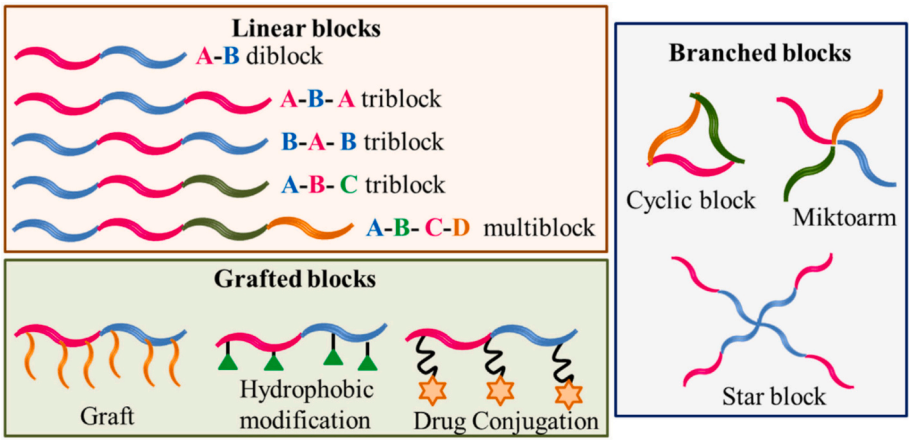

Fig. 3. Schematic designer representations of some block copolymer macromolecular structures. Adapted from [3].

and interior. These distinctive features make hyperbranched polymers highly promising for various biomedical applications, including drug and gene delivery as well as bioimaging [62,63].

Along similar lines *star*-shaped polymers have attracted significant attention from polymer scientists due to their exceptional physicochemical properties, which arise from their unique macromolecular architecture. Compared to conventional linear polymers of similar molecular weight, star polymers exhibit lower solution viscosity due to reduced arm entanglements, as well as enhanced responsiveness to stimuli and stronger interactions, owing to the higher density of functional groups. Additionally, the potential for multifunctionality, stemming from both their internal and external functional groups, has further fueled interest in star polymers. Consequently, star polymers have been explored in a wide range of biomedical applications, including diagnosis, drug and gene delivery, tissue engineering, and antibacterial biomaterials [64–66].

Another polymerization technique is the ring-opening polymerization (ROP) which is one of the three main pathways leading to the creation of polymers, alongside chain (radical and ionic) polymerization and condensation polymerization [67]. Di Leone and co-workers describe how lipid, polymer and hybrid membranes, formed from mixtures of lipids and copolymers on a solid substrate, offer an improved environment for promoting the selective and functional binding of a model redox protein, cytochrome *c* (cyt c). They synthesized an amphiphilic diblock poly(dimethyl siloxane)$_{90}$-*block*-poly(2-methyl-2-oxazoline)$_{10}$ copolymer with a carboxyl functional group on the hydrophilic domain's terminus (PDMS$_{90}$-b-PMOXA$_{10}$-COOH). More specifically, monocarbinol-functionalized PDMS-OH was prepared through anionic ring-opening polymerization of hexamethylcyclotrisiloxane, followed by end-group modification using 2-allyloxyethanol. PDMS-OH was then activated with trifluoromethanesulfonic anhydride and extended through cationic ring-opening polymerization with 2-methyl-2-oxazoline monomer. The polymer was subsequently quenched with triethylamine/water to yield hydroxy-functionalized PDMS-b-PMOXA-OH, and end-group modification with succinic anhydride produced the final carboxyl-functionalized PDMS-b-PMOXA-COOH. In the hybrid membrane, the lipid component preferentially enhanced the insertion of cyt c into lipid domains, while the copolymer facilitated conjugation with cyt c in the polymer domains via carboxylic functionalization. In summary, their findings encourage the continued advancement of complex and versatile hybrid bio-interfaces by highlighting the molecular factors being important for combining biomolecules with planar synthetic membranes, particularly when these membranes are hybrid and include both lipid and polymer domains [68]. Another interesting study was conducted by Son et al. where they synthesized a series of homopolymers, poly(cyclohexyloxy ethyl glycidyl ether)s (PCHGEs), and *block* copolymers, poly(ethylene glycol)-*block*-poly(cyclohexyloxy ethyl glycidyl ether)s (mPEG-b-PCHGE) by anionic ring-opening polymerization. These synthetic block copolymers formed polymeric micelles in aqueous solutions with a spherical shape accompanied by small sizes, and narrow distribution of diameters. Paclitaxel (PTX) was utilized as a hydrophobic model drug for encapsulation in the hydrophobic component of the micelles. The polymeric micelles showed excellent stability under physiological conditions and good drug release profile. Furthermore, in vivo experiments were performed and showed that these micelles could potentially be used as drug delivery carriers [69].

Yang's team developed an interesting polymeric system with a *star*-like macromolecular architecture consisting of an amphiphilic polycarbonate/PEG copolymer. This polymeric system had the ability to transform the formed micelles into vesicles. The synthesis of the *star*-shaped amphiphilic block copolymer involved initiating the ring-opening polymerization of trimethylene carbonate (TMC) from methyl cholate, employing a combination of metal-free organocatalytic living ring-opening polymerization and subsequent chain-end derivatization strategies. Afterwards, this *star*-shaped polymeric system self-assembled in aqueous solution into vesicles with dimensions in the nanoscale and were able to encapsulate the anticancer drug doxorubicin (DOX). DOX-loaded vesicles showed a better drug loading capacity than micelles prepared from a diblock copolymer analogue. Additional studies were performed to evaluate the in vivo antitumor activity. Upon injection into 4 T1 tumor-bearing mice, the DOX-loaded vesicles demonstrated a remarkable accumulation in tumor tissue and superior anti-tumor efficacy compared to free DOX, all while avoiding significant body weight loss or cardiotoxicity [70].

Nitroxide-mediated radical polymerization (NMP) stands out as a potent technique for sophisticated polymer synthesis. Nevertheless, NMP has certain limitations, especially in its ability to mediate the polymerization of non-vinyl monomers or facilitate the polymerization of vinyl monomers at low temperatures [71]. An interesting study was conducted by Qiao et al. who synthesized a novel series of *comb*-like terpolymers with a polymethacrylate backbone, featuring short pendant poly(ethylene)oxide (PEO) side chains (Mn = 300 g mol^{-1}, 5 EO units), carboxylic acid groups, and a small number of styrene (S) units [P(PEOMA300-co-MAA-co-S)-SG1], utilizing nitroxide-mediated polymerization. These terpolymers were employed as initiators for the emulsion polymerization of n-butyl methacrylate (BMA) and styrene at 85 °C. The cloud points of the terpolymers were adjustable across a broad temperature range, from 20 to 90 °C, by manipulating either the solution pH or the composition of the copolymer. These alterations in the solution properties of the stabilizer block significantly impacted the morphology of the resulting particles [72].

Atom-transfer radical polymerization (ATRP) stands out as an extensively utilized CRP method. The first case of applying this approach was documented by Matyjaszewski and collaborators, marking a

milestone in the synthesis of block copolymers based on polystyrene (PS) [73]. Recently, Ding and co-workers developed

a series of ABA triblock copolymers, consisting of poly(phenoxyallene) (PPOA) and poly (methoxymethyl methacrylate) (PMOMMA) segments, incorporating double bonds. The synthesis involved a combination of traditional free radical polymerization, atom transfer radical polymerization (ATRP), and the site transformation strategy.

A novel bifunctional initiator, incorporating azo and Br-containing ATRP initiating groups, was utilized to commence the conventional free radical homopolymerization of phenoxyallene with a cumulated double bond. This process resulted in the formation of a PPOA-based macroinitiator (Br-PPOA-Br), featuring ATRP initiating groups at both ends. Following this, the macroinitiator Br-PPOA-Br initiated the atom transfer radical polymerization (ATRP) of methoxymethyl methacrylate (MOMMA), resulting in the formation of triblock copolymers with a structure of PMOMMA-b-PPOA-b-PMOMMA. These copolymers were later hydrolyzed to yield amphiphilic triblock copolymers with a composition of PMAA-b-PPOA-b-PMAA.

In aqueous media, the amphiphilic triblock copolymers demonstrated self-assembly, forming wormlike and large compound micelles [74]. Huang's team developed a novel block copolymer consisting of monomethoxy poly(ethylene glycol)-b-poly(methyl methacrylate-*co*-methacrylic acid) [mPEG-b-P(MMA-co-MAA)] via ATRP polymerization. The copolymer chains formed polymeric micelles during their self-assembly in aqueous media and revealed excellent colloidal stability. Methotrexate (MTX) was utilized as a hydrophobic drug molecule to be encapsulated into the polymeric hydrophobic core. The drug-loaded micelles exhibited a satisfactory drug loading capacity and showed a gradual drug release profile (26%) in simulated gastric fluid (SGF, pH 1.2) over 48 h. Also, the drug release profile at physiological conditions (pH 7.4) was extremely high (98%). For this reason, these synthesized pH-responsive polymeric micelles could be promising drug delivery vehicles [75]. Cao and *co*-workers designed and synthesized a novel hyperbranched amphiphilic block copolymer utilizing deactivation-enhanced ATRP polymerization (DE-ATRP). Specifically, PEG2000-Br was utilized as a macroinitiator to trigger the initiation of acid-cleavable divinyl (ACD) monomers connected via acetal groups, creating the hydrophilic (PEG) and hydrophobic (ACD) components. The synthesized hyperbranched copolymers self-assembled in aqueous media forming polymeric micelles with dimensions in the range of 70–100 nm and were evaluated in terms of drug encapsulation and drug release profile. Doxorubicin (DOX) was used as the model hydrophobic molecule. The drug loading capacity for the DOX-loaded polymeric micelles was approximately 8.2 wt%. Also, the drug-loaded micelles revealed an important level of cytotoxicity against HeLa cells [76].

Reversible Addition-Fragmentation Chain Transfer (RAFT) polymerization stands as a potent technique in the field of advanced polymeric materials, leveraging the distinctive chemistry of thiocarbonylthio (TCT) compounds to regulate the radical chain growth of vinyl polymers. The current emphasis on RAFT has led to the development of novel initiation and external control strategies, laying the groundwork for the synthesis of well-defined polymers tailored for challenging applications [77–80]. An interesting approach was made by Herfurth and *co*-workers utilizing RAFT polymerization technique in order to synthesize a new series of hydrophobically end-capped linear triblock copolymers, along with three-arm and four-arm star block copolymers, using *N*,*N*-dimethylacrylamide (DMA) and N,N-diethylacrylamide (DEA). The monomers were subjected to sequential reversible addition-fragmentation chain transfer (RAFT) polymerization via the R-approach. This process involved the use of bi, tri-, and tetrafunctional chain transfer agents (CTAs) containing hydrophobic dodecyl moieties. The polymerization proceeded in a well-controlled manner, reaching nearly quantitative conversion [81]. Arshad's team synthesized an amphiphilic diblock copolymer by RAFT polymerization. Firstly, they synthesized the homopolymer *N*,*N*-dimethylacrylamide (DMA) and utilized it as macro-CTA for copolymerization with a modified stearic acid monomer (SAM). Using a gamut of characterization techniques such as proton nuclear magnetic spectroscopic (^{1}H NMR), gel permeation chromatography (GPC), Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy and transmission electron microscopy they conducted the molecular and physicochemical characterization of these block copolymers. The amphiphilic diblock copolymers were capable of encapsulating carbamazepine (CBZ) into the hydrophobic core of their micelles accompanied by a remarkable drug encapsulation efficiency of 69%. The spherical shape of these micelles was also elucidated by TEM technique. In vitro drug release profile showed a gradual drug release rate within 70 h [82]. Our research team developed a novel series of double responsive amphiphilic hyperbranched polyelectrolyte *co*-polymers consisting of the poly(oligo(ethylene glycol)methyl methacrylate)-*co*-poly(2-(diisopropylamino)ethyl methacrylate), P(OEGMA-*co*-DIPAEMA) via RAFT polymerization method. These hyperbranched polyelectrolyte copolymers were able to form polyplexes with a linear nucleic acid in aqueous media. The complexation behavior and the cytotoxicity studies were accomplished by various techniques. By dynamic and electrophoretic light scattering (DLS, ELS) they were investigated extensively. The sizes of these polyplexes appeared to be influenced by the hydrophobicity of the copolymers as well as by the N/P ratio. MTT assay studies were also conducted using HEK-293 non-cancerous cell lines and showed that the copolymers exhibited a sufficient non-toxic character [62].

5. Formulation protocols for lipid/copolymer nanoparticles

Before the formulation of a lipid/copolymer hybrid nanoparticle, it is very important to study the interactions between the two major components of the system, i.e., lipid and copolymer. Several techniques have been used for preformulation studies. Thermal analysis techniques are of paramount importance because they can quantify the interactions and the enthalpy/energy of these interactions. Through thermal analysis techniques, it is possible to identify the best weight/M ratio between the components [83].

Furthermore, the conditions of hybridization are also a key parameter for the preparation of a functional nanoparticulate system. The chemical compatibility, the hydrophobic mismatch, the chemical composition and copolymer architecture, the polymer crystallinity, the lipid phases, and the lipid main transition are the innate parameters of the system [84].

Several preparation protocols have appeared for the preparation of LPHNs. Electroformation, nanoprecipitation, emulsification solvent evaporation and film rehydration are used widely as formulation protocols [4,6,85].

Firstly, electroformation is also called the “two-step method”. In this protocol, the lipid- and polymer-based nanosystems are prepared separately in the first step. The second step is the integration by electrostatic interactions between the lipid- and polymer-based nanoparticles. The preparation of pure nanoparticles can be achieved by well-established techniques, i.e., the thin-film hydration method, high-pressure homogenization, bath or probe sonication, etc. This protocol is useful when charged copolymers and/or lipids are utilized in the final formulation [4,6].

Another technique is the nanoprecipitation method. In this method, an organic solution or dispersion is emulsified in an aqueous solution/dispersion (with or without a surfactant). Next, the organic solvent is eliminated by stirring (either with or without vacuum), which enables the creation of nanoparticles. Namely, lipids are added to the aqueous medium for dispersion after copolymers have been initially dissolved in water or any other organic solvent. To precipitate the copolymer, the organic solvent is added to the aqueous phase and heat is applied. The heating temperature is a crucial parameter for the formulation process. A uniform size distribution is accomplished because of hydrophobic interactions between the copolymer core and the lipid. The dispersion is continually stirred, sonicated, or homogenized to decrease particle size.

Excess organic solvent and polymer are removed during centrifugation [4,85].

In the emulsification solvent evaporation method, the original W/O emulsion is self-assembled by adding the aqueous phase dropwise to the copolymer and lipid-containing oil phase. The solvent is then evaporated using vacuum, high temperature, or constant stirring. The final emulsion is formulated by adding it to an aqueous phase that includes a second lipid, possibly phospholipid, to create a secondary W/O/W emulsion [4,85].

In the film rehydration method, lipids and copolymers are dissolved in the same organic solvent. The organic solvent is removed in vacuum, and the hydration of the hybrid film is achieved by adding water or buffer at a constant temperature under stirring. This protocol needs further size reduction methods to achieve nanoparticulate populations with a narrow size distribution [86].

The aqueous heat method is also used for the LPHNs preparation. Sometimes, a size reduction method is needed to achieve particles with narrow size distribution [83].

Each method prepares different structures of lipid/copolymer nanoparticles. For example, emulsification solvent evaporation method leads to the formation of core–shell-type hollow lipid polymer nanoparticles. On the other hand, the film rehydration method leads to polymer-grafted liposomes [4,87].

6. Characterization techniques for lipid/copolymer nanoparticles

As it can be expected, the overall physicochemical and structural characteristics of lipid/copolymer hybrid nanoparticles (LPHNs) are closely related to the performance of the corresponding drug delivery systems [88–91]. For this reason, the full characterization of these hybrid systems is of outmost importance in order to understand how they function, to evaluate their potential for successful application, as well as to improve their efficiency [91]. Fortunately, researchers nowadays have an extensive arsenal of suitable techniques available at their disposal. These characterization techniques are capable of providing valuable information in regard to particle size, morphological properties, surface charge, interior structure, drug loading and release, entrapment efficiency, etc. [90]. In the following the most commonly used methods utilized for the investigation of the basic properties of LPHNs will be discussed, while a summary of the available characterization techniques, methods and assays with respect to the nanoparticles' basic properties is given in Table 1, along with representative examples of relevant studies from the literature.

6.1. Physicochemical and morphological characterization

Among the key physicochemical properties that determine the behavior of LPHNs are the nanoparticle size, size distribution, surface charge and morphology. The size of the nanoparticles is one of the most crucial factors affecting systemic circulation lifetime and their ability to passively accumulate in tumor tissues. It is generally accepted that nanoparticles with a size range of 10 to 150 nm are highly beneficial and favorable for systemic drug delivery [88]. Moreover, particle size is of particular importance because it directly determines the amount of drug loaded into the core structure of the particles, along with many other parameters, like the drug release profile, the targeting ability, the cellular uptake potential, the biological distribution efficiency, and the tissue distribution [91]. Apart from the size itself, the size distribution of the produced LPHNs is equally important since it can influence therapeutic efficacy. Meaning that in a polydisperse population of particles, only those that have the appropriate size can successfully target the desired tissue, while the rest might miss it or affect surrounding regions, leading to potential undesirable side effects [91]. The hydrodynamic size and size distribution, or in other words polydispersity index, of nanoparticles is usually measured by dynamic light scattering (DLS)

Table 1
Summary of the experimental techniques and other methods commonly used for the characterization of LPHNs basic properties.

LPHNs basic properties	Characterization techniques and protocols	References
Physicochemical and Morphological Characterization		
Particle size and size distribution	Dynamic light scattering (DLS), nanoparticle tracking analysis (NTA)	[92–94] [95]
Surface charge/zeta potential	Electrophoretic light scattering (ELS)	[96–98]
Morphology (i.e., shape, size, size distribution, surface) and structure	Scanning electron microscopy (SEM), transmission electron microscopy (TEM) and cryo-TEM, or atomic force microscopy (AFM)	[99,100] [96,98,101] [102–104] [99,105,106]
Internal structure and bilayer/membrane thickness	Small angle neutron scattering (SANS), small angle X-ray scattering (SAXS), wide-angle X-ray scattering (WAXS)	[102,107] [100,108,109] [110]
Surface or interface chemical composition	X-ray photoelectron spectroscopy (XPS)	[105,111,112]
Crystallinity	X-ray diffraction (XRD), differential scanning calorimetry (DSC)	[106,112,113] [114–116]
Membrane phase transition	Differential scanning calorimetry (DSC)	[99,111,117]
Chemical composition and drug-excipient interaction	Nuclear magnetic resonance (NMR), or Fourier transform infrared spectroscopy (FTIR)	[97,112,118] [119–121]
In Vitro Assays		
In vitro stability	Interaction with saline (e.g., PBS) and/or serum solutions	[92,115,122] [109,123,124]
Drug loading/encapsulation and release efficiency	UV–Vis spectrometry or high-performance liquid chromatography (HPLC) Dialysis method and UV–Vis, fluorescence or HPLC	[100,101,104,113] [125–127]
Cellular uptake, cytotoxicity, and apoptosis	Confocal laser scanning microscopy (CLSM), flow cytometry, MTT/MTS and ATP assays, caspase, annexin V, TUNEL assays, flow cytometry	[128–130] [99,131,132] [100,115,133–135] [135–140] [95,112]
In and Ex Vivo Studies		
Pharmacokinetics and biodistribution	Animal models and blood or tissue sampling/analysis utilizing HPLC, fluorescence imaging	[120] [99,104,141]
Toxicity	Blood analysis for toxicity markers, histology after H&E staining, hemolysis assay	[94,105,137] [135,142] [98,101,118,143]
Therapeutic efficacy	Determination of tumor inhibition/regression, metastasis suppression, or therapeutic outcome	[96,130,138] [141,144] [104,105,119]
In Silico Methods		
Structure/formulation, stability, or cellular uptake	Molecular dynamics, or artificial neural networks	[121,145,146] [147]
Drug incorporation, or receptor binding affinity	Molecular docking	[121] [148]
Pharmacokinetics	Physiologically based pharmacokinetics modeling	[149]

[92–94], while nanoparticle tracking analysis (NTA) can also provide the same information [95].

Another prominent characteristic of nanoparticles is their surface charge, which constitutes a measure of the electrokinetic potential between particle surface and the bulk solution [88,91]. It also influences the stability of the particles, as well as cellular uptake and intracellular trafficking. Previous studies have shown that zeta potential values larger than 30 mV (both negative and positive) lead to strong repulsive forces between the particles, thus hindering their aggregation. On the other hand, when the zeta potential is lower than ±5 mV agglomeration usually occurs. Apart from the innate surface charge of the nanoparticles, pH, ionic strength, and the nature of the solution also influence the zeta potential. In general, the zeta potential is recognized as a measure of the stability and durability of nanoparticles [88–91]. The most prevalent technique for the determination of zeta potential is electrophoretic light scattering (ELS) [96–98].

As far as the morphology of the LPHNs is concerned, various microscopy techniques including scanning electron microscopy (SEM), transmission electron microscopy (TEM) and cryo-TEM, as well as atomic force microscopy (AFM), are extensively utilized in order to elucidate the conformational/structural features of the nanoparticles [96,98–106]. Of course, all these microscopic techniques can be used for the determination of the shape, size and size distribution of the particles as well. Additionally, SEM can provide information about the surface morphology of the particles, while TEM and cryo-TEM are better suited for the investigation of the core-shell structure, especially with the use of appropriate negative stains that increase electron contrast and highlight specific particle components [88–91]. At the same time, AFM provides 3D surface morphology at the nanometer scale, allowing detailed height profiles of individual nanoparticles, while it can also measure mechanical properties like stiffness, elasticity, and adhesion forces of the nanoparticle surface.

A more detailed description of the internal structure of the LPHNs can be obtained through different scattering techniques like small angle neutron scattering (SANS) and small or wide angle X-ray scattering (SAXS/WAXS), which can determine various structural features, including nanoparticle size distribution, particle shape, structure, etc. [100,102,107–110]. Especially, SAXS can be utilized for the measurement of the thickness of the lipid shells of core-shell LPHNs [108]. Valuable information concerning the chemical structure of the surface and/or the interface of the hybrid particles can be obtained by means of X-ray photoelectron spectroscopy (XPS) [105,111,112], while the crystallinity of the formed LPHNs and the encapsulated drugs can be explored by X-ray diffraction (XRD) measurements [104,112,113], or differential scanning calorimetry (DSC) [114–116]. Moreover, DSC analysis can provide the phase transition temperature of liposomal bilayer membranes [99,111,117] Finally, nuclear magnetic resonance (NMR) and Fourier transform infrared (FTIR) are two of the most widespread spectroscopic methods for probing the chemical composition of the LPHNs' components [97,112,118], while FTIR has also been used for the study of drug-excipient interactions [119–121].

6.2. *In vitro assays, in vivo studies and in silico methods*

For the efficient utilization of LPHNs as drug delivery vehicles, a critical parameter that needs to be considered is their stability when introduced into in vivo conditions. In order to simulate these conditions in vitro, the colloidal stability of the formed nanoparticles is tested against physiological salinity by incubation in appropriate buffer solutions, like phosphate buffer saline (PBS) [92,115,122], or against biological media such as fetal bovine serum (FBS) [109,123,124], and suitable monitoring of any possible changes of their physicochemical characteristics. Of course, another essential characteristic of any potential drug delivery system is their drug loading and release efficiency. LPHNs can serve as a robust drug delivery platform able to carry hydrophobic drugs with high loading yields and controlled release rates [88]. The drug loading capacity and encapsulation efficiency are usually quantified by means of UV–Vis spectrometry or high-performance liquid chromatography (HPLC) [100,101,104,113]. As far as the drug release profile of the hybrid nanoparticles is concerned, several factors including drug-polymer interaction, drug solubility, polymer degradation rate, and particle size have a significant role to play [88]. Experimentally drug release studies are commonly performed through dialysis methods, where a dialysis membrane bag containing the drug-loaded nanoparticles is placed in a large volume of the release medium at 37 °C under moderate agitation. This way, the drug molecules continuously diffuse out of the nanoparticles into the release medium and are accordingly collected at specific time intervals for quantification using analytical tools, like UV–Vis, fluorescence or HPLC [125–127].

In an attempt to estimate the therapeutic efficacy of encapsulated drugs and to understand the behavior of nanocarriers in the biological environment, cell-based in vitro experiments are regularly performed [91]. More specifically, cellular uptake is evaluated utilizing appropriate fluorescent probes such as fluorescein isothiocyanate (FITC), rhodamine-B, coumarin-6, cyanine, Nile red, and trypan blue for the labeling of the nanoparticles and subsequent incubation with cells. Afterwards, the cells are imaged using fluorescence techniques like confocal laser scanning microscopy (CLSM) [128–130], so as to visualize particle internalization and distribution, or quantified by flow cytometry based on fluorescence intensity [99,131,132]. Equally important for the treatment effectiveness is the ability of the drug loaded PLHNs to induce the death of target cells (e.g., cancer cell lines). Consequently, cytotoxicity is commonly assessed using the well-known 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTT/MTS) [133–135] and adenosine triphosphate (ATP) [100,115] cell viability assays, while cell apoptosis can be estimated using caspase [135,136], annexin V [137,138], or terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) [139,140] based assays, or alternately by flow cytometry studies with the use of suitable markers [95,112].

As a final step, in vivo and ex vivo experiments, and animal models are carried out in order to investigate the performance of the PLHNs under the conditions occurring in living organisms. Usually in vivo studies include pharmacokinetics, biodistribution, toxicity, and therapeutic efficacy investigation. Pharmacokinetic and biodistribution studies are frequently combined since they both entail specific animal models (mostly rats), regular blood and/or tissue sampling at various time intervals after the administration of the drug loaded LPHNs and appropriate analysis through liquid chromatography methods like HPLC in order to quantify drug concentration [120], or spectroscopic techniques such as fluorescence (after appropriate labeling) so as to track their distribution in vivo [99,104,141]. Along the same lines, drug-related toxicity is evaluated in a similar manner utilizing again animal models and suitable blood analysis to determine the concentration of various toxicity markers including blood urea nitrogen (BUN), creatinine, creatine kinase (CK), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) [94,105,137], or major organ (i.e., heart, liver, kidneys, spleen, and lungs) histological evaluation usually with the aid of relative staining methods like hematoxylin and eosin (H&E) staining [135,142]. Another way to evaluate the LPHNs' toxicity is through the ex vivo hemolysis assay of human blood samples [98,101,118,143], which examines the potential rupture or lysis of red blood cells and thus assesses the hemocompatibility of the tested drug delivery systems. Lastly, the actual therapeutic outcome or anti-cancer/tumor efficacy of the applied drug loaded nanoparticles is verified by determining parameters relative to tumor inhibition/regression [96,130,138], metastasis suppression [141,144], or therapeutic effect [104,105,119].

Apart from the plethora of available experimental techniques, several in silico methods can be also used for the characterization of LPHNs behavior. These methods help predict and optimize their overall

physicochemical properties, stability, drug release behavior, and even interactions with biological systems, without performing time-consuming and complicated experiments [150]. Moreover, they enable the prediction of suitable conditions for further investigations, thus complementing experiments, while at the same time they help create new formulations with improved efficacy [150]. In particular, molecular dynamics simulations have been employed for the prediction/optimization of the structure and/or formulation of the nanoparticles [121,145], as well as for the investigation of their stability [121,146], or cellular uptake [145]. Similarly, molecular docking is used for the prediction of the preferred orientation of molecules (like drugs or targeting ligands) and can hence predict either the best possible drug-polymer formulation for optimum incorporation [121], or the binding affinity to specific receptors [148]. Other characteristic examples of in silico studies include the optimization of controlled release nanoparticle formulation with the aid of artificial neural networks [147], and the use of specialized physiologically-based pharmacokinetics modeling (PBPK) software for predicting pharmacokinetic behavior [149].

7. Recent developments from the literature

As detailed below, there are several developments, where different copolymers and lipids have been used in combination to examine the influential parameters and the properties of LPHNs for different applications. Table 2 summarizes the selected biomaterials including the copolymer's topology that were preferred except for the ones incorporating PLGA as the main copolymer. Due to the impact of PLGA hybrid formulations on pharmaceutical technology, they are mentioned separately as the main subject of Section 7.2.

7.1. Lipid/copolymer hybrid nanoparticles (LPHNs)

Shen et al. (2014) prepared hybrid nanoparticles of different lipid to polymer molar ratios (1, 5, 10, and 20% copolymer) to interpret the impact of triblock poly(2-methyl oxazoline-b-dimethyl siloxane-b-2-methyl oxazoline) copolymers of different mass on DOPC vesicles. The physicochemical, thermotropic, and bilayer properties were examined and compared to pure DOPC liposomes suggesting specific trends upon polymer's increase and a unique behavior depending on the different hydrophilic to hydrophobic ratio. In particular, the variant copolymer length led to different interactions between the biomaterials reflecting on a peculiar behavior regarding permeability. Namely, the presence of copolymers into the membrane in a small amount favored a denser packing arrangement. By increasing the % copolymer, the bilayer permeability seems to be dependent on the copolymer's hydrophobic segment length due to different hydrophobic interactions between the copolymer and lipid chains. In general, hybrid systems ameliorate the stability in simulated physiological conditions and the integrity of the membrane. However, the copolymer of smaller hydrophilic chains and longer hydrophobic anchor could be distinguished for its significant cooperativity with DOPC [151].

Dao and colleagues investigated thoroughly the interactions between phospholipids and poly(dimethylsiloxane-ethylene oxide) copolymers paying a lot of attention to membrane properties of vesicles at nano- and micro-scale. Regarding hybrid nanoparticles, they proved the existence of stable rafts in hybrid vesicles with graft copolymer as the major component, testing two different phospholipids DPPC and POPC (a solid or a liquid one in ambient temperature, respectively) [152]. In 2017, they demonstrated the magnitude of the copolymer's architecture (graft or triblock), mass (triblock copolymers of the same composition but different length) and membrane thickness on the morphology and the ability of the biomaterials to homogenously disperse [153]. In more details, the authors highlighted that significant properties of the vesicles, regarding their membrane organization, thermodynamic and mechanical properties, are differentiated between the giant vesicles and the nanoparticles, due to the different membrane curvature, as a result of the different size. As it was analyzed by Dao et al. [153], the membrane surface curvature affects the molecular packing, like the stretching grade of the hydrophobic parts and the surface-to-volume ratio, the membrane mechanics, like membrane tension, rigidity and tension, as well as the phase behavior, like the nanodomain formation, and therefore conclusions drawn from giant vesicles (of low curvature) cannot be directly extrapolated to nanoparticles (of high curvature), while the observed physical constraints and lateral structures are fundamentally different. Hybrid lipid/copolymer giant vesicles are out of the scope of this review though and more details can be found in the literature [110,153,169–174].

The formation of hybrid nanoparticles composed of poly (dimethylsiloxane)-b-poly(ethylene oxide) diblock copolymer and POPC lipid having the copolymer as the predominant biomaterial has been presented. The design parameters probed were the lipid/copolymer ratio, the copolymer's mass, and hydrophilic to hydrophobic ratio. All the hybrid systems were stable for over a year, while the different biomaterials seem to be finely mixed in vesicular structures with no obvious domains according to cryo-TEM and SANS techniques. It was indicated that this is attributed to the similar architecture of the two components. Slight deviations from the main morphology and membrane permeability are illustrated in the highest and lowest copolymer's mass, respectively [154].

Flandez and colleagues (2020) prepared hybrid systems of DPPC and four different diblock copolymers in two lipid to polymer ratios, particularly a low and a high copolymer concentration equal to 0.001 mg/ml or 0.2 mg/ml. The copolymers had a fixed hydrophilic comonomer; particularly poly(ethylene oxide) and differed in their hydrophobic one, which was either PCL or poly(lactic acid) or a mixture of them with a random topology leading to copolymers with variant hydrophilic to hydrophobic ratio. Due to different glass transition temperatures, the copolymers had a disparate impact on the lipid bilayer. Different glass transition temperature values of hydrophobic polymer segments incorporated into the lipid bilayer directly translate into different mechanical characteristics of the membrane. As it is also depicted by the Flandez et al. [155] results, polymers with higher glass transition temperature, like PLA, exist in a more rigid, glassy state of reduced conformational flexibility at physiological temperature due to the presence of methyl side groups along its backbone. Their incorporation promotes membrane stiffening, decreases lipid lateral mobility, and can induce packing constraints within the bilayer. Conversely, lower glass transition temperature polymers, like PCL (as a result of its more flexible streamline aliphatic backbone and reduced steric hindrance due to the absence of side groups), remain in a more flexible, rubbery state, allowing greater conformational adaptation within the bilayer and preserving membrane fluidity. As a result, the thermal behavior of the polymer, represented by its glass transition temperature, creates a clear structure–property relationship between its structural characteristics, its backbone architecture, and polymer chain mobility with membranes properties like bilayer packing, membrane mechanics and ultimately the mechanical and physicochemical characteristics of the later formatted polymer-lipid hybrid vesicles. The later properties include membrane permeability, lipid chain mobility, mode of drug encapsulation, as well as control of drug release kinetics and overall membrane stabilization. For example, the differences of the hydrophobic block (PLA or PCL) of the PEG-based block copolymers had different impacts on mechanical properties and the permeability of water and calcein in the resultant hybrid DPPC vesicles [155].Namely, the hybrid nanoplatforms exhibited varied behavior regarding their physicochemical characteristics and membrane mechanics in a copolymer dependent manner. Furthermore, it was highlighted that the systems with higher poly(lactic acid) content corresponded to a more ordered bilayer and a slower release profile of calcein used as a hydrophilic model drug [155]. In a similar manner, the group had studied a few years earlier the self-assembly of DPPC and PCL-PEO-PCL triblock copolymers with different length of the hydrophilic block in varied lipid to polymer

Table 2
Recent developments of LPHNs and their properties.

Source	Biomaterials			Morphology	Physicochemical Characteristics	Type of cargo	Application
	Lipids	Copolymers	Other materials				
[151]	DOPC	**triblock** $PMOXA_{15}$-$PDMS_{67}$-$PMOXA_{15}$ and $PMOXA_6$-$PDMS_{33}$-PMOX A_6	–	Vesicles *(morphology was not examined)*	**Size**: 120–140 nm for hybrids of $PMOXA_{15}$-$PDMS_{67}$-$PMOXA_{15}$ and 80 nm for $PMOXA_6$-$PDMS_{33}$-PMOX A_6 hybrid systems	–	Material Science
[152]	DPPC or DOPC	**graft** $PDMS_{26}$-g-$(PEO_{12})_2$	–	Spherical and faceted vesicles	**Size**: 47 nm **Membrane thickness**: 3.4–5.6 nm	–	Material Science
[153]	DPPC	1. **graft** [$PDMS_{26}$-g-$(PEO_{12})_2$] and **triblock** copolymers 2. PEO_8-b-$PDMS_{22}$-b-PEO_8, 3. PEO_{12}-b-$PDMS_{43}$-b-PEO_{12}, 4.PEO_{17}-b-$PDMS_{67}$-b-PEO_{17}	–	Variety of morphologies (from wormlike micelles to vesicles)	**Membrane thickness**: 5.0–12.0 nm	–	Material Science
[154]	POPC	**diblock** PDMS-b-PEO of different molecular masses	–	Vesicles, a small number of elongated structures	**Size**: 100 nm	Fluorescein dye	Material Science
[155]	DPPC	**diblock** PEO_{114}–PCL_{88}, PEO_{114}–P(CL_{42}:LA_{85} 1:2), PEO_{114}–P(CL_{14}:LA_{138} 1:10), PEO_{114}–PLA_{328}	–	Large Unilamellar Vesicles	**Size**: 265–275 nm (at low copolymer concentration), 285–360 nm (at high copolymer concentration)	Calcein dye as model drug	Drug delivery system in cancer treatment
[156]	DPPC	PCL_{12}-PEO_{45}-PCL_{12}, PCL_{16}-PEO_{104}-PCL_{16}	–	Large Unilamellar Vesicles	**Size**: 100–200 nm **Zeta potential**: 2-(−16) mV	Calcein dye	Material Science
[157]	POPC	**block** PEG_{12}-b-PCL_6, PEG_{12}-b-PCL_9 or PEG_{16}-b-PLA_{38}	–	Large Unilamellar Vesicles	**Size**: 110–150 nm **PDI**: 0.1–0.2	Calcein dye as model drug	Drug Delivery
[99]	DPPC	**diblock** PEG-PLA	–	Spherical Vesicles	**Size**: ≈50 nm (unloaded LPHNs), 56–76 nm (loaded LPHNs) **PDI**: 0.4–0.5 **Zeta potential**: −3-(−1) mV	Rhodamine-6G (fluorescent dye) as hydrophilic model drug	Drug delivery system for cancer therapy
[102]	DPPC	PEG-P(8C-Chol) in varied amount of P(8C-Chol) regime	–	Rounded and faced vesicles or Vesicles and elongated micelles *(Polymer dependent)*	**Size**: 120 nm **Membrane thickness**: 5.1 nm	–	Drug Delivery
[128]	Soybean PC: Chol, mPEG-DSPE	stearoyl-PEG-poly (methacryloyl sulfadimethoxine)	–	Spherical vesicles	**Size**: 150 nm (unloaded LPHNs), 160 nm (loaded LPHNs) **PDI**: 0.2 **Zeta potential**: 0-(−6) mV **EE: 16%**	Gemcitabine	Drug delivery system for cancer therapy with pH responsive properties
[97]	DPPC	mPEG-b-P(MAA-g-His)-NH_2	CTPP-chol	Spherical Vesicles	**Size**: 130 nm **PDI**: 0.3 **Zeta potential**: −12 mV	Ceramide	Drug delivery system with mitochondria-targeted ability and pH responsive properties
[123]	DPPC	(mPEG-b-P(MAAc)-chol	PEG conjugated to lysine (crosslinker)	Vesicles	**Size**: 140 nm **PDI**: 0.2 **Zeta potential**: −22 mV	Doxorubicin	Drug delivery system for cancer therapy with pH responsive properties
[117]	HSPC, chol	**random** and **diblock** copolymers composed of 2-(methacryloyloxy)ethyl phosphorylcholine and polyhedral oligomeric silsesquioxane methacrylate with acyl chains of different length [C2, C6, C8]	–	Spherical Vesicles	**Size**: 230–270 nm **PDI**: 0.4–0.6 **Zeta potential**: −3-(−2) mV	Doxorubicin	Drug delivery system for cancer therapy with pH responsive properties

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Table 2 (*continued*)

Source	Biomaterials			Morphology	Physicochemical Characteristics	Type of cargo	Application
	Lipids	Copolymers	Other materials				
[112]	HPC:chol	**graft** CS-g-m-PEG-NH_2	–	Spherical Vesicles	**Size**: 180 nm **PDI**: 0.2 **Zeta potential**: 20 mV **EE: 73%**	Lentinan	Drug delivery system for cancer therapy (Nose-to-brain delivery)
[158]	egg yolk PC	MD-MAA_{50}-LT, MD-MAA_{20}-LT	–	Polymer-grafted liposomes *(morphology was not examined)*	**Size**: 90 nm for MD-MAA_{50}-LT LPHNs, 140 nm for MD-MAA_{20}-LT LPHNs **Zeta potential**: −11 mV for MD-MAA_{50}-LT LPHNs, −21 mV for MD-MAA_{20}-LT LPHNs	Pyranine dye as hydrophilic model drug	Drug delivery system with pH- and thermo-responsive properties
[159]	DPPC	**block** PBD(1200)-b-PEO(600)	USPIONS (Superparamagnetic nanoparticles)	Large Unilamellar Vesicles	**Size**: 20 and 100 nm (two size populations)	Calcein dye as hydrophilic model drug	Drug delivery system for hyperthermia treatment with magnetic and thermo-responsive properties
[160]	DPPC or DOPC	**block** PBD(1200)-b-PEO (1000)	–	Unilamellar vesicles *(morphology was not examined)*	**Size**: 50–100 nm	Calcein dye as hydrophilic model drug	Phospholipase A2 – triggered drug delivery system with modified release properties
[106]	DPPC	**block** PBD-b-PEO	citrate gold nanoparticles	Vesicles *(morphology was not examined)*	–	–	Nanomedicine for potential photothermal therapy
[161]	Egg yolk PC, DOPE, chol, PEG-carbonyl-mPEG-DSPE	**block** copolymer of octadecyl vinyl ether and 2-(2-ethoxy) ethoxyethyl vinyl ether	–	Polymer-grafted liposomes *(morphology was not examined)*	**Size**: 120 nm	$MnSO_4$ (magnetic resonance imaging contrast agent), rhodamine, doxorubicin	Drug delivery system with thermo-responsive properties for cancer diagnosis and photothermal therapy
[139]	Egg yolk PC, DOPE, chol, PEG-carbonyl-mPEG-DSPE	**block** copolymer of octadecyl vinyl ether and 2-(2-ethoxy) ethoxyethyl vinyl ether	–	Polymer-grafted liposomes *(morphology was not examined)*	**Size**: 140 nm **PDI**: 0.1	$MnSO_4$ (magnetic resonance imaging contrast agent), rhodamine, doxorubicin	Drug delivery system with thermo-responsive properties for cancer diagnosis and photothermal therapy
[108]	DPPC	**triblock** PEO_{100}-PPO_{70}-PEO_{100} (Pluronic F127)	–	Unilamellar and spherical vesicles	**Size**: 100 nm (unloaded LPHNs), 100–112 nm (loaded LPHNs) **PDI**: 0.2–0.3 **Zeta potential**: −4-(+3) mV **EE**: >90%	• xanthene dye • erythrosine B • hydrophobic derivatives (photosensitizers)	Photodynamic cancer therapy
[162]	DPPC	**triblock** PEO_{100}-PPO_{70}-PEO_{100} (Pluronic F127)	–	Spherical Vesicles	**Size**: 28 nm **Zeta potential**: 2 mV	Hypericin (photosensitizer)	Photodynamic cancer diagnosis and therapy
[163]	PC	**triblock** PEO_{100}-PPO_{70}-PEO_{100} (Pluronic F127)	–	Spherical Vesicles	**Size**: 50–65 nm (unloaded LPHNs), 70 nm (loaded LPHNs) **PDI**: ≈0.3 **Zeta potential**: 0–1 mV **EE**: 80%	beclomethasone dipropionate	Drug delivery system for respiratory treatment

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Table 2 (*continued*)

Source	Biomaterials			Morphology	Physicochemical Characteristics	Type of cargo	Application
	Lipids	Copolymers	Other materials				
[111]	DPPC, DSPE-PEG_{2000}-mannose	**graft** polyaspartamide-polycaprolactone	–	Spherical (core -shell structure)	**Size**: 70 nm (unloaded LPHNs), 90 nm (loaded LPHNs) **PDI**: ≈0.2 **Zeta potential**: −20 mV *- 15% size increase after freeze-drying -*	Roflumilast	Drug delivery system for respiratory treatment *(microparticles* via *spray drying)*
[127]	DPPC, DSPE-PEG_{2000}-mannose	**graft** polyaspartamide-poly (lactic-*co*-glycolic acid)	–	Spherical (core -shell structure)	**Size**: 120 nm (unloaded LPHNs), 130 nm (loaded LPHNs) **PDI**: 0.3 **Zeta potential**: ≈ − 13 mV **EE**: 96% *- size increase after freeze-drying -*	Rapamycin	Drug delivery system for respiratory treatment *(microparticles* via *spray drying)*
[143]	DSPC, chol	**diblock** poly[(DEAEMA-*co*-BMA)-b-ManEMA] (40% BMA, 60% DEAEMA)	–	Polymer-grafted liposomes *(morphology was not examined)*	**Size**: 70–120 nm *(pH dependent)* **Drug loading**: 12%	Streptomycin	Drug delivery system for antibiotic therapy with targeted release and pH responsive properties
[164]	DPPC:chol	PEG-PMMI-CholC6	–	Vesicles *(morphology was not examined)*	**Size**: 214 nm **PDI**: 0.3 **Zeta potential**: −5 mV **EE**: 77%	Rapamycin	Drug delivery system for cancer therapy
[141]	soybean PC, chol	**diblock** PEG-b- poly[(1,4-butanediol)-diacrylate-β-N,N-diisopropylethylenediamine]	methoxy polyethylene glycol-peptide-vitamin E succinate	Micelle–liposome double layer structure (compartmentalized structure)	**Size**: 100 nm **Zeta potential**: −12 mV **EE**: 97% (Paclitaxel), 70% (thioridazine), 96% (PD-1/PD-L1 inhibitor HY19991)	• Paclitaxel • Thioridazine • PD-1/PD-L1 inhibitor HY19991	Multifunctional nanoparticles for cancer therapy with dual-responsive properties
[140]	cationic DOTAP	**diblock** mPEG-PCL	–	Spherical Micelles	**Size**: 46 nm **PDI**: 0.2 **Zeta potential**: 44 mV	Interleukin-12	Non-viral vector for cancer immunotherapy
[136]	cationic DOTAP	**diblock** mPEG-PCL	cRGD-R9 peptide ligand	Spherical Micelles	**Size**: 270 nm (unloaded LPHNs), 380 nm (loaded LPHNs) **PDI**: 0.4 (unloaded LPHNs), 0.2 (complex) **Zeta potential**: 17 mV (unloaded LPHNs), 13 mV (complex)	Bim mRNA	Non-viral vector for cancer therapy (for metastatic phenomena as well)
[118]	cationic DDAB	**triblock** PLA-PEG-PLA	–	Spherical (core-shell structure)	**Size**: 40 nm (unloaded LPHNs), 50 nm (complex) **PDI**: 0.2 **Zeta potential**: 25 mV (unloaded LPHNs), 25 mV (complex) **EE**: 80%	Insulin-like growth factor type I siRNA	Non-viral vector for cancer therapy
[134]	cationic DDAB	**diblock** copolymer mPEG-PCL	–	–	**Size**: ≈85 nm **PDI**: ≈0.3 (optimum unloaded LPHNs	Insulin-like growth factor type I siRNA	Non-viral vector for cancer therapy

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Table 2 (*continued*)

Source	Biomaterials			Morphology	Physicochemical Characteristics	Type of cargo	Application
	Lipids	Copolymers	Other materials				
[165]	DC-Chol, DSPE-$mPEG_{2000}$	cationic copolymers derived from esters of acrylic and methacrylic acid (Eudragit E100 or RS100)	–	Spherical with a PEGylated lipid shell and pDNA stripes	and complex) **Zeta potential**: 25 mV (unloaded LPHNs), 12 mV (complex) **EE**: 95% **Size**: 150–200 nm **PDI**: 0.2 **Zeta potential**: 3–5 mV **EE**: 73% (Eudragit E100 LPHNs), 97% (Eudragit RS100 LPHNs)	pDNA expressing RFP (model nucleic acid)	Non-viral vector for gene therapy
[109]	DPPC:chol: DOPE:DOTAP + DSPE-PEG_{2000}	**diblock** PEG-PLA	–	Unilamellar vesicles (core-shell structure)	**Size**: 150 nm **PDI**: ≈0.2 (optima LPHNs) **Zeta potential**: 38 mV (unloaded LPHNs), 25 mV (complex) **EE**: 96% (nucleic acid), 85% (cisplatin caprylate)	• ABCC3 siRNA • cisplatin caprylate	Drug and gene co-delivery for cancer therapy and against drug resistance
[166]	soybean PC, chol	PEG_{2000}-b-PCL_{2000} or PEG_{5000}-b-PCL_{5000}	–	Spherical	**Size**: 100 nm **Zeta potential**: ≈ −4 mV **EE**: 70–80% (polymer dependent)	Paclitaxel	Drug delivery system for cancer therapy
[124]	DSPE-mPEG	**graft** $PBAE_{3100}$-ss-$mPEG_{2000}$ copolymer	–	Spherical	**Size**: 95 nm **PDI**: 0.3 **Zeta potential**: −4 mV **Drug loading**: 19% **EE**: 77% (optima LPHNs)	Doxorubicin	Drug delivery system for cancer therapy with dual responsive properties
[130]	DOTAP, DSPE-PEG_{2000}, DPPC, Chol	PLA-TK-PEG	TPGS	Spherical (core-shell structure)	**Size**: 140 nm **PDI**: 0.3 **EE**: 46% (losartan), 14% (paclitaxel)	• Losartan • Paclitaxel	Drug delivery system for cancer therapy with stimuli responsive properties
[93]	DMPC, chol	**diblock** mPEG-PDL in different MW	–	Vesicles *(morphology was not examined)*	**Size**: 120–190 nm (unloaded LPHNs), 100–180 nm (loaded LPHNs) *copolymer dependent* **PDI**: 0.2 **Zeta potential**: −10 mV **EE**: 40%	Ovalbumin	Protein delivery
[167]	DPPC:chol, DOPC	**diblock** PBD-b-PEO	–	Multilamellar nanoparticles with possible nanodomains	**Size**: 100–200 nm **Zeta potential**: −17 or − 32 mV *lipid dependent*	• Paclitaxel • DSM265	Drug Delivery
[107]	DPPC	**diblock** PBD_{43}-b-PEO_{20}	–	Worm-like structures (vesicles and mainly micelles)	**Size**: 30–50 nm **Membrane thickness**: 5.0–7.0 nm *(lipid to polymer dependent)*	–	Material Science
[168]	POPC:POPS	**diblock** PEG-b-PCMA	–	Vesicles	**Size**: 150–180 nm (for 90:10 lipid to polymer weight ratio), 120–150 nm (for 75:25 lipid to polymer weight ratio)	–	Drug delivery system for oral administration with mucus penetrating ability

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Table 2 (*continued*)

Source	Biomaterials			Morphology	Physicochemical Characteristics	Type of cargo	Application
	Lipids	Copolymers	Other materials				
[103]	POPC, POPC: POPS, POPC: POEPC	**diblock** PCMA -b-PDEAEMA	–	Multilamellar Vesicles or occasionally Multivesicular Vesicles	*Extrusion filter dependent* **Zeta potential**: −39 mV (for 90:10 lipid to polymer weight ratio), −12 mV (for 75:25 lipid to polymer weight ratio) **Size**: 170–230 nm **PDI**: 0.1–0.2 **Membrane thickness**: 5.8–6.7 nm **Zeta potential**: 1–30 mV	–	Drug delivery systems with lysosomal leakage ability

ratios. Even though both copolymers affect liposomes in a concentration dependent manner, a good cooperativity between the biomaterials was observed. This cooperativity relates to the interactions at the molecular level between the lipids and copolymers leading to a higher packing degree of the hybrid bilayer compared to the lipid one, which was induced as a result of the presence of both copolymers. Besides, the difference in copolymer's hydrophobicity seems to have a great impact on the integrity of the membrane [156].

Khan et al. (2020) presented hybrid systems utilizing block copolymers of low molecular weight with the scope of preventing polymer/lipid bilayer's thickness mismatch. The copolymers consisted of PEG and either PCL or polylactic acid that could self-assemble into hybrid vesicular morphologies by interacting with phospholipids; particularly POPC. This ability is not an inherent property of these copolymers as they declared. The hybrid particles exhibited good stability, encapsulation capability, and a homogenous membrane that seems to be fine-tuned based on copolymer's composition (Fig. 4) [157].

The co-assembly of DPPC and PEG-PLA block copolymer was examined for its physicochemical, thermodynamic, and morphological behavior as well as the in vitro and in vivo efficacy as drug delivery system in anticancer treatment. This study pointed out the magnitude of the hybrid nature of the nanoparticles and the crucial role of lipid to polymer ratio by examining five lipid to polymer molar ratios (100:0, 75:25, 50:50, 25:75, 0:100). The calorimetric results confirmed the hybrid nature of the systems exhibiting one endothermic peak, which was broader than the neat DPPC one. The successful model drug incorporation did not affect much the dimensions of the hybrid systems compared to pure liposomes or polymersomes. Additionally, the lipid to polymer ratio is deemed as a critical design parameter influencing API's loading and release capability, stability in serum, cell internalization, and pharmacokinetics. Among the different vesicular hybrids, the DPPC: PEG-PLA (25,75) was distinguished [99].

A functional biomaterial of poly(ethylene glycol)-b-poly(cholesteryl-2-oxo-1,3,6-dioxazocane-6-carboxylate) block copolymer was introduced in DPPC liposomes via post-insertion protocol to produce a hybrid drug delivery systems with an integral and robust morphology and no coexistence of micellar phenomena. ITC, cryo-TEM, DLS, and SANS techniques permitted the investigation of the appropriate number of hydrophobic regimes for hybrid nanoparticles' self-assembly and stabilization in storage conditions. The appropriate number of 4–5 cholesterol conjugated moieties per copolymer chain favored the formation of hybrid systems by reducing the inter- and intra-molecular interactions – that could lead to phase separation and micelle formation - via hydrophobic forces between the hydrocarbon chains and the cholesteryl moiety. Having this in mind, the copolymer's topology is an important

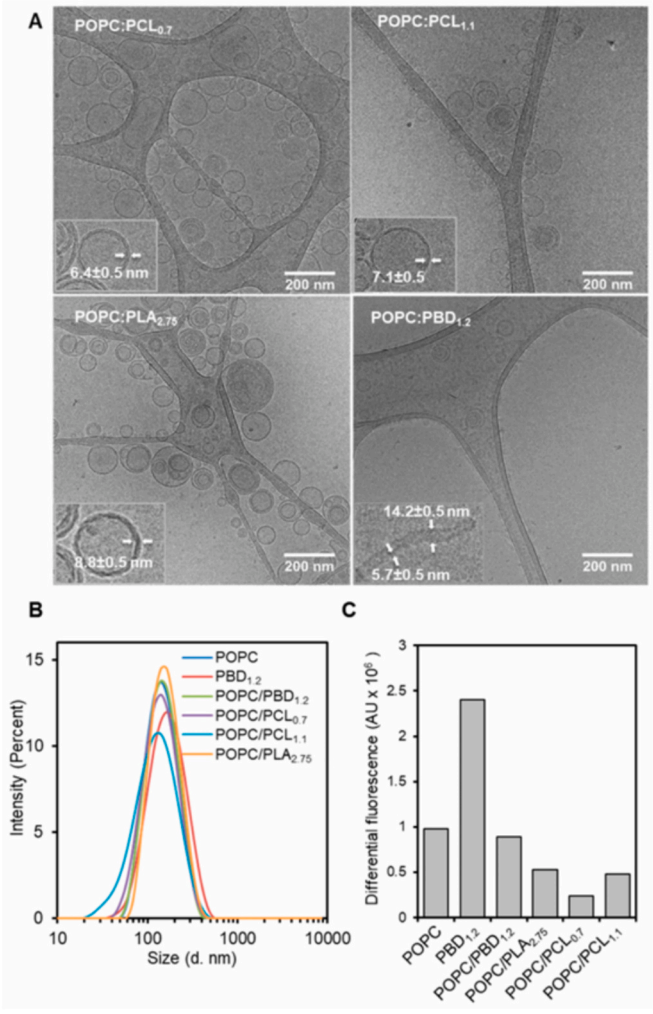

Fig. 4. Molecular assemblies of hybrid vesicles. (A) Cryogenic-transmission electron microscopy (cryo-TEM) images of hybrid vesicles formed by solvent dispersion and extrusion method in phosphate-buffered saline (PBS). Average membrane thickness is indicated in the bottom left inset of each image; (B) Intensity-weighted hydrodynamic diameter of hybrid vesicles; and (C) Differential Fluorescence of calcein of lysed hybrid vesicles with Triton X-100 after background subtraction of dialyzed hybrid vesicles. Adapted from [157].

design parameter [102].

Based on preformulation studies, Bersani et al. (2014) fabricated PC:Chol vesicles with stearoyl-poly(ethylene glycol)-poly(methacryloyl sulfadimethoxine) copolymer and a PEGylated lipid in fixed copolymer/PEGylated lipid ratio, while the lipid to polymer ratio was varied. The hybrid systems had a particle size of approximately 150 nm, spherical shape and a negative zeta potential at pH 7.4. Besides, they were stable at physiological conditions but increased their size at pH 6.5. The optimum formulation, namely 1:3:100 (PEGylated lipid:copolymer:lipids) molar ratio, was further examined for its toxicity, stability, and efficacy in cancer cell lines, while the experiments were repeated with gemcitabine as the cargo. The drug loading did not seem to have an impact on the physicochemical characteristics, whereas the pH-sensitivity and the advantageous features of the hybrid nanocarrier were confirmed in vitro [128].

Cationic DPPC liposomes with the ability to target mitochondria due to a ligand compound, were interacted electrostatically with mPEG-b-poly(methacrylic acid-g-histidine) block copolymer to fabricate a pH responsive ceramide-loaded hybrid nanoplatform for targeted cancer therapy. The cationic lipid was synthesized using cholesterol and (3-carboxypropyl) triphenyl phosphonium. The vesicular morphology of the hybrid complex was confirmed via TEM having an average size of 130 nm and a negative charge (−12 mV). The polymeric coating favored the selectivity of the system, while the charge modifications of histidine in varied pH offered the asset for rapid leakage of cationic liposomes from endosomes and effective targeting and accumulation in mitochondria organelles; a fact that was ascertained by in vitro experiments [97].

Hybrid polymer/liposomal nanoplatforms were prepared utilizing a pH-responsive and negatively charged block copolymer, namely methoxy poly(ethylene glycol)-*block*-poly(methacrylic acid)-cholesterol and DPPC phospholipid (Fig. 5). The nanoparticles were crosslinked via PEGylated lysine and the concentration of the crosslinker was optimized. The optimum formula exhibited a homogenous size distribution with particle size equal to 140 nm, negative zeta potential, pH-responsive properties, and good stability in physiological conditions. According to confocal microscopy technique, the successful internalization in cancer cell lines of doxorubicin loaded hybrid nanocarriers and the controlled drug release in the acidic environment of lysosomes took place, highlighting the biomedical potential of these pH-sensitive hybrid nanocarriers [123].

Random copolymers of methoxy diethylene glycol methacrylate, lauroxy tetraethylene glycol methacrylate, and methacrylic acid were synthesized and investigated for their pH- and thermo-triggered conformational changes as well as utilized for lipid/copolymer nanovesicles formation with spatiotemporal release properties. Physicochemical evaluation and release studies of a fluorescence dye as model drug in different conditions suggested the stimuli-responsive character of the hybrid systems. The simultaneous pH and temperature triggered release capability of MD-MAA_{50}-LT:PC nanoparticles was confirmed via in vitro experiments in cancer cell lines. However, further research is needed for amelioration of the cell internalization [158].

Chatterjee and Ooya (2020) investigated the adequacy of random and diblock polymeric guests to maintain or even augment the in vivo stability of HSPC:Chol liposomes. For this purpose, 2-(methacryloyloxy) ethyl phosphorylcholine and polyhedral oligomeric silsesquioxane methacrylate with acyl groups having different length were combined to create copolymers via RAFT polymerization. LPHNs of 250 nm and almost neutral charge were formulated and compared to one another as well as pure and PEGylated liposomes. The physicochemical, morphological, and thermodynamic characteristics of the hybrid systems advocate the copolymers' incorporation in the bilayer's surface influencing the lipid packing density in function of copolymers architecture. Specifically, the calorimetric features suggest that the random topology of copolymers favored a more rigid bilayer compared to HSPC:Chol bilayers integrating block copolymers that were more fluid. The protein

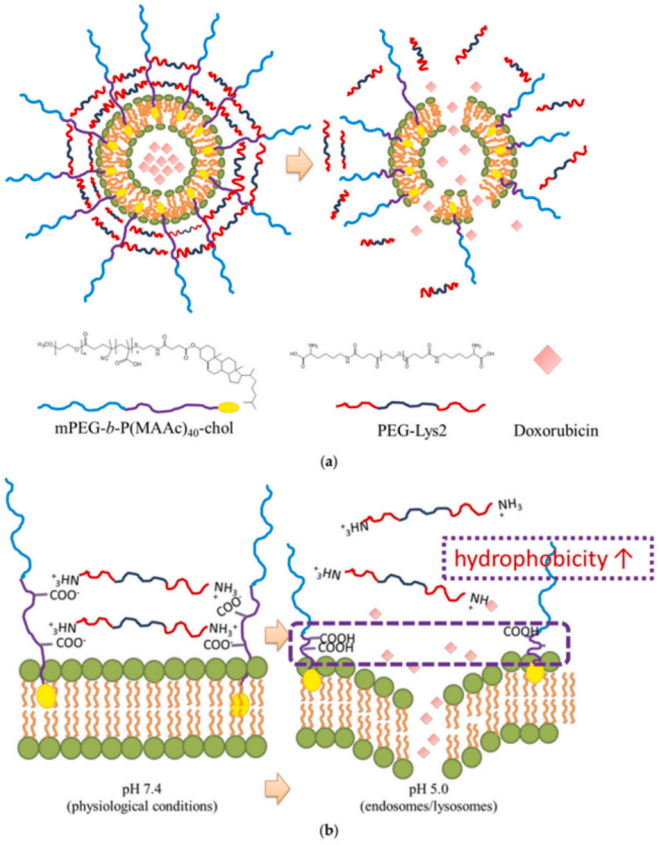

Fig. 5. Concepts of crosslinking polymer–liposomes (PC liposomes). (a) The crosslinked polymer–liposomes were composed of the pH-responsive copolymer mPEG-b-$P(MAAc)_{40}$-chol, crosslinker PEG-Lys2, and phospholipids; (b) At pH 7.4, the crosslinked polymer–liposomes were stabilized by the electrostatic forces between the anionic pH-responsive copolymers and the cationic crosslinker molecules. At pH 5.0, the protonation of the carboxylic groups of the pH-responsive copolymers occurred. The electrostatic forces were therefore destroyed, increasing the hydrophobicity of mPEG-b-$P(MAAc)_{40}$-chol and releasing the anticancer drug doxorubicin. Adapted from [123].

adsorption on unloaded and doxorubicin loaded nanoparticles was also evaluated revealing the stability of the nanocarriers in serum and the impact of the hydrocarbon chain's length on the protein corona formation [117].

Bixner and colleagues (2016) successfully prepared hybrid vesicles of DPPC and polybutadiene(1200)-b-poly(ethylene oxide)(600) integrating a superparamagnetic nanoparticle. The lipid/copolymer nanoparticles (30% *w*/w phospholipid) were formulated by two different methods, namely solvent inversion accompanied by extrusion and rehydration followed by sonication. The former one showed lipid domains according to DSC technique, while the last one led to homogenous vesicles and ameliorated the release rate of a model drug. The good release rate of the hybrids compared to pure and mixed polymersomes highlighted their possible utilization for hyperthermia treatment [159].

The exploitation of nanodomains was reported in DPPC or DOPC:PBD-b-PEO nano- or micro-systems for the modified release of hydrophilic APIs. Moreover, this study demonstrated the utility of physiological degradation enzymes in the formation of a stimuli-responsive hybrid nanostructure. The different lipid to polymer ratio and the intrinsic lipid conformation in physiological temperature (due to different T_m) were proven as vital design parameters to fine-tune the size and the drug pharmacokinetics of the nanoparticles. Indeed, the % weight of phospholipid, the type of lipid, and the hybrid nature of the systems led to specific trends in terms of size, total drug release and rate, and resistance to breakdown phenomena as well [160].

Recently, the membrane mechanics of DPPC hybrid nanoparticles with poly(butadiene-b-ethylene-oxide) block copolymer were investigated suggesting creation of rafts by increasing copolymer amount up to 65%. This research focuses on the formation of inorganic/organic superstructures with plasmonic features for photothermal purposes. The gold nanoparticles that were utilized seem to prefer interacting with PBD-b-PEO rich, soft amphiphilic block copolymer regions within the membrane and not with the rigid DPPC phospholipid regions, whereas the overall interactions could be fine-tuned by the inorganic-to-organic and lipid-to-polymer ratios thus promoting the clustering of the citrate-stabilized AuNPs [106].

The objective of Aoki and co-authors research (2015) involved the development of a multifunctional hybrid platform for antitumor diagnosis and therapy. A lipid mixture (egg PC, DOPE, chol, PEG-carbonyl-mPEG-DSPE) and a block copolymer of (2-ethoxy) ethoxy ethyl vinyl ether and octadecyl vinyl ether, were mixed and nanoparticles were prepared by thin film hydration method. The copolymer shows configurational adaptability in a temperature dependent manner suitable for photothermal therapy. Furthermore, the hybrid nanoparticles were combined with an imaging agent and doxorubicin. The ambitious theranostic nanoplatform with an average size of 120 nm was probed for its effectiveness achieving to accumulate in the targeted site via intravenous administration and to decrease the size of tumors in mice after mild heating [161]. Further investigation of the hybrid nanomedicine was published the same year for another tumor model in mice refining the heating protocol and revealing respective promising results [139].

Additionally, hybrid DPPC/Pluronic F127 nanoparticles were fabricated against cancer exploiting photodynamic therapy. The phospholipid and the triblock copolymer were used in varied proportions and the – first introduced - formulation protocol involved a solid dispersion process followed by bath sonication. The successful incorporation of the triblock copolymer and the spherical morphology of the hybrid nanostructures with a polymeric coating were confirmed by a gamut of advanced techniques including DSC, DLS, SAXS, and TEM. Afterwards, the hybrid vesicles were loaded with photosensitizers of different hydrophobicity exhibiting an encapsulation efficiency more than 90% and a good stability under various conditions. The different formulations were also examined for their efficacy in vitro and their skin permeation capability with encouraging results [108].

In a similar manner, DPPC/Pluronic F127 (2,1 weight ratio) nanoparticles were developed for a photoactive molecule's entrapment. Their physicochemical characteristics included an average size of 30 nm and a slightly positive zeta potential. In comparison to nanostructures of the individual biomaterials, the loaded hybrid system exhibited better photophysical features and good stability of their cargo according to spectroscopic techniques and singlet oxygen concentration measurements. In vitro proof-of-concept in cancer and normal cell lines highlighted the effectiveness of the hybrid nanoplatform, but also the need for optimization due to a non-ideal selectivity in the phototoxicity assays [162].

Pluronic F127 was also used for the modification of PC liposomes by De Leo et al. (2018). Hybrid vesicles were formulated by a micelle-to-vesicle transition protocol and compared to pure and PEGylated liposomes to bring out the ideal nanovector of beclomethasone dipropionate as a respiratory nanomedicine. According to preformulation studies the lipid concentration was equal to 10 mg/ml, whereas the copolymer was tested at two different levels – 0.2 and 0.5 mg/ml. Further experiments were conducted at the higher copolymer concentration. Although the systems were biocompatible and stable, the PEGylated one was found to be superior as in vitro and in vivo studies indicated [163].

Craparo and colleagues developed microparticles via spray drying comprising of hybrid lipid/copolymer nanostructures for combating respiratory diseases. In 2022 a roflumilast delivery nanoplatform was formed via self-assembly of a graft copolymer; namely polyaspartamide-polycaprolactone, DPPC phospholipid, and a mannose-functionalized PEGylated lipid. In 2024 the components of the hybrid system were polyaspartamide-poly(lactic-*co*-glycolic acid) graft copolymer, the same lipid mixture, and rapamycin. The lipid to polymer ratio in both studies was fixed at 2:3 or 1:1 *v*/v, respectively. The morphology and the interactions between the biomaterials were thoroughly examined by a gamut of different techniques. In both cases the nanoparticles were biocompatible and permitted controlled release of the chosen API in vitro. Lastly, the active and selective endocytosis was confirmed [111,127].

The limitations of antibiotics in pulmonary treatment due to non-selectivity was attempted to be overcome by a hybrid lipid/copolymer nanostructure loaded with streptomycin, which is characterized by poor pharmacokinetics. In this manner, the authors created a DSPC:chol:poly [(DEAEMA-*co*-BMA)-b-ManEMA] nanoplatform, which had the ability for active targeting of macrophages via mannose ligands. The nanocarrier had a loading capacity of 0.8 mg/ml and decreased in size following a pH dependent manner due to the protonated form of poly (DEAEMA-*co*-BMA) in an acidic environment. Most importantly, the system exhibited a controlled release into the endosomal environment showing a 60% burst release as well. Drug release was 4.5 times greater at pH 5.8 than at 7.4. Additionally, the nanoparticles were selectively toxic and mainly biocompatible with a much better efficacy compared to free drug and PEGylated liposomes [143].

PEG-poly(monomethylitaconate)-CholC6 copolymer co-assembled with DPPC:chol in a 4:1 lipid to polymer weight ratio to form pH-responsive hybrid vesicles for rapamycin delivery to cancer cells. The hybrid nanoparticles were compared to pure liposomes with or without oleic acid that permeated pH-sensitivity. The ratios of the biomaterials were optimized with statistical tools. Even though both pH-responsive systems could release their cargo in a pH dependent manner and be selectively toxic in vitro, indeed only the lipid/copolymer system could preserve its stability and efficacy in blood [164].

An interesting approach in hybrid lipid/copolymer nanovectors was introduced by Lang et al. (2019) for cancer therapeutics. They formulated a multifunctional system comprised of a block copolymer with pH responsiveness which could self-assemble inside a polymer/lipid vesicle of methoxy polyethylene glycol-peptide-vitamin E succinate, cholesterol, and soybean PC. The nanocarrier had an average particle size of 100 nm and was loaded with three different APIs. In vitro and in vivo results indicated targeted release and effectiveness of LPHNs at about 90–98% against tumors and metastatic incidents [141].

Men et al. (2018) created a spherical lipid/copolymer micelle for gene therapy of cancer. For this purpose, they utilized a positively charged lipid DOTAP and a mPEG-PCL copolymer. The carrier with a nanoparticulate size of 50 nm was used for immunotherapy with encouraging results via intravenous administration [140]. The same group optimized the characteristics of the vector in 2021 by adding a peptide conjugate ameliorating the transfection efficacy. In this case, the system had a colloidal concentration of 7.75 mg/ml and exhibited an increased size of 270 nm and a positive zeta potential. According to a systematic investigation in vitro and in vivo, the nanoplatform proved to be biocompatible and effective against metastasis compared to market available lipid and polymer non-viral gene vectors (Fig. 6) [136].

Monirinasab and colleagues (2018) reported the delivery and protection of siRNA by a hybrid lipid/copolymer nanostructure for anti-tumor therapy purposes. Upon preformulation studies, they fabricated DDAB/PLA-PEG-PLA nanoparticles of a 0.13 lipid to polymer ratio and a N/P ratio equal to 60. The final spherical complex had a slightly positive charge, 50 nm size and an encapsulated cargo of 80%. The success of this formulation involved its stability in cellular environment, the biocompatibility, and the efficiency in gene silencing compared to pure nucleic acid or respective lipoplex [118].

The necessity for less toxic lipid non-viral vectors led to the development of a hybrid gene nanocarrier comprising of the aforementioned cationic lipid and mPEG-PCL diblock for gene knockdown in the battle against cancer. The prepared nanoparticles had an average size of 85 nm, a homogenous population, and a positive charge, which was

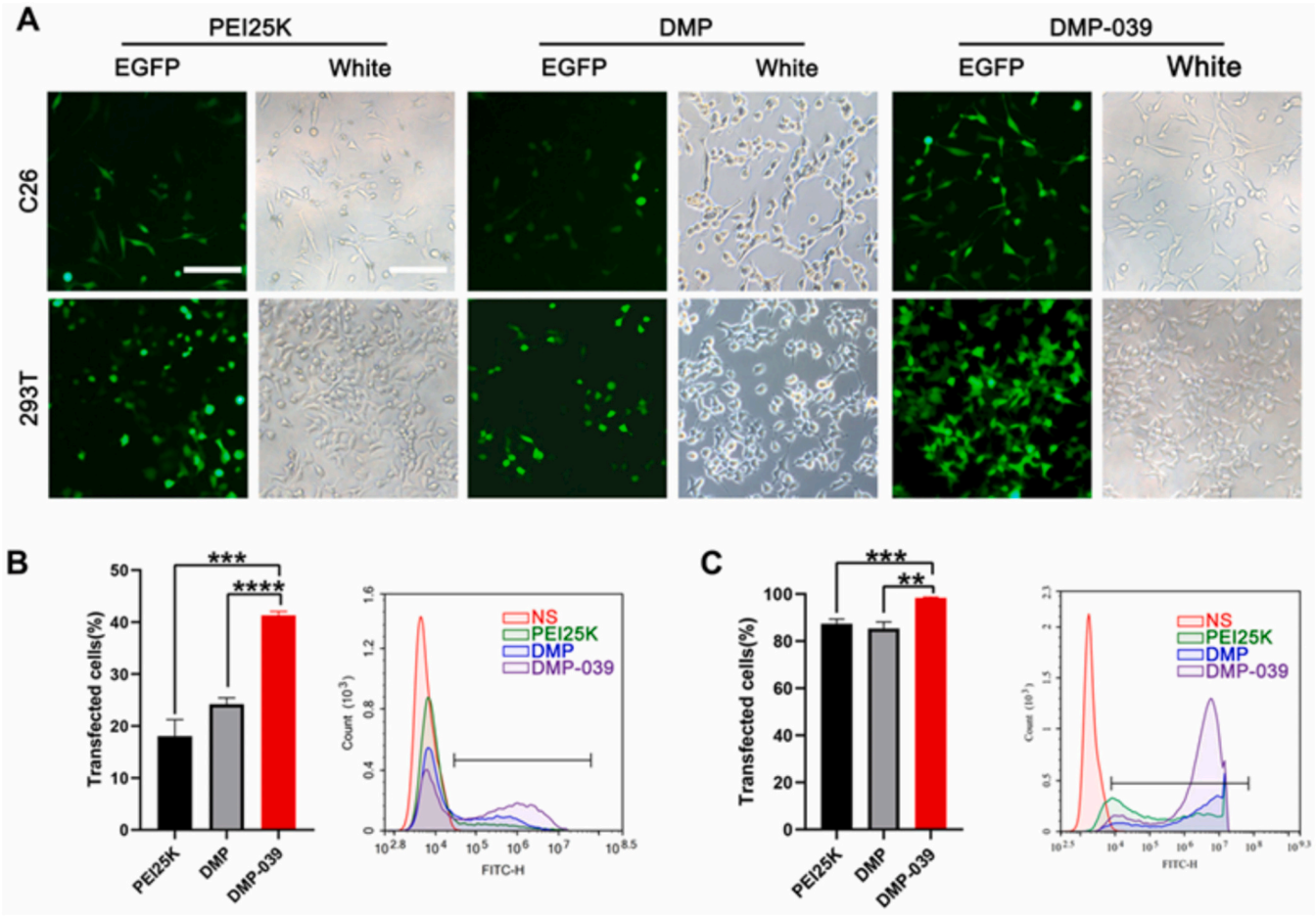

Fig. 6. In vitro gene transfection by the DMP-039/mRNA complex. (A) Fluorescence microscopy images of C26 and 293 T cells (scale bar: 100 μm). (B) The efficiency of transfection of C26 cells (***$P < 0.001$, ****$P < 0.0001$) and (C) 293 T cells (**$P < 0.01$, ***$P < 0.001$) analyzed by flow cytometry. Adapted from [136].

reduced to 12 mV by incorporating siRNA in an optimum N/P ratio equal to 40. Indeed, in vitro cytotoxicity studies demonstrated the superiority of the hybrid system not only in transfection efficacy but also in biocompatibility compared to respective lipid nanostructures [134].

In line with the abovementioned work, the nanoprecipitation method and lipid to polymer ratio 1:10 *w*/w were selected by Santhanes et al. (2024) to fabricate non-viral vectors as well. In this case, market available cationic copolymers derived from esters of acrylic and methacrylic acid were used. The lipid part comprised mainly of DC-Chol but also of DSPE-mPEG$_{2000}$ as a surfactant. Using several techniques, they indicated that spherical stripped nanoparticles incorporating a model nucleic acid were prepared. The different types of cationic copolymers used (Eudragit E100 or RS100) having different mass and pH responsiveness, led to variant physicochemical properties, encapsulation ability, and in vitro gene expression, while hybrid carriers of Eudragit E100 exhibited targeting capability and biocompatibility in vivo [165].

Hybrid nanostructures in a core-shell morphology were prepared to be co-loaded with an anticancer drug and a nucleic acid for enhanced antitumor therapy. The vesicles comprised of a mixture of lipids; in particular DPPC, DOPE, cholesterol, DOTAP, and DSPE-PEG$_{2000}$, and PEG-PLA block copolymer. Preformulation studies highlighted the optimum ratios including the lipid to polymer weight ratio (3:1). The selected formulation had a suitable particle size (150 nm) and ameliorated the drug loading in comparison with respective liposomes, while provoked protection of its cargo against serum proteins, a sustained release profile, better pharmacokinetics, and an increased therapeutic effect in both in vitro and in vivo assays compared to the free drug or the nanocarrier without the siRNA [109].

Self-assembly of the block copolymer mPEG-b-PCL with soybeanPC and cholesterol at a 1:17:2 M ratio was presented as antitumor nanomedicine. The nanoparticles were tested in two different lengths of PEG and were correlated to respective PEGylated ones for their properties as paclitaxel vectors in a drug to lipid ratio 1:10. The spherical nanostructure of PEG$_{2000}$ based system was distinguished for its capability of protecting the cargo during time and in physiological conditions, entering and accumulating in cancer cells, and enhancing API's pharmacokinetics. Besides, its efficacy could be measured by the limitation of tumor growth in vivo at about 20% and 40% more than the other nanocarriers and the free drug, respectively (Fig. 7) [166].

Doxorubicin loaded hybrid nanoparticles were prepared and optimized having the ability for targeted release in tumor microenvironmental conditions due to pH- and redox- responsive features. The stimuli-responsive properties are owed to a diblock grafted copolymer, namely PBAE-ss-mPEG. By investigating the characteristics of several formulations with different amount of DSPE-mPEG lipid, the optimum lipid to polymer molar ratio was found equal to 7:93. It was also demonstrated that the hybrid carrier was superior to the pure polymeric one in several aspects: a biocompatible drug delivery system with appropriate physicochemical features (size equal to 100 nm), more efficient drug loading and cellular internalization, as well as stability towards serum proteins adsorption and selectivity in cytotoxicity assays [124].

A sophisticated lipid/copolymer complex nanosystem was introduced by Ni et al. (2022) exploiting the cancer microenvironmental conditions. The hybrid nanoassemblies consisted of a DOTAP:poly(D,L-lactide)-thioketal-polyethylene glycol micelle with ROS-sensitive

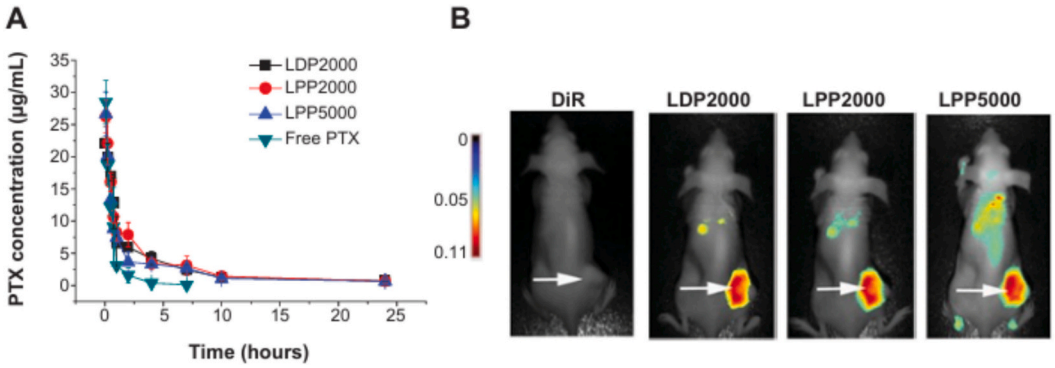

Fig. 7. (A) The PTX concentration–time curves in rat blood plasma after intravenously administrated free PTX and PTX-loaded Pegylated nanoparticles at a PTX dose of 10 mg/ml. (B) representative images of breast tumor–bearing BalB/c mice 24 h after injection of free Dir- and Dir-labeled nanoparticles. Arrows indicate the tumors. Adapted from [166].

properties in the interior of a DPPC:Chol:DSPE-PEG_{2000} vesicle. The compartmentalized structure was further decorated with PEGylated lipid, while paclitaxel and losartan were loaded. In vitro and in vivo results indicated enhanced anticancer efficacy and the targeted release capability of the system due to the synergistic effect of losartan and ROS sensitivity, provoking the morphological conversion of the nanoparticles in situ (Fig. 8) [130].

A graft copolymer of chitosan with mPEG-NH_2 was synthesized, characterized, and optimized properly via statistical analysis to cover the surface of HPC:chol nanoparticles for intranasal administration and brain targeting. The hybrids were loaded with lentinan – a nature derived anticancer polysaccharide, with a loading and encapsulation capability of 9% and 73%, respectively. The graft copolymer exhibited increased solubility, biodegradability, and mucoadhesive properties upgrading the features of the hybrid systems: increased retention time and permeation through nasal mucus, targeted and prolonged release, and thus superior anticancer efficacy in vitro and in vivo [112].

Hussain and colleagues (2020) formulated polymer/lipid vesicles as potential protein vehicles or adjuvants. The selected biomaterials were DMPC, chol, and diblock mPEG-b-poly(δ-decalactone) copolymers of different molar mass. The selection of the copolymers is based on the necessity of an alternative solution to PEGylation due to its disadvantages. Based on preformulation research, the copolymer's concentration was fixed at 4% *w*/w. They reported the successful fabrication of hybrid nanoparticles via microfluidics. The particle size of the colloidal dispersions varied regarding the different length of the comonomers (120–190 nm), while the loaded nanoparticles had a decreased size. The systems were stable at physiological conditions, non-toxic, and capable of loading the model protein with no defects on its structure. The encapsulation efficacy is deemed as formulation process dependent [93].

Kambar and Leal (2023) fabricated multilamellar hybrid nanoparticles by microfluidics. DPPC:chol or DOPC *co*-assembled successfully with poly(butadiene-*block*-ethylene oxide) having appropriate physicochemical characteristics for drug delivery purposes. For this goal, preformulation studies were conducted to improve the formulation conditions. The group demonstrated the multilamellarity of lipid/copolymer nanostructures in a 4:1 lipid to polymer molar ratio by X-ray and microscopy techniques. They, also, highlighted the superiority of the hybrids compared to pure biomaterials' nanoplatforms due to efficient solubilization of BSC Class II and IV drugs and stability of the loaded vehicles [167].

An investigation of lipid/copolymer nano- and micro- structures consisted of DPPC and poly(butadiene-*block*-ethylene oxide) in two different lipid to polymer molar ratios pointed out the different features of the nano- and micro-sized objects. The hybrid nanoplatforms did not show any phase separation phenomena, whilst the coexistence of different shaped structures and a non-vesicular morphology were observed via the use of DLS, SANS and TEM. Morphology and bilayer thickness are demonstrated to be dependent on lipid to polymer ratio [107].

An interesting strategy for oral administration was reported incorporating hybrid lipid/copolymer nanostructures into microbeads. The formulation protocol was optimized using different extrusion filters and lipid to polymer ratios, while the selected functional biomaterials were POPC, POPS, and PEG-b-PCMA diblock. The nanoplatforms were investigated for their physicochemical properties and membrane stability. Afterwards, the most promising system (75:25 lipid to polymer weight ratio) was evaluated for its ability to penetrate the intestinal mucus in vitro exhibiting superior results compared to pure and

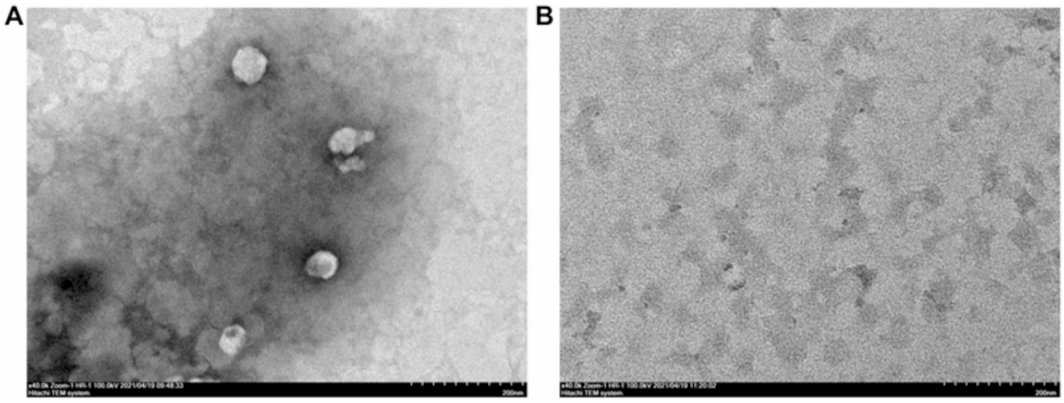

Fig. 8. TEM images of M/LST without (A) and with (B) H_2O_2. Adapted from [130].

PEGylated liposomes. The final beads could protect their cargo in stomach and release the nanoparticles in the intestinal region according to in vivo experiments [168].

Hybrid nanoparticles of POPC and copolymers were developed and charged lipids were occasionally added to the formulation. CMA was utilized for the synthesis of diblock copolymers via RAFT polymerization with 2-diethyl(aminoethyl) methacrylate in two different ratios. DEAEMA has been selected for its conformational adaptability to pH changes. Preformulation studies in varying lipid composition, comonomer ratio, and lipid to polymer weight ratio brought out the optimum

Table 3
Biomaterials and formulation protocols used in the most recent developments of PLGA containing LPHNs.

Source	Biomaterials			Formulation Protocol	Application
	Lipids	Copolymers	Others		
[119]	DPPC	PLGA (75:25)	–	double emulsification solvent evaporation	Drug delivery system
[113]	soybean PC	PLGA, poly(methyl vinyl ether-*co*-maleic anhydride)- D-α-Tocopheryl polyethylene glycol succinate copolymer, Poloxamer 188	glyceryl monostearate	emulsification-solvent evaporation	Drug delivery system
[96]	DSPE-PEG	PLGA-mPEG	cRGD peptide (ligand attached to the copolymer), perfluorohexane (contrast agent)	modified emulsion evaporation	Theranostics
[101]	DLPC, PEG-DSPE	PLGA (50:50)	fructose (preservative)	emulsification-solvent evaporation	Drug delivery system
[133]	soyPC, DSPE–PEG_{2000}	PLGA (50:50 and 65:35 – a mixture of 60:40 weight ratio)	PVA (emulsifier), Trehalose (cryoprotectant)	modified double emulsion solvent evaporation	Drug delivery system
[125]	HSPC, DSPE–PEG_{2000}	PLGA (50:50)	Trehalose (cryoprotectant)	modified two step emulsion–solvent evaporation	Drug delivery system
[144]	DPPC	NH_2-PLGA-NH_2 (50:50)	1-butyl-3-methylimidazolium-L-lactate (microwave sensitizer), hyaluronic acid (ligand), PVA (emulsifier)	double emulsification	Multifunctional drug delivery system
[138]	DOTAP	PLGA	Hyaluronic acid (ligand) (0.04% *w/v*)	Single emulsion-solvent evaporation	Drug delivery system
[174]	soy PC	PLGA (50:50)	PVA (emulsifier and stabilizer)	modified double emulsion solvent evaporation	Peptide delivery system
[142]	soyPC: chol	PLGA (75:25)	FA-PEG_{2000}-Chol (ligand- optimum ratio 1%), PVA (emulsifier)	emulsion solvent evaporation	Peptide delivery system
[122]	Lipids extracted from *mycobacterium smegmatis*	PLGA	–	Emulsion- solvent evaporation method	Drug delivery system
[129]	DPPC	PLGA (50:50)	–	emulsion/solvent diffusion	Non-viral vector
[175]	Lipidoid 5	PLGA (75:25)	Trehalose (cryoprotectant) (5% w/v)	double emulsion solvent evaporation	Non-viral vector
[132]	hydrogenated PC, DSPE-PEG2K-MAL	PLGA (50:50)	Transferrin (ligand)	one-step nanoprecipitation	Drug delivery system
[176]	soyPC	PLGA (50:50)	FA-PEG-HZ-GO (folate/pH sensitive ligand)	one-step nanoprecipitation	Drug delivery system
[135]	PC, DSPE-PEG_{2000}	PLGA (50:50)	Folate attached to the PEGylated lipid (ligand)	Nanoprecipitation	Drug delivery system
[126]	PC, DSPE–PEG_{2000}	PLGA (50:50)	–	modified single step nanoprecipitation	Drug delivery system
[177]	PC, DSPE–PEG_{2000}	PLGA	kNGR peptide (ligand) attached to the lipid (34% conjugation efficacy)	modified nanoprecipitation	Drug delivery system
[98]	soyPC, DSPE-PEG_{2000}	PLGA (50:50)	–	One-step nanoprecipitation	Drug delivery system
[137]	soyPC, DSPE-PEG	PLGA, Pluronic F-68	–	nanoprecipitation	Drug delivery system
[95]	PC, DSPE-PEG_{2000}-COOH	PLGA (50:50)	–	nanoprecipitation	Drug delivery system for photothermal therapy
[92]	soyPC	PLGA	Brij 78 (stabilizer), PEG (cryoprotectant for freeze-drying)	modified one-step nanoprecipitation	Drug delivery system
[115]	PC, DSPE-PEG_{2000}	PLGA (50:50)		**i.** Microfluidic co-flow nanoprecipitation compared to **ii.** bulk nanoprecipitation	Drug delivery system
[94]	DLPC, DSPE-PEG_{2000}	PLGA (50:50)	RWrNM (ligand attached to PEGylated lipid)	nanoprecipitation	Drug delivery system
[105]	DOPE, cationic DMAPAP	PLGA (50:50 or 85:15)	–	modified one-step nanoprecipitation	Non-viral vector
[100]	DPPE	PLGA (75:25)	Hyaluronic acid (ligand attached to DPPE)	nanoprecipitation	Drug delivery

formulations for further in vitro toxicity research due to nanoparticulate size, appropriate charge, spherical shape, and storage stability. Selected systems with an increased PDEAEMA content were found to be superior in transfection efficacy and lysosomal leakage compared to liposomes [103].

7.2. LPHNs incorporating PLGA copolymer

Reviewing the literature, most of the experimental works refer to hybrid lipid/copolymer nanostructures consisting of PLGA. For this purpose, our collected data for PLGA/lipid nanoparticles are focused on the most recent literature (2019–2024) (Table 3).

Besides, PLGA is usually used in an equal lactic/glycolic comonomer ratio due to faster rate of degradation in vivo [105]. However, the scientific works of Das and colleagues (2019) and Thanki et al. (2019) selected PLGA in a comonomer ratio 75:25 for topical use. In the former case, PLGA was amalgamated with DPPC to deliver quercetin for transdermal administration [119]. In the last case, they prepared non-viral vectors to be administered via pulmonary route and optimized the formulation having two critical attributes: lipid content and lipid to siRNA ratio. Both parameters have an impact on encapsulation efficiency, while experiments of one factor at a time showed that increasing each variable reduced the burst release and ameliorated the prolonged release kinetics [175].

A PLGA mixture (60:40 *w*/w) was selected for gemcitabine loaded lipid/copolymer nanostructures. The systems showed a monodispersed size population equal to 200 nm and a negative zeta potential (−18 mV). In comparison with the market available product the hybrid nanoparticles having a 45% encapsulation efficiency exhibited a higher toxic effect in vitro and a better anticancer efficacy in vivo via intraperitoneal administration with less side effects due to improved pharmacokinetics [133].

Furthermore, PLGA 50:50 and 85:15 in two lipid to polymer concentration levels (2 mg/ml or 5 mg/ml PLGA) were used to form gene therapeutics. The selected lipids were the fusogenic DOPE and the cationic DMAPAP. The nanovectors comprised mainly of hybrid particles with a spherical compartmentalized morphology and a homogeneous surface according to TEM, cryoTEM, SEM, X-ray photoelectron spectroscopy, and AFM, while their size ranged from 290 to 350 nm exhibiting a high positive zeta potential around 60 mV as well. Compared to lipoplexes the hybrid systems in a PLGA concentration equal to 5 mg/ml were less toxic and more efficient in vivo for oral usage [105].

A non-viral hybrid vector was formulated via emulsion/solvent diffusion process as cystic fibrosis therapeutics. DPPC:PLGA nanoparticles had an average size of 170 nm and a negative zeta-potential, while they managed to maintain these properties in biorelevant media with only small differences. Moreover, the hybrid nanoparticles encapsulated successfully about 94% of a nucleic acid encoding CFTR gene and exhibited mucus-penetrating and modified release ability ex vivo and in vitro, respectively [129].

Interestingly, PLGA/lipid hybrid studies presented in the current review mainly concern nanocarriers for anticancer therapy (65%) and/or with modified release capability (65%). It is of great importance that many works include in vivo findings as well (58%). The lipid of choice in most cases (77%) is a zwitterionic phosphatidylcholine, such as soybean PC, and the preferred assistant lipid for half of these research papers is DSPE-PEG so as to either provide stealth properties to the nanoparticles or to facilitate ligand transfer.

Huang et al. (2019) utilized both biomaterials in a PEGylated version of LPHNs, namely DSPE-PEG and PLGA-mPEG, while a peptide (as a ligand) and platinum (IV) were attached to the copolymer and the lipid,

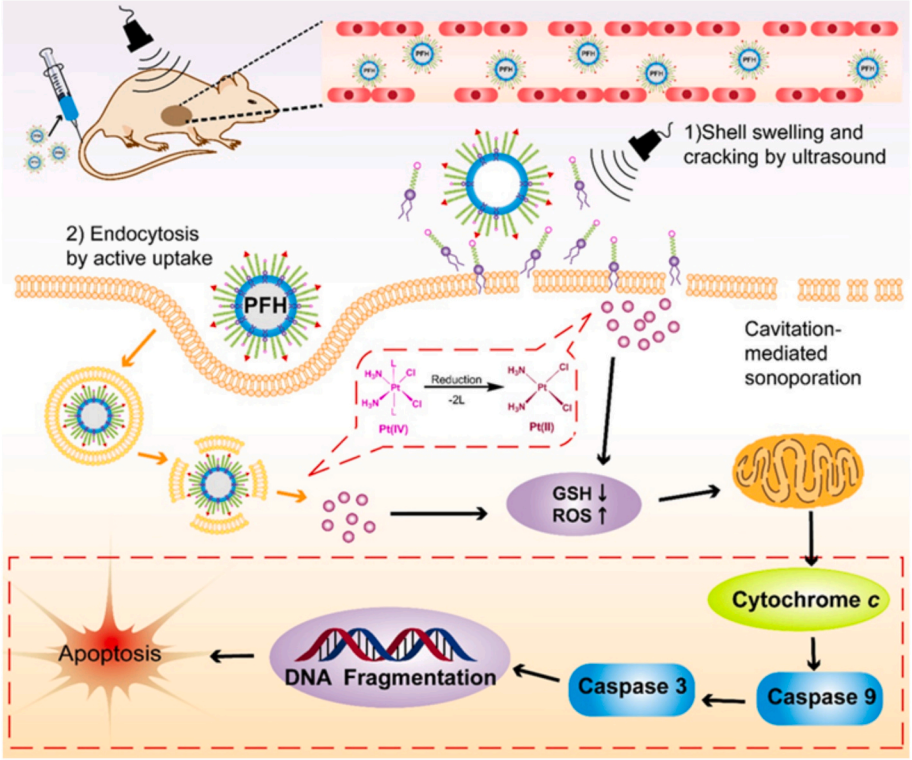

Fig. 9. The schematic illustration of Pt(IV) NP-cRGD assisted by US for tumor treatment. The Pt(IV) NP-cRGD are rapidly accumulated at the tumor sites via the EPR effect and active targeting and then uptaken by tumor cells through two main pathways: (1) Prodrugs are taken up by passive diffusion and sonoporation after ultrasound exposure and interact favorably with and adsorb onto the cancer cell membrane; (2) Pt(IV) NP-cRGD are selectively taken up via receptor-mediated endocytosis. Next, the prodrug and nanoparticles that escape from the endosome respond to endogenous GSH and release the drug. Finally, Pt(IV) NP-cRGD with US influence induces ROS overproduction and cause cell apoptosis to maximize the antitumor efficiency. Adapted from [96].

respectively. They prepared a hybrid smart nanoplatform for theragnostic purposes having dual-responsive properties as well (Fig. 9). The ligand's amount was found to be a critical factor for targeted cytotoxicity with the optimum value equal to 1%. Additionally, combining the nanocarrier with ultrasound led to increased size and modified morphology of the nanoparticles, from round-shaped to irregular ones, whereas improved cell internalization and targeted release alongside with decreased side effects and increased effectiveness compared to traditional cisplatin treatment [96].

Most formulations were fabricated by emulsification/solvent evaporation technique or nanoprecipitation (Table 3). However, a sorafenib delivery system for cancer treatment was developed by a microfluidics method with scale up potential. The authors compared the technique to bulk nanoprecipitation. Even though both nanoparticles exhibited similar physicochemical and morphological characteristics accompanied with similar encapsulation capability and release kinetic model, indeed the nanocarrier formed by microfluidic device had more controllable properties and higher anticancer ability in vitro [115].

To improve the pharmacokinetic profile and enable PC/PLGA nanoparticles to exhibit additional characteristics, other copolymers could also be utilized. For example, poly(methyl vinyl ether-*co*-maleic anhydride)-D-α-Tocopheryl polyethylene glycol succinate copolymer showed mucoadhesive capability and increased permeation of cabazitaxel for oral administration [113], while PLGA/lipid nanoparticles having a small proportion of hyaluronic acid could effectively prolong the release of anticancer APIs and be superior in vitro as well as in vivo [138,144]. The use of hyaluronic acid as a ligand for targeted cancer treatment and enhanced API encapsulation was favored by Andreana et al. (2024) as well. The polymer was attached to DPPE and mixed systems with PLGA (75:25) and pentamidine as the cargo (drug to polymer ratio 0.08:1 *w*/w) were formed via the nanoprecipitation technique [100].

In general, the addition of ligands is a valuable and wide sread approach for active targeting. Among others, folate is a popular one. Li et al. (2020) utilized folate conjugated with PEG and cholesterol to formulate hybrid nanocarriers of approximately 250 nm size to successfully deliver peptides highlighting the optimum ligand amount equal to 1% [142]. Another study deals with the use of a folate and pH sensitive ligand attached to PC:PLGA nanoparticles for co-delivery of highly encapsulated (EE > 80% for both of them) anticancer APIs. The ligand ameliorated the efficacy and led to a modified release profile according to in vitro and in vivo data [176]. Similar results came from Yang and colleagues (2020) concerning the valuable role of folate in hybrid systems for multidrug resistance cancer treatment. In this case, the lipid to polymer and the drugs to polymer weight ratios were equal to 2:8 and 1:10, respectively [135]. The use of transferrin improved the cellular uptake and the anticancer efficacy of hybrid nanoparticles, while the authors optimized the lipid to polymer and lipid to PEGylated lipid ratios. At optimum ratios the nanoparticles had a monodisperse size population - about 200 nm - and a negative zeta-potential, aproximately 50 mV [132]. Additionally, the formation of paclitaxel loaded PC:DSPE–PEG_{2000}:PLGA nanoparticles was investigated by multiple techniques and protocols (in vitro, ex vivo, and in vivo). The core-shell structure and the hybrid nature favored the controlled API release, while active targeting via kNGR peptide enabled antitumor efficacy and selectivity [177]. More recently, RWrNM was selected as ligand for targeted release in inflammations via nasal administration. Thus, DLPC:DSPE-PEG_{2000}:PLGA (4:1.5:10 weight ratio) nanoplatforms for dexamethasone delivery were fabricated and probed in vitro and in vivo [94].

Markowski and colleagues (2022) optimized component ratios for IV administration of ursolic acid. Various drug to copolymer ratios led to similar %EE but different size, selecting 1:10 (*w*/w) ratio as the most appropriate one. The spherical nanoparticles were prepared by one-step nanoprecipitation method in a lipid to polymer ratio equal to 1:6 (w/w) and two different PC:PEGylated lipid ratios (Fig. 10). The optimal loaded formulations were reproducible and had around 150 nm size and a negative charge [98]. Moreover, Tahir et al. (2019) examined drug hydrophobicity and drug to polymer ratio's impact on the physicochemical characteristics, encapsulation capability and % release of

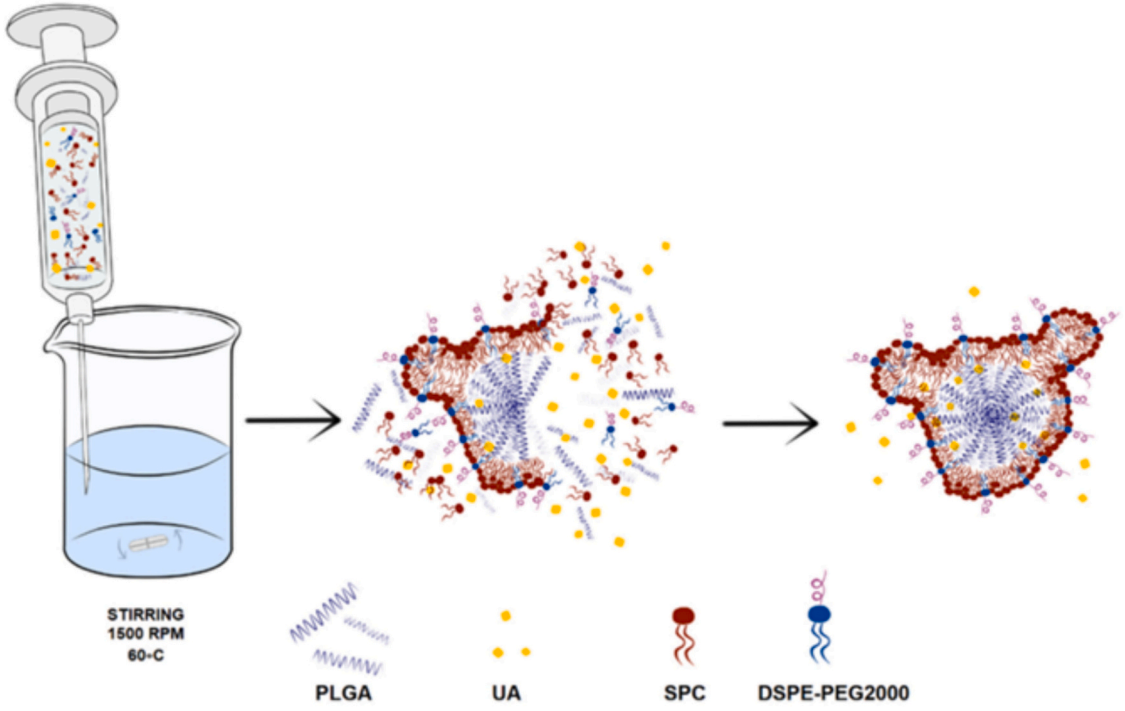

Fig. 10. Schematic diagram representing preparation of UA-S85-PLGA-PEG_{2000} hybrid nanoparticles. In the first stage, PLGA, lipids, and UA are injected into Milli-Q ultrapure water under constant stirring and heating. Hybrid UA-loaded PLGA/lipid particles are formed spontaneously with UA entrapment during particle formation. Adapted from [98].

hybrid particles using doxorubicin in two states. The nanoparticles loaded with the API in the base form demonstrated more promising results in vitro in comparison compared to the acid form, while in this case the chosen lipid to copolymer ratio was 2:10 (w/w) [126].

Co-loading is an asset for hybrid drug delivery systems and the selected strategy for several works to increase the effectiveness of pharmacotherapy. Hybrid PC:PLGA nanoparticles were developed and co-loaded with darunavir and ritonavir for HIV oral treatment. A surfactant was used as a stabilizer, namely Brij 78. Brij to PC ratio and freeze drying were deemed as important parameters for nanoplatforms performance in vitro [92]. Gefitinib (EE: 77%) and apatinib (EE: 95%) were co-loaded in DLPC:DSPE-PEG:PLGA vectors of 100 nm for cancer therapy [101]. Alternatively, Patel and colleagues (2020) co-administrated two nano-sized formulations loaded with mycophenolic acid and quercetin [137]. Exploiting the compartmentalization of HSPC: DSPE-PEG:PLGA hybrids, Fraix et al. (2020) succeeded to co-load an API and a NO photodonor. The compounds exhibited no cross influential phenomena and different release kinetics [125].

Other applications using PLGA:PC nanoparticles that succeeded to have an added-value for the pharmaceutical technology involve the delivery of fragile molecules -namely peptides- via oral administration [174] or photosensitizers for photothermal treatment [95] with promising in vitro results. More recently, PLGA was mixed with lipids extracted from Mycobacterium having also immunogenic ability to deliver berberine or coptisine against tuberculosis in vitro [122].

7.3. Our contribution to the field

Our research group has been investigating hybrid systems comprised of phospholipids and copolymers focusing on the rational design of drug delivery systems using multiple complimentary techniques for their characterization and the examination of the design parameters affecting their performance. Our scientific interest in lipid/copolymer nanovectors regards not only block copolymers but also novel copolymers of different architecture including gradient and random one, while we have exploited the capability of several copolymers to show stimuli-responsive properties.

Namely, a physicochemical and morphological evaluation of hybrid DPPC nanoparticles incorporating a gradient copolymer in different lipid to polymer ratios was conducted that indicated the thermoresponsiveness of the systems due to structural changes according to static light scattering results. A faster release of an anti-inflammatory drug in a temperature dependent manner compared to pure polymersomes was observed [178]. In 2021, analogous hybrid nanoparticles were formed utilizing the gradient copolymer in two different comonomer ratios (providing different levels of hydrophobicity) and a steady lipid to polymer molar ratio equal to 9:1. The systems were examined via SANS methodology giving information about the influence of copolymer's hydrophobicity and concentration, and temperature on the hybrid bilayer structure. It was highlighted that at ambient temperature hybrid nanoparticles integrating the gradient copolymer of higher hydrophobic content have a crucial role on the lamellarity compared to the vesicles comprised of the copolymer with the lower hydrophobic content that affected only the physicochemical features. [179].

Another SANS study by Papagiannopoulos et al. (2020) illustrated the successful formation of unilamellar hybrid DPPC:PEO-b-PCL nanoparticles and the capability to control lamellarity of the structures. The hybrid systems were found stable in temperature and concentration variations, while it was demonstrated that the length of the diblock copolymer's hydrophobic segment is a critical parameter for the structure of the nanoparticles [180].

Pippa and colleagues (2016) explored the role of a pH-responsive polymeric guest into liposomes. DPPC and poly(n-butylacrylate)-b-poly(acrylic acid) at two different comonomer ratios were mixed at six different lipid to polymer molar ratios (from 9:0.0 to 9:3.0) to form hybrid bilayers and nanoparticles. The systems were investigated for their thermodynamic and physicochemical behavior as well as their stability as a function of lipid to polymer and comonomer ratios by DSC and DLS techniques. The obtained data exhibited non-cooperative type hybrid bilayers accompanied by different thermal behavior in correlation to the comonomer ratio, as well as enhanced stability due to copolymer content increase in the hybrid systems [181].

Naziris et al. (2021) successfully formulated stimuli-responsive lipid/copolymer nanocarriers for cancer treatment by examining the functionality of two different block copolymers. In the first study, a thermo-responsive block copolymer; poly(N- isopropylacrylamide)-b-poly(lauryl acrylate), was used and in the other one a pH-responsive one; PDMAEMA-b-PLMA. The anticancer drug of choice was in a constant concentration level equal to 1.2 mg/ml or 6% *w*/w, respectively. In both cases, egg PC was the selected lipid and the lipid to polymer molar ratio was equal to 9:0.5. Hybrid systems were compared with respective liposomal ones regarding their biophysical, physicochemical and morphological features. Drug loaded nanoparticles were further evaluated for their drug release profile in a stimuli-dependent manner. Additionally, the in vitro biocompatibility of the nanovectors was confirmed. Lastly, PC:PDMAEMA-b-PLMA were further assessed for their in vitro and in vivo effectiveness against glioblastoma showing promising results [182,183].

Recently, Chountoulesi et al. (2024) focused on the morphological diversity of hybrid lipid/copolymer nanostructures and the crucial role of different design parameters on their morphology. Changes in lipid composition, block copolymer type, charge, lipid to polymer ratio, colloidal concentration, and formulation protocol have a great impact on nanoparticles co-assembly, and thus in their internal and external morphology highlighting its fundamental role on hybrids' functionality and highlighting particle morphology as a critical quality attribute [184].

Triantafyllopoulou and colleagues added another parameter into the equation: the one of copolymer's random monomer sequence in several studies. Their investigation indicated the successful formation of hybrid phospholipid/copolymer structures and highlighted the most influential design parameters including lipid composition, hydrophobic to hydrophilic balance, lipid to polymer ratio, and especially random copolymer topology. For this purpose, different copolymers and phospholipids (DSPC and DOPC) were selected to fabricate several hybrids nanosystems with different features. The selected copolymers were P(OEGMA$_{950}$-co-DIPAEMA), P(OEGMA$_{950}$-co-LMA), and P(OEGMA$_{500}$-co-LMA) that showed differences in their comonomer ratio and/or the hydrophilic chain length as well as the nature of the hydrophobic segment and the pH and thermo-responsive ability. The hybrid systems were examined in a bilayer and in a nanoparticulate form for their interactions leading to unique self-assembly. More importantly, it was highlighted that exceptional co-assembly reflects differences in the overall behavior of the hybrid systems including their thermotropic, physicochemical, morphological, and toxicological properties as well as their membrane mechanics. For the rational design of hybrid nanocarriers the necessity for utilizing several complimentary techniques was indicated due to the structural and compositional complexity of the nanosystems, such as the ones utilized in the abovementioned studies, in particular DSC, mDSC, DLS, SLS, cryo-TEM, HR-US, fluorescence spectroscopy using different probes (namely pyrene and Laurdan), in vitro MTS and MTT assays (Fig. 11) [185–187].

In this manner, a step-by-step design road map was presented for the development of a biocompatible lipid/copolymer nanocarrier. DSPC and P(OEGMA-co-DIPAEMA) copolymer were selected to form a hybrid drug delivery system with potential stimuli-responsive capability for anticancer treatment with promising in vitro results due to exceptional encapsulation efficiency of the hydrophobic API, methotrexate, and the in vitro potency due to statistically significant selectivity on cancer cell lines [188].

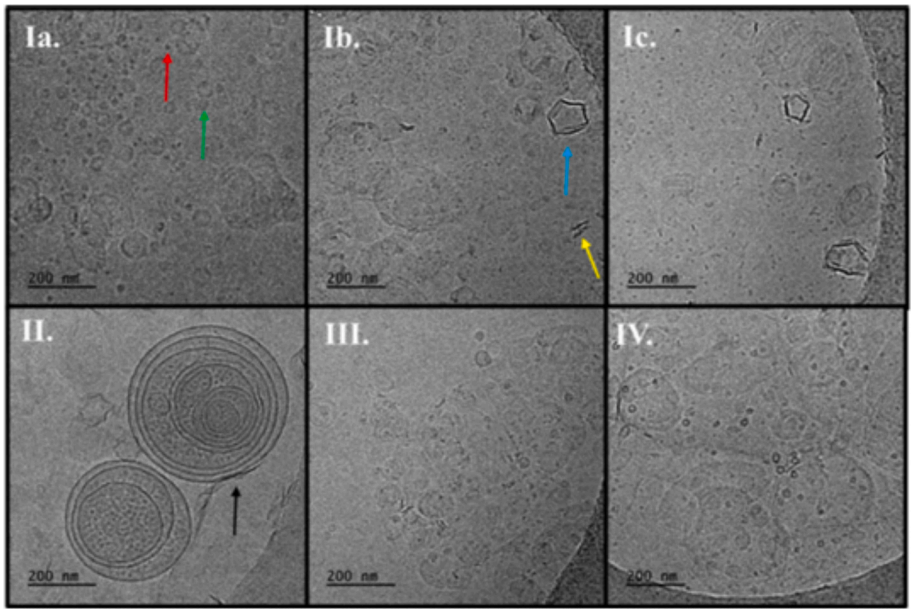

Fig. 11. Cryo-TEM images of DSPC:P(OEGMA-co-LMA) hybrid systems with different comonomer ratio (varying % PLMA) and/or different oligoethylene glycol side chain length (OEGMA950 or OEGMA500): (I) DSPC:copolymer-1 in different lipid to polymer weight ratios: a. 9:1, b. 7:3, and c. 5:5 ratio, (II) DSPC:copolymer-2, (III) DSPC:copolymer-3, and (IV) DSPC:copolymer-4. The colored arrows point out a different morphology; namely small spherical particles (red arrow), spherical, or irregular shape particles with distinct membranes (green arrow), rods (yellow arrow), nearly pentagonal-shaped particles (blue arrow), and spherical or irregular-shaped vesicles (black arrow). Adapted from [187]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

8. Applications for drug delivery

According to the previously discussed results, LPHNs could be promising candidates for drug delivery applications. In most cases, they are selected as drug carriers when modified drug release (delayed, targeted, etc.) is the goal. Their tailor-made properties enable the targeted release capability via surface functionalization or in situ morphological conversion due to stimuli-responsive mechanisms. There are several cases where sustained release is also achieved. Their versatility due to the amphiphilic and hybrid nature of the nanoparticulate systems offers the ability to incorporate challenging chemical compounds, including APIs of BCS Class II and IV. Co-loading is also a popular strategy for delivery of APIs and/or other compounds and use as multifunctional advanced drug delivery systems. From this scope, hybrid nanoparticles could be ideal candidates for diseases that have not been treatable yet or there are limitations concerning their effective treatment so as to meet patients' unmet needs. For example, they could be utilized in antimicrobial therapy, HIV, or/and diseases of central nervous system, such as Alzheimer's disease, multiple sclerosis etc. Referring to nanoparticles, there are several studies emphasizing in such treatments, however, there are limited studies regarding hybrid lipid/copolymer systems (Fig. 12).

So far research is mainly focused on cancer treatment exploiting the LPHNs advantages to overcome the existing barriers. Their size permits their passive accumulation on tumor sites through EPR effect and enables them to be suitable vectors for targeted therapy. Furthermore, the peculiar environment in tumors could be also deployed for tailor-made nanomedicine, such as redox- and pH-responsive systems [124,130]. For this reason, anticancer drugs are the main category of APIs that are utilized for hybrid lipid/copolymer nanoparticle loading. Additionally, hybrid nanoparticles with thermoresponsive characteristics or loaded with photosensitizers or inorganic compounds are ideal for photothermal therapy, while co-loading with conventional APIs or probes

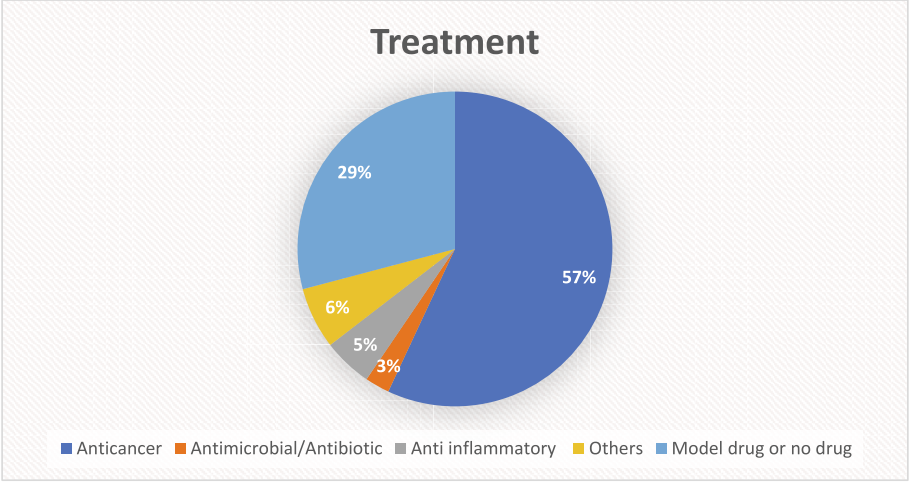

Fig. 12. The selected treatment of recent hybrid lipid/copolymer developments.

could accomplish synergistic effects or the formation of theragnostic devices, respectively [106,125,139,161]. These sophisticated and even multifunctional formulations could contribute to great progress in antitumor therapy via synergistic treatment or/and targeted and efficient therapy with less toxic effects, increased potency in tumor sites, and lower incidents of resistance in pharmacotherapy.

According to Fig. 13, is obvious that hybrid nanoparticles are emphasizing mostly in APIs delivery. However, the capability of lipid/copolymer hybrids to sufficiently protect their cargo and modify its release profile raises opportunities for protein, nucleic acid, and adjuvants to be encapsulated in lipid/copolymer nanoplatforms and utilized as advanced therapeutics or even for vaccination. Additionally, the ability for compartmentalized structures favors the co-delivery of APIs and genes [137,141]. In this way, gene therapy could become more efficient, and biotechnological formulations could be upgraded as well. Unfortunately, there are not a lot of such studies yet.

Considering the route of administration, LPHNs could be used in a gamut of different dosage forms but in most cases being injectable is preferred (Fig. 14). Transdermal administration is a suitable alternative due to the phospholipids' capability to interact with the stratum corneum, improve the insertion of their cargo in blood, and thus enhance the effectiveness of pharmacotherapy [119]. Furthermore, there are several formulations dealing with hybrid nanoparticles with mucoadhesive properties or other features transfusing targeted release capability due to the used copolymers or ligands. These developments are mainly focused on oral administration or local delivery via nasal or pulmonary cavity [112,113,163].

9. Quality by design approach for design and development of LPHNs

The hybrid nature and the complex interactions between the components of LPHNs result in the necessity for their examination via several characterization techniques and biological assays to conclude about their effectiveness and safety for pharmaceutical utilization. The Quality by Design (QbD) approach for the design, development, and scale-up of LPHNs is mandatory for their fast clinical translation. Critical Quality Attributes (CQAs) should be taken into consideration in the development of lipid/copolymer systems [175,184,188,189]. Several CQAs should be examined. Firstly, the physicochemical properties of the vector, including the size, size distribution, and zeta potential. Physicochemical features are fundamental for the characterization and evaluation of nanoparticles and could give a lot of valuable information regarding their physical properties and their interrelation with drug encapsulation/release and interactions with biological environments. They involve the measurement of the size and the size distribution or in other words the PDI value in multiple conditions. In particular, the dimensions of the nanoplatforms should be investigated for unloaded and loaded nanoparticles the day of their preparation as well as in storage and in vivo conditions to determine the physical stability of the vectors. Protein corona formation should be also considered when IV administration is selected. Occasionally, their size is examined in different pH and temperature conditions. Referring to physical stability, it is also connected to the surface charge according to DLVO theory, whereas steric stabilization could be also conducted due to surface decoration [190,191]. The surface charge and the zeta potential are strongly associated with the physical stability of the LPHNs. Electrostatic interactions (attractive or repulsive) are also involved in DLVO theory and explain the physicochemical behavior of the LPHNs dispersion over time and can be evaluated by the zeta potential values. Membrane mechanics, including fluidity, can be evaluated by thermal analysis, and spectroscopic techniques are widely used in the stage of the preformulation studies but are also considered as CQAs. As far as the membrane is concerned, soft matter and especially hybrid lipid/copolymer vectors should be investigated thoroughly for their membrane associated features due to the potential incompatibility of the used biomaterials and the possibility of domains formation. Heterogeneity of the membrane and phase separation inside it could lead to unstable systems, while nanodomain's formation might be advantageous for modified release [192,193]. For this purpose, membrane fluidity and membrane mechanics in general, the thermodynamic characteristics, such as the thermal transition temperature and the enthalpy of the mixed systems, could give valuable information regarding the temperature range that hybrids could be used as well as their overall functionality and effectiveness. Additionally, the detailed morphology (internal and external) and shape of the nanostructures are strongly associated with the interactions with cells, especially the immune cells, and tissues at the biological level [193]. As mentioned before, the morphology of LPHNs is quite complex, including vesicular, micellar, and compartmentalized structures [194].

Furthermore, considering the design parameters influencing the CQAs, the utilized materials including the loading of API/macromolecules are important for the co-assembly of the nanosystem. Besides their chemical structure/composition, the colloidal concentration, the lipid to polymer ratio, the drug to excipients ratio, the copolymer architecture, and the hydrophobic to hydrophilic balance have a crucial role in their overall performance. The use of ligands and their ratio is also fundamental for the hybrid nanoparticle characteristics and their potency. Referring to the fabricating protocols, the preparation process is critical as well as dependent on different parameters that are either general or dedicated to the process (including temperature, the organic or/and aqueous solvent type, the stirring rate and duration, etc.), while the size reduction method is also a factor that influences the nanoparticles' properties [151,160,187,195,196].

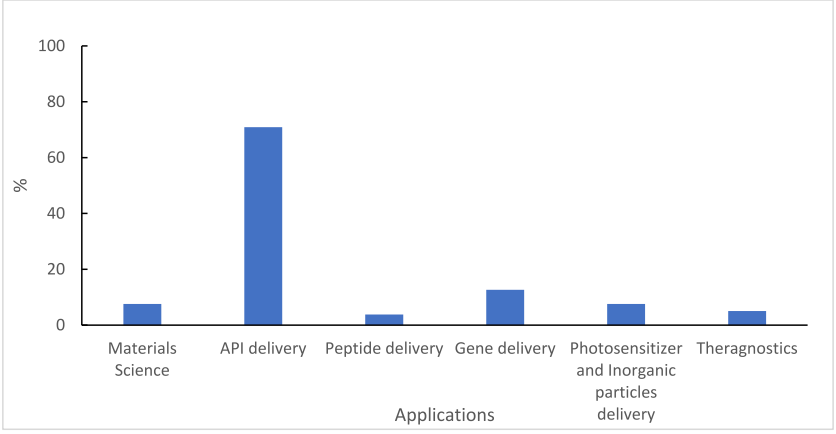

Fig. 13. Applications of hybrid lipid/copolymer vectors.

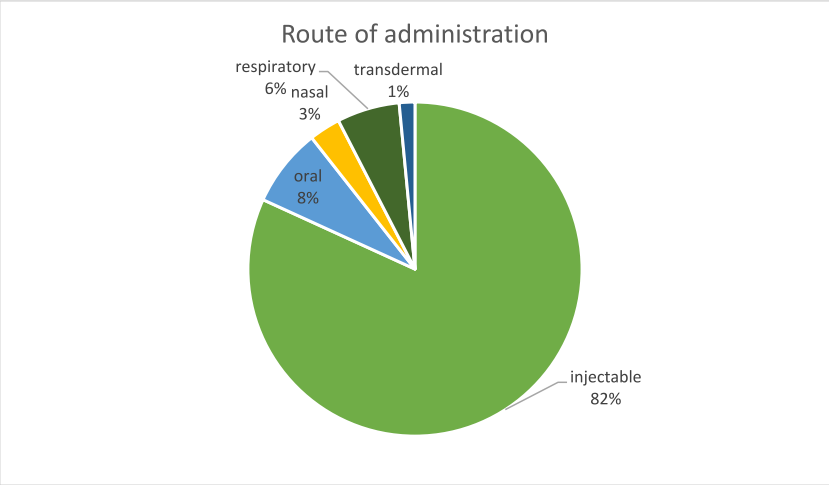

Fig. 14. The preferable route of administration for LPHNs.

Even though there are quality and material attributes as well as process parameters that are crucial for all the nanoparticulate systems and especially for lipid/copolymer ones, formulation scientists should have in mind that the determination of the critical quality attributes, the critical process parameters, and the critical material attributes is a case-by-case concept due to the plethora of different preparation protocols and biomaterials as well as the complexity of such systems. In this manner, the targeted quality product profile is of paramount importance, so that the route of administration, the indication, the intended release profile, and the targeting strategy (active or passive) should be examined.

10. Limitations and challenges of the LPHNs

Even though there has been great progress in nanomedicine and especially in hybrid lipid/copolymer nanoparticles, there are still limitations and challenges for their clinical use. There is an increasing number of publications in recent years about the design, development, and evaluation of LPHNs. Considering that most of the recent studies discussed in the current review utilize a gamut of different methodologies to characterize the nanoparticles and the fact that only a small amount of them has clinical data, it seems that there are several promising cases that are rationally designed. However, the code for fast clinical translation has not been cracked yet. The optimization of the characteristics of LPHNs is strongly associated with the preformulation studies; the selection of the suitable preparation protocol and the identification of CQAs are the basis for their development with added value for the pharmaceutical use. In general, nanosized drug delivery platforms are complex systems, their properties being dictated by the interactions of the biomaterials utilized, the API, and the surrounding environment [197].

Taking into consideration that lipid/copolymer nanovectors are even more complex due to their hybrid nature, great effort should be devoted to accomplishing the intended results. For this purpose, several diverse techniques should be applied to characterize such systems. The unique lipid/copolymer interactions in each formulation can affect the co-assembly, and thus several functionalities and characteristics of the vector; among others the structure of the hybrid systems including the external and internal morphology as well as their physicochemical and membrane mechanical features (dimensions, surface charge, ligand targeting, fluidity etc.). These characteristics are fundamental for physical stability, intrinsic toxicity, effectiveness, and most importantly the fate of the nanoparticles in vivo.

All these characteristics are strongly associated with their biological behavior and their pharmacokinetic profile. Namely, the formation of protein corona, which influences clearance by the mononuclear phagocyte system (MPS). The active or passive targeting of tumor issues is also influenced by the physicochemical, morphological, and stability characteristics of the LPHNs. Finally, the adjuvant properties and immunogenicity can also be altered by the CQAs of the LPHNs [198].

However, the need for their extensive investigation by multiple complementary techniques and the difficulties in scaling up and batch-to-batch reproducibility are critical issues that should be considered by the pharmaceutical industry for investing in their clinical application. According to the QbD approach, the batch-to-batch variability should be near to zero in order to achieve identical batches during the manufacturing processes on the industrial scale. The batch consistency is very sensitive to the changes of materials and parameters of preparation processes. For this reason, the in-depth understanding of the properties and the interactions between the lipids and polymers is of paramount importance to build up a robust and scalable preparation protocol. The multi-stage fabrication process is a limitation for the scale-up of LPHNs, but in recent years the introduction of techniques like microfluidics has been very important for the quick scale-up of LPHNs [199,200].

Additionally, fast clinical translation could be even more challenging in nanoparticulate systems due to the lack of knowledge on interspecies extrapolation of in vivo studies and the population variability. The intrinsic toxicity of nanoparticulate systems and especially cationic ones is another major issue, while the in vivo pharmacokinetics and the high potency of targeted drug delivery systems need further investigation to be considered as safe pharmacotherapy [189,201–208]. Additionally, the long-term nanotoxicity based on clinical studies and pharmacovigilance data and the immunogenic properties are also regulatory challenges for nanomedicines generally and off-patented nanomedicines and, consequently, for LPHNs, too [209].

In the same context, focusing on copolymers, their tailor-made and novel properties might upgrade the functionality of hybrid nanoparticles and solve several existing problems of conventional lipid-based drug delivery systems. However, the approval of new biomaterials and especially polymers for clinical use is a time- and money- consuming procedure [206,208]. This is probably the main reason why the use of PLGA in LPHNs studies prevails by searching the literature. It is an FDA approved family of polymers for pharmaceutical use. In this manner, there is a lot of scientific data for lipid/PLGA core-shell structures, but only a few regarding mixtures of lipids with copolymers of different architecture and/or composition. Reviewing the literature, 68% of these works concern diblock and triblock copolymers, while only 23% of the studies, most of them reported by our research group, deal with graft, random, and gradient copolymers. There are, also, scientific projects where the copolymer topology has not been explored. In our opinion,

copolymer architecture and composition are crucial design parameters that should be further examined. In general, there is a gap in literature regarding LPNHs comprised of lipids with copolymers other than the ones of block type. Probing the interactions and properties of hybrid systems with copolymers of a more complex topology –statistical, hyperbranched etc.– could give valuable information for material science, biomimetics, and pharmaceutical technology.

11. Future perspectives of LPHNs as nanomedicines

LPHNs have been introduced as promising drug delivery candidates for pharmaceutical applications exploiting the advantages of lipids and polymers in combination as well as the unique features of new functional materials. Their technological versatility, their physicochemical and morphological characteristics, and the interactions between their components can ameliorate the ADMET profile of the encapsulated API. Their added value is obvious, based on the increasing number of publications in recent years and the academic teams that are working in this field worldwide. On the other hand, many challenges are still here to act as a barrier for their scale-up in pharmaceutical industries, fast clinical translation, and approval from regulatory agencies.

In our opinion, there should be a close and strong collaboration between different scientific fields, including materials and polymer scientists, pharmacists, engineers, and clinicians, as well as the pharmaceutical industry and regulatory organizations. The study of the properties of lipids and polymers in depth, as well as the techniques for their development into drug delivery systems and the subsequent drug loading and release studies, should be the cooperative subject of material and formulation scientists. In addition, the development of methods for scale-up and standardization of characterization protocols for in-process and batch release control will be the subject of collaboration between the academic community and the pharmaceutical industry. To the best of the authors' knowledge, this is the first report emphasizing phospholipid/copolymer hybrid drug delivery nanoplatforms from a technological perspective. Embracing the quality by design-oriented formulation strategy, this work could, hopefully, contribute to the in-depth knowledge and application potential of LPHNs.

CRediT authorship contribution statement

Efstathia Triantafyllopoulou: Writing – original draft, Methodology, Investigation. **Dimitrios Selianitis:** Writing – original draft, Investigation. **Maria Chountoulesi:** Writing – original draft, Methodology, Investigation. **Maria Karayianni:** Writing – original draft, Investigation. **Georgia Valsami:** Writing – review & editing, Supervision. **Stergios Pispas:** Writing – review & editing, Supervision, Conceptualization. **Natassa Pippa:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All relevant data included in the manuscript.

References

[1] Siepmann J, Faham A, Clas SD, Boyd BJ, Jannin V, Bernkop-Schnürch A, et al. Lipids and polymers in pharmaceutical technology: lifelong companions. Int J Pharm 2019;(558):128–42. https://doi.org/10.1016/j.ijpharm.2018.12.080.
[2] García-Pinel B, Porras-Alcalá C, Ortega-Rodríguez A, Sarabia F, Prados J, Melguizo C, et al. Lipid-based nanoparticles: application and recent advances in cancer treatment. Nanomaterials (Basel) 2019;9(4):638. https://doi.org/10.3390/nano9040638.
[3] Kuperkar K, Patel D, Atanase LI, Bahadur P. Amphiphilic block copolymers: their structures,and self-assembly to polymeric micelles and polymersomes as drug delivery vehicles. Polymers (Basel) 2022;14(21):4702. https://doi.org/10.3390/polym14214702.
[4] Dave R, Patel R, Patel M. Hybrid lipid-polymer nanoplatform: a systematic review for targeted colorectal cancer therapy. Eur Polym J 2023;186:111877. https://doi.org/10.1016/j.eurpolymj.2023.111877.
[5] Go YK, Leal C. Polymer–lipid hybrid materials. Chem Rev 2021;121:13996–4030. https://doi.org/10.1021/acs.chemrev.1c00755.
[6] Bangera PD, Kara DD, Tanvi K, Tippavajhala VK, Rathnanand M. Highlights on cell-penetrating peptides and polymer-lipid hybrid nanoparticle: overview and therapeutic applications for targeted anticancer therapy. AAPS PharmSciTech 2023;24(5):124. https://doi.org/10.1208/s12249-023-02576-x.
[7] Azmi ID, Moghimi SM, Yaghmur A. Cubosomes and hexosomes as versatile platforms for drug delivery. Ther Deliv 2015;6(12):1347–64. https://doi.org/10.4155/tde.15.81.
[8] Mulet X, Boyd BJ, Drummond CJ. Advances in drug delivery and medical imaging using colloidal lyotropic liquid crystalline dispersions. J Colloid Interface Sci 2013;393:1–20. https://doi.org/10.1016/j.jcis.2012.10.014.
[9] Israelachvili JN. Intermolecular and surfaces forces. 2nd ed. Cambridge, MA, USA: Academic Press; 1991.
[10] Holmberg K, Jonsson B, Kronberg B, Lindman B. Surfactants and polymers in aqueous solutions. Chichester, UK: Wiley; 2002.
[11] Hyde S, Andersson S, Larsson K, Blum Z, Landh T, Lidin S, et al. Lipid self-assembly and function in biological systems. In: Mackay AL, editor. The Languange of shape; the role of curvature in condensed matter: Physics, chemistry and Biology. 337. Amsterdam, The Netherlands: Elsevier B. V.; 1997. p. 199–236.
[12] Karami Z, Hamidi M. Cubosomes: Remarkable drug delivery potential. Drug Discov Today 2016;21(5):789–801. https://doi.org/10.1016/j.drudis.2016.01.004.
[13] Chountoulesi M, Pispas S, Tseti IK, Demetzos C. Lyotropic liquid crystalline nanostructures as drug delivery systems and vaccine platforms. Pharmaceuticals 2022;15(4):429. https://doi.org/10.3390/ph15040429.
[14] Li J, Wang X, Zhang T, Wang C, Huang Z, Luo X, et al. A review on phospholipids and their main applications in drug delivery systems. Asian J Pharm Sci 2015;10(2):81–98. https://doi.org/10.1016/j.ajps.2014.09.004.
[15] Nsairat H, Khater D, Sayed U, Odeh F, Al Bawab A, Alshaer W. Liposomes: structure, composition, types, and clinical applications. Heliyon 2022;8(5):e09394. https://doi.org/10.1016/j.heliyon.2022.e09394.
[16] Zhang Y, Sun C, Wang C, Jankovic KE, Dong Y. Lipids and lipid derivatives for RNA delivery. Chem Rev 2021;121(20):12181–277. https://doi.org/10.1021/acs.chemrev.1c00244.
[17] van der Veen JN, Kennelly JP, Wan S, Vance JE, Vance DE, Jacobs RL. The critical role of phosphatidylcholine and phosphatidylethanolamine metabolism in health and disease. Biochim Biophys Acta Biomembr 2017;1859(9):1558–72. https://doi.org/10.1016/j.bbamem.2017.04.006.
[18] van Hoogevest P, Wendel A. The use of natural and synthetic phospholipids as pharmaceutical excipients. Eur J Lipid Sci Technol 2014;116(9):1088–107. https://doi.org/10.1002/ejlt.201400219.
[19] Kulkarni CV, Wachter W, Iglesias-Salto G, Engelskirchen S, Ahualli S. Monoolein: A magic lipid? Phys Chem Chem Phys 2011;13:3004–21. https://doi.org/10.1039/C0CP01539C.
[20] Gyanani V, Goswami R. Key design features of lipid nanoparticles and electrostatic charge-based lipid nanoparticle targeting. Pharmaceutics 2023;15(4):1184. https://doi.org/10.3390/pharmaceutics15041184.
[21] Kauffman KJ, Dorkin JR, Yang JH, Heartlein MW, DeRosa F, Mir FF, et al. Optimization of lipid nanoparticle formulations for mRNA delivery in vivo with fractional factorial and definitive screening designs. Nano Lett 2015;15(11):7300–6. https://doi.org/10.1021/acs.nanolett.5b02497.
[22] Hashemzadeh H, Javadi H, Darvishi MH. Study of structural stability and formation mechanisms in DSPC and DPSM liposomes: a coarse-grained molecular dynamics simulation. Sci Rep 2020;10(1):1837. https://doi.org/10.1038/s41598-020-58730-z.
[23] Kulkarni JA, Myhre JL, Chen S, Tam YYC, Danescu A, Richman JM, et al. Design of lipid nanoparticles for in vitro and in vivo delivery of plasmid DNA. Nanomedicine 2017;13(4):1377–87. https://doi.org/10.1016/j.nano.2016.12.014.
[24] Koltover I, Salditt T, Rädler JO, Safinya CR. An inverted hexagonal phase of cationic liposome-DNA complexes related to DNA release and delivery. Science 1998;281(5373):78–81. https://doi.org/10.1126/science.281.5373.78.
[25] Eygeris Y, Gupta M, Kim J, Sahay G. Chemistry of lipid nanoparticles for RNA delivery. Acc Chem Res 2022;55(1):2–12. https://doi.org/10.1021/acs.accounts.1c00544.
[26] Cheng MHY, Leung J, Zhang Y, Strong C, Basha G, Momeni A, et al. Induction of bleb structures in lipid nanoparticle formulations of mRNA leads to improved

transfection potency. Adv Mater 2023;35(31):e2303370. https://doi.org/10.1002/adma.202303370.
[27] Scriboni AB, Couto VM, Ribeiro LNM, Freires IA, Groppo FC, de Paula E, et al. Fusogenic liposomes increase the antimicrobial activity of vancomycin against staphylococcus aureus biofilm. Front Pharmacol 2019;10:1401. https://doi.org/10.3389/fphar.2019.01401.
[28] Lahooti B, Akwii RG, Zahra FT, Sajib MS, Lamprou M, Alobaida A, et al. Targeting endothelial permeability in the EPR effect. J Control Release 2023;361:212–35. https://doi.org/10.1016/j.jconrel.2023.07.039.
[29] Knop K, Hoogenboom R, Fischer D, Schubert US. Poly(ethylene glycol) in drug delivery: pros and cons as well as potential alternatives. Angew Chem Int Ed Eng 2010;49(36):6288–308. https://doi.org/10.1002/anie.200902672.
[30] Ryals RC, Patel S, Acosta C, McKinney M, Pennesi ME, Sahay G. The effects of PEGylation on LNP based mRNA delivery to the eye. PLoS One 2020;15(10): e0241006. https://doi.org/10.1371/journal.pone.0241006.
[31] Suzuki T, Suzuki Y, Hihara T, Kubara K, Kondo K, Hyodo K, et al. PEG shedding-rate-dependent blood clearance of PEGylated lipid nanoparticles in mice: faster PEG shedding attenuates anti-PEG IgM production. Int J Pharm 2020;588: 119792. https://doi.org/10.1016/j.ijpharm.2020.119792.
[32] Judge A, McClintock K, Phelps JR, Maclachlan I. Hypersensitivity and loss of disease site targeting caused by antibody responses to PEGylated liposomes. Mol Ther 2006;13(2):328–37. https://doi.org/10.1016/j.ymthe.2005.09.014.
[33] Shi D, Beasock D, Fessler A, et al. To PEGylate or not to PEGylate: immunological properties of nanomedicine's most popular component, polyethylene glycol and its alternatives. Adv Drug Deliv Rev 2022;180:114079. https://doi.org/10.1016/j.addr.2021.114079.
[34] Zhang T, Yin H, Li Y, Yang H, Ge K, Zhang J, et al. Optimized lipid nanoparticles (LNPs) for organ-selective nucleic acids delivery *in vivo*. iScience 2024;27(6): 109804. https://doi.org/10.1016/j.isci.2024.109804.
[35] Kim M, Jeong M, Hur S, Cho Y, Park J, Jung H, et al. Engineered ionizable lipid nanoparticles for targeted delivery of RNA therapeutics into different types of cells in the liver. Sci Adv 2021;7(9):eabf4398. https://doi.org/10.1126/sciadv.abf4398.
[36] Samaridou E, Heyes J, Lutwyche P. Lipid nanoparticles for nucleic acid delivery: current perspectives. Adv Drug Deliv Rev 2020;154-155:37–63. https://doi.org/10.1016/j.addr.2020.06.002.
[37] Semple SC, Chonn A, Cullis PR. Influence of cholesterol on the association of plasma proteins with liposomes. Biochemistry 1996;35(8):2521–5. https://doi.org/10.1021/bi950414i.
[38] Krause MR, Regen SL. The structural role of cholesterol in cell membranes: from condensed bilayers to lipid rafts. Acc Chem Res 2014;47(12):3512–21. https://doi.org/10.1021/ar500260t.
[39] Kulkarni JA, Witzigmann D, Leung J, Tam YYC, Cullis PR. On the role of helper lipids in lipid nanoparticle formulations of siRNA. Nanoscale 2019;11:21733–9. https://doi.org/10.1039/C9NR09347H.
[40] Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. Nat Rev Mater 2021;6:1078–94. https://doi.org/10.1038/s41578-021-00358-0.
[41] Guéguen C, Ben Chimol T, Briand M, Renaud K, Seiler M, Ziesel M, et al. Evaluating how cationic lipid affects mRNA-LNP physical properties and biodistribution. Eur J Pharm Biopharm 2024;195:114077. https://doi.org/10.1016/j.ejpb.2023.08.002.
[42] Kedmi R, Ben-Arie N, Peer D. The systemic toxicity of positively charged lipid nanoparticles and the role of toll-like receptor 4 in immune activation. Biomaterials 2010;31(26):6867–75. https://doi.org/10.1016/j.biomaterials.2010.05.027.
[43] Landesman-Milo D, Peer D. Toxicity profiling of several common RNAi-based nanomedicines: a comparative study. Drug Deliv Transl Res 2014;4(1):96–103. https://doi.org/10.1007/s13346-013-0158-7.
[44] Lou G, Anderluzzi G, Schmidt ST, Woods S, Gallorini S, Brazzoli M, et al. Delivery of self-amplifying mRNA vaccines by cationic lipid nanoparticles: the impact of cationic lipid selection. J Control Release 2020;325:370–9. https://doi.org/10.1016/j.jconrel.2020.06.027.
[45] Blakney AK, McKay PF, Yus BI, Aldon Y, Shattock RJ. Inside out: optimization of lipid nanoparticle formulations for exterior complexation and in vivo delivery of saRNA. Gene Ther 2019;26(9):363–72. https://doi.org/10.1038/s41434-019-0095-2.
[46] Zhi D, Bai Y, Yang J, Cui S, Zhao Y, Chen H, et al. A review on cationic lipids with different linkers for gene delivery. Adv Colloid Interf Sci 2018;253:117–40. https://doi.org/10.1016/j.cis.2017.12.006.
[47] Rietwyk S, Peer D. Next-generation lipids in RNA interference therapeutics. ACS Nano 2017;11(8):7572–86. https://doi.org/10.1021/acsnano.7b04734.
[48] Sun D, Lu ZR. Structure and function of cationic and ionizable lipids for nucleic acid delivery [published correction appears in pharm res. 2024 jul;41(7): 1543-1545. Doi: 10.1007/s11095-024-03714-1]. Pharm Res 2023;40(1):27–46. https://doi.org/10.1007/s11095-022-03460-2.
[49] Carrasco MJ, Alishetty S, Alameh MG, Said H, Wright L, Paige M, et al. Ionization and structural properties of mRNA lipid nanoparticles influence expression in intramuscular and intravascular administration. Commun Biol 2021;4(1):956. https://doi.org/10.1038/s42003-021-02441-2.
[50] Sabnis S, Kumarasinghe ES, Salerno T, Mihai C, Ketova T, Senn JJ, et al. A novel amino lipid series for mRNA delivery: improved endosomal escape and sustained pharmacology and safety in non-human primates. Mol Ther 2018;26(6):1509–19. https://doi.org/10.1016/j.ymthe.2018.03.010.
[51] Hald Albertsen C, Kulkarni JA, Witzigmann D, Lind M, Petersson K, Simonsen JB. The role of lipid components in lipid nanoparticles for vaccines and gene therapy. Adv Drug Deliv Rev 2022;188:114416. https://doi.org/10.1016/j.addr.2022.114416.
[52] Zou Y, Zhou Q, Zhao Y, Zhi D, Chen H, Wang R, et al. Structure-activity relationships of pH-responsive and ionizable lipids for gene delivery. Int J Pharm 2022;617:121596. https://doi.org/10.1016/j.ijpharm.2022.121596.
[53] Tenchov R, Bird R, Curtze AE, Zhou Q. Lipid nanoparticles—from liposomes to mRNA vaccine delivery, a landscape of research diversity and advancement. ACS Nano 2021;15(11):16982–7015. https://doi.org/10.1021/acsnano.1c04996.
[54] Severino P, Andreani T, Macedo AS, Fangueiro JF, Santana MH, Silva AM, et al. Current state-of-art and new trends on lipid nanoparticles (SLN and NLC) for oral drug delivery. J Drug Deliv 2012;2012:750891. https://doi.org/10.1155/2012/750891.
[55] Mehta M, Bui TA, Yang X, Aksoy Y, Goldys EM, Deng W. Lipid-based nanoparticles for drug/gene delivery: an overview of the production techniques and difficulties encountered in their industrial development. ACS Mater Au 2023; 3(6):600–19. https://doi.org/10.1021/acsmaterialsau.3c00032.
[56] Gao Y, Zhou D, Lyu J, Xu Q, Newland B, Matyjaszewski K, et al. Complex polymer architectures through free-radical polymerization of multivinyl monomers. Nat Rev Chem 2020;4(4):194–212. https://doi.org/10.1038/s41570-020-0170-7.
[57] Li X, Mastan E, Wang W-J, Li B-G, Zhu S. Progress in reactor engineering of controlled radical polymerization: a comprehensive review. Reaction Chemistry & Engineering 2016;1(1):23–59. https://doi.org/10.1039/C5RE00044K.
[58] Beyazit S, Bui BTS, Haupt K, Gonzato C. Molecularly imprinted polymer nanomaterials and nanocomposites by controlled/living radical polymerization. Prog Polym Sci 2016;62:1–21. https://doi.org/10.1016/j.progpolymsci.2016.04.001.
[59] Feng H, Lu X, Wang W, Kang N-G, Mays JW. Block copolymers: synthesis, self-assembly, and applications. Polymers 2017;9(10):494. https://doi.org/10.3390/polym9100494.
[60] Karayianni M, Pispas S. Block copolymer solution self-assembly: recent advances, emerging trends, and applications. J Polym Sci 2021;59(17):1874–98. https://doi.org/10.1002/pol.20210430.
[61] Xu Z, Dong Q, Li W. Architectural design of block copolymers. Macromolecules 2024;57(5):1869–84. https://doi.org/10.1021/acs.macromol.3c01730.
[62] Selianitis D, Katifelis H, Gazouli M, Pispas S. Novel multi-responsive hyperbranched polyelectrolyte polyplexes as potential gene delivery vectors. Pharmaceutics 2023;15(6):1627. https://doi.org/10.3390/pharmaceutics15061627.
[63] Zhang H, Pan Y, Li Y, Li C, Wang Y, Jiang W, et al. Hierarchical self-assembly of hyperbranched polymer-based topological supramolecular amphiphiles. Chem Eur J 2024:e202402231. https://doi.org/10.1002/chem.202402231.
[64] Hartmann F, Dockhorn R, Pusse S, Niebuur B-J, Koch M, Kraus T, et al. Design and self-assembly of second-generation dendrimer-like block copolymers. Macromolecules 2024;57(15):7098–111. https://doi.org/10.1021/acs.macromol.4c00944.
[65] Stefanovic S. Development of functional star-shaped polypeptides for biomedical applications: Royal College of Surgeons in Ireland. 2023.
[66] Lim C, Blocher McTigue WC. Form equals function: influence of coacervate architecture on drug delivery applications. ACS Biomater Sci Eng 2024. https://doi.org/10.1021/acsbiomaterials.4c01105.
[67] Palmiero UC, Sponchioni M, Manfredini N, Maraldi M, Moscatelli D. Strategies to combine ROP with ATRP or RAFT polymerization for the synthesis of biodegradable polymeric nanoparticles for biomedical applications. Polym Chem 2018;9(30):4084–99. https://doi.org/10.1039/C8PY00649K.
[68] Di Leone S, Avsar SY, Belluati A, Wehr R, Palivan CG, Meier W. Polymer–lipid hybrid membranes as a model platform to drive membrane–cytochrome c interaction and peroxidase-like activity. J Phys Chem B 2020;124(22):4454–65. https://doi.org/10.1021/acs.jpcb.0c02727.
[69] Son I, Lee Y, Baek J, Park M, Han D, Min SK, et al. PH-responsive amphiphilic polyether micelles with superior stability for smart drug delivery. Biomacromolecules 2021;22(5):2043–56. https://doi.org/10.1021/acs.biomac.1c00163.
[70] Yang C, Liu SQ, Venkataraman S, Gao SJ, Ke X, Chia XT, et al. Structure-directing star-shaped block copolymers: supramolecular vesicles for the delivery of anticancer drugs. J Control Release 2015;208:93–105. https://doi.org/10.1016/j.jconrel.2015.03.027.
[71] Cobo I, Li M, Sumerlin BS, Perrier S. Smart hybrid materials by conjugation of responsive polymers to biomacromolecules. Nat Mater 2015;14(2):143–59. https://doi.org/10.1038/nmat4106.
[72] Qiao X, Dugas P-Y, Charleux B, Lansalot M, Bourgeat-Lami E. Nitroxide-mediated polymerization-induced self-assembly of amphiphilic block copolymers with a pH/temperature dual sensitive stabilizer block. Polym Chem 2017;8(27): 4014–29. https://doi.org/10.1039/c7py00595d.
[73] Acar MH, Matyjaszewski K. Block copolymers by transformation of living anionic polymerization into controlled/"living" atom transfer radical polymerization. Macromol Chem Phys 1999;200(5):1094–100.
[74] Ding A, Lu G, Guo H, Huang X. ATRP synthesis of polyallene-based amphiphilic triblock copolymer. Polym Chem 2017;8(45):6997–7008. https://doi.org/10.1039/C7PY01666B.
[75] Huang L-m, Li L-d, Shang L, Zhou Q-h, Lin J. Preparation of pH-sensitive micelles from miktoarm star block copolymers by ATRP and their application as drug nanocarriers. React Funct Polym 2016;107:28–34. https://doi.org/10.1016/j.reactfunctpolym.2016.08.005.
[76] Cao H, Chen C, Xie D, Chen X, Wang P, Wang Y, et al. A hyperbranched amphiphilic acetal polymer for pH-sensitive drug delivery. Polym Chem 2018;9 (2):169–77. https://doi.org/10.1039/C7PY01739A.

[77] Tomara M, Selianitis D, Pispas S. Dual-responsive amphiphilic p (DMAEMA-co-LMA-co-OEGMA) terpolymer Nano-assemblies in aqueous media. Nanomaterials 2022;12(21):3791. https://doi.org/10.3390/nano12213791.
[78] Selianitis D, Pispas S. Multi-responsive poly (oligo (ethylene glycol) methyl methacrylate)-co-poly (2-(diisopropylamino) ethyl methacrylate) hyperbranched copolymers via reversible addition fragmentation chain transfer polymerization. Polym Chem 2021;12(45):6582–93. https://doi.org/10.1039/D1PY01320C.
[79] Leer K, Reichel LS, Wilhelmi M, Brendel JC, Traeger A. Tailoring gene transfer efficacy through the arrangement of cationic and anionic blocks in triblock copolymer micelles. ACS Macro Lett 2024;13:158–65. https://doi.org/10.1021/acsmacrolett.3c00633.
[80] Saha R, Haldar S, Pradhan SS, Jana K, Sarkar K. Superior gene transfection efficiency in triple negative breast cancer by RAFT-mediated amino acid-based cationic diblock copolymers. J Mater Chem B 2023;11(16):3617–34. https://doi.org/10.1039/D2TB02681C.
[81] Herfurth C, Laschewsky A, Noirez L, von Lospichl B, Gradzielski M. Thermoresponsive (star) block copolymers from one-pot sequential RAFT polymerizations and their self-assembly in aqueous solution. Polymer 2016;107:422–33. https://doi.org/10.1016/j.polymer.2016.09.089.
[82] Arshad M, Pradhan RA, Ullah A. Synthesis of lipid-based amphiphilic block copolymer and its evaluation as nano drug carrier. Mater Sci Eng C 2017;76:217–23. https://doi.org/10.1016/j.msec.2017.03.109.
[83] Pippa N, Lagopati N, Forys A, Chountoulesi M, Katifelis H, Chrysostomou V, et al. Aqueous heat method for the preparation of hybrid lipid–polymer structures: from preformulation studies to protein delivery. Biomedicines 2022;10(6):1228. https://doi.org/10.3390/biomedicines10061228.
[84] Schulz M, Binder WH. Mixed hybrid lipid/polymer vesicles as a novel membrane platform. Macromol Rapid Commun 2015;36(23):2031–41. https://doi.org/10.1002/marc.201500344.
[85] Hadinoto K, Sundaresan A, Cheow WS. Lipid-polymer hybrid nanoparticles as a new generation therapeutic delivery platform: a review. Eur J Pharm Biopharm 2013;85(3 Pt A):427–43. https://doi.org/10.1016/j.ejpb.2013.07.002.
[86] Gameiro M, Mano JF, Gaspar VM. Emerging lipid–polymer hybrid nanoparticles for genome editing. Polym Chem 2024;15:3436–68. https://doi.org/10.1039/D4PY00298A.
[87] Bochicchio S, Lamberti G, Barba AA. Polymer-lipid pharmaceutical nanocarriers: innovations by new formulations and production technologies. Pharmaceutics 2021;13(2):198. https://doi.org/10.3390/pharmaceutics13020198.
[88] Zhang L, Zhang L. Lipid–Polymer hybrid nanoparticles: synthesis, characterization and applications. Nano Life 2010;01:163–73. https://doi.org/10.1142/S179398441000016X.
[89] Shah S, Famta P, Raghuvanshi RS, Singh SB, Srivastava S. Lipid polymer hybrid nanocarriers: insights into synthesis aspects, characterization, release mechanisms, surface functionalization and potential implications. Colloid Interface Sci Commun 2022;46:100570. https://doi.org/10.1016/j.colcom.2021.100570.
[90] Sengel-Turk CT, Gumustas M, Uslu B, Ozkan SA. A novel approach for drug targeting. In: Design of Nanostructures for Theranostics applications. Elsevier; 2018. p. 69–107. https://doi.org/10.1016/B978-0-12-813669-0.00003-8.
[91] Sengel-Turk CT, Paksoy AO, Alpturk O. The state of the art in core–shell-type lipid–polymer hybrid nanocarriers and beyond. Polym Bull 2024;81:4771–800. https://doi.org/10.1007/s00289-023-04951-x.
[92] Elkateb H, Tatham LM, Cauldbeck H, Niezabitowska E, Owen A, Rannard S, et al. Optimization of the synthetic parameters of lipid polymer hybrid nanoparticles dual loaded with darunavir and ritonavir for the treatment of HIV. Int J Pharm 2020;588:119794. https://doi.org/10.1016/j.ijpharm.2020.119794.
[93] Hussain MT, Tiboni M, Perrie Y, Casettari L. Microfluidic production of protein loaded chimeric stealth liposomes. Int J Pharm 2020;590:119955. https://doi.org/10.1016/j.ijpharm.2020.119955.
[94] Tao W, Xie P, Huang C, Wang Y, Huang Y, Yin Z. Construction of PLGA nanoparticles modified with RWrNM and DLPC and their application in acute rhinosinusitis. Drug Deliv Transl Res 2024;14(4):1063–76. https://doi.org/10.1007/s13346-023-01450-4.
[95] Pramual S, Lirdprapamongkol K, Jouan-Hureaux V, et al. Overcoming the diverse mechanisms of multidrug resistance in lung cancer cells by photodynamic therapy using pTHPP-loaded PLGA-lipid hybrid nanoparticles. Eur J Pharm Biopharm 2020;149:218–28. https://doi.org/10.1016/j.ejpb.2020.02.012.
[96] Huang H, Dong Y, Zhang Y, Ru D, Wu Z, Zhang J, et al. GSH-sensitive Pt(IV) prodrug-loaded phase-transitional nanoparticles with a hybrid lipid-polymer shell for precise theranostics against ovarian cancer. Theranostics 2019;9(4):1047–65. https://doi.org/10.7150/thno.29820.
[97] Shueng PW, Yu LY, Hou HH, Chiu HC, Lo CL. Charge conversion polymer-liposome complexes to overcome the limitations of cationic liposomes in mitochondrial-targeting drug delivery. Int J Mol Sci 2022;23(6):3080. https://doi.org/10.3390/ijms23063080.
[98] Markowski A, Jaromin A, Migdał P, Olczak E, Zygmunt A, Zaremba-Czogalla M, et al. Design and development of a new type of hybrid PLGA/lipid nanoparticle as an ursolic acid delivery system against pancreatic ductal adenocarcinoma cells. Int J Mol Sci 2022;23(10):5536. https://doi.org/10.3390/ijms23105536.
[99] Zahiri M, Taghdisi SM, Abnous K, Zolfaghari R, Ramezani M, Alibolandi M. Marriage of phospholipid and block copolymer in lipopolymersome hybrid structure for efficient tumor accumulation. Int J Pharm 2020;591:120030. https://doi.org/10.1016/j.ijpharm.2020.120030.
[100] Andreana I, Chiapasco M, Bincoletto V, Digiovanni S, Manzoli M, Ricci C, et al. Targeting pentamidine towards CD44-overexpressing cells using hyaluronated lipid-polymer hybrid nanoparticles. Drug Deliv Transl Res 2024;14:2100–11. https://doi.org/10.1007/s13346-024-01617-7.
[101] Yu F, Zhang F. A feasible strategy of fabricating hybrid drugs co-loaded polymer-lipid nanoparticles for the treatment of nasopharyngeal cancer therapy. Process Biochem 2021;111:78–86. https://doi.org/10.1016/j.procbio.2021.08.027.
[102] Mineart KP, Venkataraman S, Yang YY, Hedrick JL, Prabhu VM. Fabrication and characterization of hybrid stealth liposomes. Macromolecules 2018;51(8):3184–92. https://doi.org/10.1021/acs.macromol.8b00361.
[103] Zong W, Thingholm B, Itel F, Schattling PS, Brodszkij E, Mayer D, et al. Phospholipid-block copolymer hybrid vesicles with lysosomal escape ability. Langmuir 2018;34(23):6874–86. https://doi.org/10.1021/acs.langmuir.8b01073.
[104] Miao R, Jin F, Wang Z, Lu W, Liu J, Li X, et al. Oral delivery of decanoic acid conjugated plant protein shell incorporating hybrid nanosystem leverage intestinal absorption of polyphenols. Biomaterials 2022;281:121373. https://doi.org/10.1016/j.biomaterials.2022.121373.
[105] Arruda DC, Lachagès AM, Demory H, Escriou G, Lai-Kuen R, Dugas PY, et al. Spheroplexes: hybrid PLGA-cationic lipid nanoparticles, for in vitro and oral delivery of siRNA. J Control Release 2022;350:228–43. https://doi.org/10.1016/j.jconrel.2022.08.030.
[106] Cardellini J, Balestri A, Comparini L, Lonetti B, Brucale M, Valle F, et al. Controlling plasmonic suprastructures through self-assembly of gold nanoparticles with hybrid copolymer-lipid vesicles. J Colloid Interface Sci 2024;654(Pt B):848–58. https://doi.org/10.1016/j.jcis.2023.10.082.
[107] Magnani C, Montis C, Mangiapia G, Mingotaud AF, Mingotaud C, Roux C, et al. Hybrid vesicles from lipids and block copolymers: phase behavior from the micro- to the nano-scale. Colloids Surf B: Biointerfaces 2018;168:18–28. https://doi.org/10.1016/j.colsurfb.2018.01.042.
[108] de Freitas CF, Calori IR, da Silva ACP, de Castro LV, Sato F, Silva Pellosi D, et al. PEG-coated vesicles from pluronic/lipid mixtures for the carrying of photoactive erythrosine derivatives. Colloids Surf B: Biointerfaces 2019;175:530–44. https://doi.org/10.1016/j.colsurfb.2018.12.031.
[109] Patel V, Lalani R, Vhora I, Bardoliwala D, Patel A, Ghosh S, et al. Co-delivery of cisplatin and siRNA through hybrid nanocarrier platform for masking resistance to chemotherapy in lung cancer. Drug Deliv Transl Res 2021;11(5):2052–71. https://doi.org/10.1007/s13346-020-00867-5.
[110] Go YK, Kambar N, Leal C. Hybrid unilamellar vesicles of phospholipids and block copolymers with crystalline domains. Polymers 2020;12(6):1232. https://doi.org/10.3390/polym12061232.
[111] Craparo EF, Cabibbo M, Scialabba C, Giammona G, Cavallaro G. Inhalable formulation based on lipid-polymer hybrid nanoparticles for the macrophage targeted delivery of roflumilast. Biomacromolecules 2022;23(8):3439–51. https://doi.org/10.1021/acs.biomac.2c00576.
[112] rivedi S, Belgamwar V. Fabrication and optimization of chitosan-g-m-PEG-NH_2 copolymer for advanced glioblastoma therapy using surface engineered lentinan loaded nanovesicles for nasal delivery. Int J Biol Macromol 2024;273(2):133125. https://doi.org/10.1016/j.ijbiomac.2024.133125.
[113] Ren T, Zheng X, Bai R, Yang Y, Jian L. Bioadhesive poly(methyl vinyl ether-co-maleic anhydride)-TPGS copolymer modified PLGA/lipid hybrid nanoparticles for improving intestinal absorption of cabazitaxel. Int J Pharm 2022;611:121301. https://doi.org/10.1016/j.ijpharm.2021.121301.
[114] Sonawane SJ, Kalhapure RS, Rambharose S, Mocktar C, Vepuri SB, Soliman M, et al. Ultra-small lipid-dendrimer hybrid nanoparticles as a promising strategy for antibiotic delivery: in vitro and in silico studies. Int J Pharm 2016;504(1–2):1–10. https://doi.org/10.1016/j.ijpharm.2016.03.021.
[115] Tahir N, Madni A, Li W, Correia A, Khan MM, Rahim MA, et al. Microfluidic fabrication and characterization of sorafenib-loaded lipid-polymer hybrid nanoparticles for controlled drug delivery. Int J Pharm 2020;581:119275. https://doi.org/10.1016/j.ijpharm.2020.119275.
[116] Wilhelm Romero K, Quirós MI, Vargas Huertas F, Vega-Baudrit JR, Navarro-Hoyos M, Araya-Sibaja AM. Design of hybrid polymeric-lipid nanoparticles using curcumin as a model: preparation, characterization, and in vitro evaluation of demethoxycurcumin and bisdemethoxycurcumin-loaded nanoparticles. Polymers 2021;13(23):4207. https://doi.org/10.3390/polym13234207.
[117] Chatterjee S, Ooya T. Copolymers composed of 2-(Methacryloyloxy)ethyl phosphorylcholine and methacrylated polyhedral oligomeric silsesquioxane as a simple modifier for liposomes. ACS Appl Polym Mater 2020;2:1909–16. https://doi.org/10.1021/acsapm.0c00129.
[118] Monirinasab H, Asadi H, Rostamizadeh K, Esmaeilzadeh A, Khodaei M, Fathi M. Novel lipid-polymer hybrid nanoparticles for SiRNA delivery and IGF-1R gene silencing in breast cancer cells. J Drug Delivery Sci Technol 2018;48:96–105. https://doi.org/10.1016/j.jddst.2018.08.025.
[119] Das L, Kaurav M, Pandey RS. Phospholipid-polymer hybrid nanoparticle-mediated transfollicular delivery of quercetin: prospective implement for the treatment of androgenic alopecia. Drug Dev Ind Pharm 2019;45(10):1654–63. https://doi.org/10.1080/03639045.2019.1652635.
[120] Omar SH, Osman R, Mamdouh W, Abdel-Bar HM, Awad GAS. Bioinspired lipid-polysaccharide modified hybrid nanoparticles as a brain-targeted highly loaded carrier for a hydrophilic drug. Int J Biol Macromol 2020;165(Pt A):483–94. https://doi.org/10.1016/j.ijbiomac.2020.09.170.
[121] Khan MA, Khan S, Kazi M, Alshehri SM, Shahid M, Khan SU, et al. Norfloxacin loaded lipid polymer hybrid nanoparticles for oral administration: fabrication, characterization, in silico modelling and toxicity evaluation. Pharmaceutics 2021;13(10):1632. https://doi.org/10.3390/pharmaceutics13101632.
[122] Pu X, Wang Y, Wang X, Sang X, Jiang M, Qi D, et al. Lipids extracted from mycobacterial membrane and enveloped PLGA nanoparticles for encapsulating

antibacterial drugs elicit synergistic antimicrobial response against mycobacteria. Mol Pharm 2024;21(5):2238–49. https://doi.org/10.1021/acs.molpharmaceut.3c01001.
[123] Chiang Y-T, Lyu S-Y, Wen Y-H, Lo C-L. Preparation and characterization of electrostatically crosslinked polymer–liposomes in anticancer therapy. Int J Mol Sci 2018;19(6):1615. https://doi.org/10.3390/ijms19061615.
[124] Men W, Zhu P, Dong S, Liu W, Zhou K, Bai Y, et al. Fabrication of dual pH/redox-responsive lipid-polymer hybrid nanoparticles for anticancer drug delivery and controlled release. Int J Nanomedicine 2019;14:8001–11. https://doi.org/10.2147/IJN.S226798.
[125] Fraix A, Conte C, Gazzano E, Riganti C, Quaglia F, Sortino S. Overcoming doxorubicin resistance with lipid-polymer hybrid nanoparticles photoreleasing nitric oxide. Mol Pharm 2020;17(6):2135–44. https://doi.org/10.1021/acs.molpharmaceut.0c00290.
[126] Tahir N, Madni A, Correia A, Rehman M, Balasubramanian V, Khan MM, et al. Lipid-polymer hybrid nanoparticles for controlled delivery of hydrophilic and lipophilic doxorubicin for breast cancer therapy. Int J Nanomedicine 2019;14:4961–74. https://doi.org/10.2147/IJN.S209325.
[127] Craparo EF, Cabibbo M, Scialabba C, Casula L, Lai F, Cavallaro G. Rapamycin-based inhaled therapy for potential treatment of COPD-related inflammation: production and characterization of aerosolizable nano into micro (NiM) particles. Biomater Sci 2024;12(2):387–401. https://doi.org/10.1039/d3bm01210g.
[128] Bersani S, Vila-Caballer M, Brazzale C, Barattin M, Salmaso S. PH-sensitive stearoyl-PEG-poly(methacryloyl sulfadimethoxine) decorated liposomes for the delivery of gemcitabine to cancer cells. Eur J Pharm Biopharm 2014;88(3):670–82. https://doi.org/10.1016/j.ejpb.2014.08.005.
[129] Comegna M, Conte G, Falanga AP, Marzano M, Cernera G, Di Lullo AM, et al. Assisting PNA transport through cystic fibrosis human airway epithelia with biodegradable hybrid lipid-polymer nanoparticles. Sci Rep 2021;11(1):6393. https://doi.org/10.1038/s41598-021-85549-z.
[130] Ni R, Huang L, Li Z, Zhang W, Wang Y, Shen Y, et al. Multifunctional ROS-responsive and TME-modulated lipid-polymer hybrid nanoparticles for enhanced tumor penetration. Int J Nanomedicine 2022;17:5883–97. https://doi.org/10.2147/IJN.S383517.
[131] Huang L, Himawan E, Belhadj S, Pérez García RO, Paquet Durand F, Schipper N, et al. Efficient delivery of hydrophilic small molecules to retinal cell lines using gel Core-containing solid lipid nanoparticles. Pharmaceutics 2021;14(1):74. https://doi.org/10.3390/pharmaceutics14010074.
[132] Sakpakdeejaroen I, Somani S, Laskar P, Mullin M, Dufès C. Regression of melanoma following intravenous injection of plumbagin entrapped in transferrin-conjugated, lipid-polymer hybrid nanoparticles. Int J Nanomedicine 2021;16:2615–31. https://doi.org/10.2147/IJN.S293480.
[133] Yalcin TE, Ilbasmis-Tamer S, Takka S. Antitumor activity of gemcitabine hydrochloride loaded lipid polymer hybrid nanoparticles (LPHNs): in vitro and in vivo. Int J Pharm 2020;580:119246. https://doi.org/10.1016/j.ijpharm.2020.119246.
[134] Khodaei M, Rostamizadeh K, Taromchi AH, Monirinasab H, Fathi M. DDAB cationic lipid-mPEG, PCL copolymer hybrid nano-carrier synthesis and application for delivery of siRNA targeting IGF-1R into breast cancer cells. Clin Transl Oncol 2021;23(6):1167–78. https://doi.org/10.1007/s12094-020-02507-3.
[135] Yang P, Zhang L, Wang T, Liu Q, Wang J, Wang Y, et al. Doxorubicin and edelfosine combo-loaded lipid-polymer hybrid nanoparticles for synergistic anticancer effect against drug-resistant osteosarcoma. Onco Targets Ther 2020;13:8055–67. https://doi.org/10.2147/OTT.S259428.
[136] Gao Y, Men K, Pan C, Li J, Wu J, Chen X, et al. Functionalized DMP-039 hybrid nanoparticle as a novel mRNA vector for efficient cancer suicide gene therapy. Int J Nanomedicine 2021;16:5211–32. https://doi.org/10.2147/IJN.S319092.
[137] Patel G, Thakur NS, Kushwah V, Patil MD, Nile SH, Jain S, et al. Mycophenolate co-administration with quercetin via lipid-polymer hybrid nanoparticles for enhanced breast cancer management. Nanomedicine 2020;24:102147. https://doi.org/10.1016/j.nano.2019.102147.
[138] Pan C, Zhang T, Li S, Xu Z, Pan B, Xu S, et al. Hybrid nanoparticles modified by hyaluronic acid loading an HSP90 inhibitor as a novel delivery system for subcutaneous and orthotopic colon cancer therapy. Int J Nanomedicine 2021;16:1743–55. https://doi.org/10.2147/IJN.S275805.
[139] Kokuryo D, Nakashima S, Ozaki F, Yuba E, Chuang KH, Aoshima S, et al. Evaluation of thermo-triggered drug release in intramuscular-transplanted tumors using thermosensitive polymer-modified liposomes and MRI. Nanomedicine 2015;11(1):229–38. https://doi.org/10.1016/j.nano.2014.09.001.
[140] Men K, Huang R, Zhang X, Zhang R, Zhang Y, He M, et al. Local and systemic delivery of interleukin-12 gene by cationic micelles for cancer immunogene therapy. J Biomed Nanotechnol 2018;14(10):1719–30. https://doi.org/10.1166/jbn.2018.2593.
[141] Lang T, Liu Y, Zheng Z, Ran W, Zhai Y, Yin Q, et al. Cocktail strategy based on Spatio-temporally controlled nano device improves therapy of breast cancer. Adv Mater 2019;31(5):e1806202. https://doi.org/10.1002/adma.201806202.
[142] Li J, Chen B, Yu T, Guo M, Zhao S, Zhang Y, et al. An efficient controlled release strategy for hypertension therapy: folate-mediated lipid nanoparticles for oral peptide delivery. Pharmacol Res 2020;157:104796. https://doi.org/10.1016/j.phrs.2020.104796.
[143] Su FY, Chen J, Son HN, Kelly AM, Convertine AJ, West TE, et al. Polymer-augmented liposomes enhancing antibiotic delivery against intracellular infections. Biomater Sci 2018;6(7):1976–85. https://doi.org/10.1039/c8bm00282g.
[144] Zhang H, Xu J, Gao B, Wang H, Huang J, Zhou J, et al. Synergistic cascade strategy based on modifying tumor microenvironment for enhanced breast cancer therapy. Front Pharmacol 2021;12:750847. https://doi.org/10.3389/fphar.2021.750847.
[145] Sun J, Zhang L, Wang J, Feng Q, Liu D, Yin Q, et al. Tunable rigidity of (polymeric core)-(lipid shell) nanoparticles for regulated cellular uptake. Adv Mater 2015;27(8):1402–7. https://doi.org/10.1002/adma.201404788.
[146] Jaglal Y, Osman N, Omolo CA, Mocktar C, Devnarain N, Govender T. Formulation of PH-responsive lipid-polymer hybrid nanoparticles for co-delivery and enhancement of the antibacterial activity of vancomycin and 18β-Glycyrrhetinic acid. J Drug Delivery Sci Technol 2021;64:102607. https://doi.org/10.1016/j.jddst.2021.102607.
[147] Li Y, Abbaspour MR, Grootendorst PV, Rauth AM, Wu XY. Optimization of controlled release nanoparticle formulation of verapamil hydrochloride using artificial neural networks with genetic algorithm and response surface methodology. Eur J Pharm Biopharm 2015;94:170–9. https://doi.org/10.1016/j.ejpb.2015.04.028.
[148] de Groot AM, Thanki K, Gangloff M, Falkenberg E, Zeng X, van Bijnen DCJ, et al. Immunogenicity testing of lipidoids in vitro and in silico: modulating Lipidoid-mediated TLR4 activation by nanoparticle design. Mol Ther Nucleic Acids 2018;11:159–69. https://doi.org/10.1016/j.omtn.2018.02.003.
[149] Kasif M, Gupta R, Singh PP, Bhardwaj P, Goyal R, Bansal KK, et al. Development of biocompatible lipid-polymer hybrid nanoparticles for enhanced oral absorption of posaconazole: a mechanistic in vitro and in silico assessment. J Drug Delivery Sci Technol 2024;101:106109. https://doi.org/10.1016/j.jddst.2024.106109.
[150] Agha A, Waheed W, Stiharu I, Nerguizian V, Destgeer G, Abu-Nada E, et al. A review on microfluidic-assisted nanoparticle synthesis, and their applications using multiscale simulation methods. Discov Nano 2023;18(1):18. https://doi.org/10.1186/s11671-023-03792-x.
[151] Shen W, Hu J, Hu X. Impact of amphiphilic triblock copolymers on stability and permeability of phospholipid/polymer hybrid vesicles. Chem Phys Lett 2014;600:56–61. https://doi.org/10.1016/j.cplett.2014.03.057.
[152] Dao TPT, Fernandes F, Er-Rafik M, Salva R, Schmutz M, Brûlet A, et al. Phase separation and nanodomain formation in hybrid polymer/lipid vesicles. ACS Macro Lett 2015;4(2):182–6. https://doi.org/10.1021/mz500748f.
[153] Dao TP, Brûlet A, Fernandes F, Er-Rafik M, Ferji K, Schweins R, et al. Mixing block copolymers with phospholipids at the nanoscale: from hybrid polymer/lipid wormlike micelles to vesicles presenting lipid nanodomains. Langmuir 2017;33(7):1705–15. https://doi.org/10.1021/acs.langmuir.6b04478.
[154] Fauquignon M, Courtecuisse E, Josselin R, Mutschler A, Brûlet A, Schmutz M, et al. Large hybrid polymer/lipid unilamellar vesicle (LHUV) at the nanoscale: an insight into the lipid distribution in the membrane and permeability control. J Colloid Interface Sci 2021;604:575–83. https://doi.org/10.1016/j.jcis.2021.06.172.
[155] Flandez K, Bonardd S, Soto-Arriaza M. Physicochemical properties of l-alpha dipalmitoyl phosphatidylcholine large unilamellar vesicles: effect of hydrophobic block (PLA/PCL) of amphipathic diblock copolymers. Chem Phys Lipids 2020;230:104927. https://doi.org/10.1016/j.chemphyslip.2020.104927.
[156] Palominos MA, Vilches D, Bossel E, Soto-Arriaza MA. Interaction between amphipathic triblock copolymers and l-α-dipalmitoyl phosphatidylcholine large unilamellar vesicles. Colloids Surf B: Biointerfaces 2016;148:30–40. https://doi.org/10.1016/j.colsurfb.2016.08.038.
[157] Khan AK, Ho JCS, Roy S, Liedberg B, Nallani M. Facile mixing of phospholipids promotes self-assembly of low-molecular-weight biodegradable block co-polymers into functional vesicular architectures. Polymers 2020;12(4):979. https://doi.org/10.3390/polym12040979.
[158] Sugimoto T, Yamazaki N, Hayashi T, Yuba E, Harada A, Kotaka A, et al. Preparation of dual-stimuli-responsive liposomes using methacrylate-based copolymers with pH and temperature sensitivities for precisely controlled release. Colloids Surf B: Biointerfaces 2017;155:449–58. https://doi.org/10.1016/j.colsurfb.2017.04.043.
[159] Bixner O, Bello G, Virk M, Kurzhals S, Scheberl A, Gal N, et al. Magneto-thermal release from nanoscale unilamellar hybrid vesicles. ChemNanoMat 2016;2:1111. https://doi.org/10.1002/cnma.201600278.
[160] Mumtaz Virk M, Reimhult E. Phospholipase A2-induced degradation and release from lipid-containing polymersomes. Langmuir 2018;34(1):395–405. https://doi.org/10.1021/acs.langmuir.7b03893.
[161] Aoki I, Yoneyama M, Hirose J, Minemoto Y, Koyama T, Kokuryo D, et al. Thermoactivatable polymer-grafted liposomes for low-invasive image-guided chemotherapy. Transl Res 2015;166(6):660–673.e1. https://doi.org/10.1016/j.trsl.2015.07.009.
[162] Pedroso de Morais F Amanda, Sonchini Gonçalves R, Souza Campanholi K, et al. Photophysical characterization of hypericin-loaded in micellar, liposomal and copolymer-lipid nanostructures based f127 and DPPC liposomes. Spectrochim Acta A Mol Biomol Spectrosc 2021;248:119173. https://doi.org/10.1016/j.saa.2020.119173.
[163] De Leo V, Ruscigno S, Trapani A, Di Gioia S, Milano F, Mandracchia D, et al. Preparation of drug-loaded small unilamellar liposomes and evaluation of their potential for the treatment of chronic respiratory diseases. Int J Pharm 2018;545(1–2):378–88. https://doi.org/10.1016/j.ijpharm.2018.04.030.
[164] Ghanbarzadeh S, Arami S, Pourmoazzen Z, Khorrami A. Improvement of the antiproliferative effect of rapamycin on tumor cell lines by poly (monomethylitaconate)-based pH-sensitive, plasma stable liposomes. Colloids Surf B: Biointerfaces 2014;115:323–30. https://doi.org/10.1016/j.colsurfb.2013.12.024.

[165] Santhanes D, Zhang H, Wilkins A, John Aitken R, Gannon AL, Liang M. Engineering pH-sensitive dissolution of lipid-polymer nanoparticles by eudragit integration impacts plasmid DNA (pDNA) transfection. Eur J Pharm Biopharm 2024;199:114299. https://doi.org/10.1016/j.ejpb.2024.114299.
[166] He X, Li L, Su H, et al. Poly(ethylene glycol)-block-poly(ε-caprolactone)-and phospholipid-based stealth nanoparticles with enhanced therapeutic efficacy on murine breast cancer by improved intracellular drug delivery. Int J Nanomedicine 2015;10:1791–804. https://doi.org/10.2147/IJN.S75186.
[167] Kambar N, Leal C. Microfluidic synthesis of multilayered lipid-polymer hybrid nanoparticles for the formulation of low solubility drugs. Soft Matter 2023;19(8): 1596–605. https://doi.org/10.1039/d2sm01443b.
[168] Taipaleenmäki E, Christensen G, Brodszkij E, et al. Mucopenetrating polymer - lipid hybrid nanovesicles as subunits in alginate beads as an oral formulation. J Control Release 2020;322:470–85. https://doi.org/10.1016/j.jconrel.2020.03.047.
[169] Dao TPT, Fernandes F, Fauquignon M, Ibarboure E, Prieto M, Le Meins JF. The combination of block copolymers and phospholipids to form giant hybrid unilamellar vesicles (GHUVs) does not systematically lead to "intermediate" membrane properties. Soft Matter 2018;14(31):6476–84. https://doi.org/10.1039/c8sm00547h.
[170] Fauquignon M, Ibarboure E, Carlotti S, Brûlet A, Schmutz M, Le Meins JF. Large and giant unilamellar vesicle(s) obtained by self-assembly of poly (dimethylsiloxane)-b-poly(ethylene oxide) diblock copolymers, membrane properties and preliminary investigation of their ability to form hybrid polymer/lipid vesicles. Polymers (Basel) 2019;11(12):2013. https://doi.org/10.3390/polym11122013.
[171] Fauquignon M, Ibarboure E, Le Meins JF. Hybrid polymer/lipid vesicles: influence of polymer architecture and molar mass on line tension. Biophys J 2022;121(1): 61–7. https://doi.org/10.1016/j.bpj.2021.12.005.
[172] Miele Y, Mingotaud AF, Caruso E, Malacarne MC, Izzo L, Lonetti B, et al. Hybrid giant lipid vesicles incorporating a PMMA-based copolymer. Biochim Biophys Acta Gen Subj 2021;1865(4):129611. https://doi.org/10.1016/j.bbagen.2020.129611.
[173] Schulz M, Olubummo A, Bacia K, Binder WH. Lateral surface engineering of hybrid lipid–BCP vesicles and selective nanoparticle embedding. Soft Matter 2014;10:831–9. https://doi.org/10.1039/C3SM52040D.
[174] Ebrahimian M, Mahvelati F, Malaekeh-Nikouei B, Hashemi E, Oroojalian F, Hashemi M. Bromelain loaded lipid-polymer hybrid nanoparticles for oral delivery: formulation and characterization. Appl Biochem Biotechnol 2022;194 (8):3733–48. https://doi.org/10.1007/s12010-022-03812-z.
[175] Thanki K, van Eetvelde D, Geyer A, et al. Mechanistic profiling of the release kinetics of siRNA from lipidoid-polymer hybrid nanoparticles in vitro and in vivo after pulmonary administration. J Control Release 2019;310:82–93. https://doi.org/10.1016/j.jconrel.2019.08.004.
[176] Wang J. Combination treatment of cervical cancer using folate-decorated, pH-sensitive, carboplatin and paclitaxel co-loaded lipid-polymer hybrid nanoparticles. Drug Des Devel Ther 2020;14:823–32. https://doi.org/10.2147/DDDT.S235098.
[177] Gupta M, Sharma V, Sharma K, et al. A kNGR peptide-tethered lipid-polymer hybrid nanocarrier-based synergistic approach for effective tumor therapy: development, characterization, ex-vivo, and in-vivo assessment. Pharmaceutics 2022;14(7):1401. https://doi.org/10.3390/pharmaceutics14071401.
[178] Pippa N, Merkouraki M, Pispas S, Demetzos C. DPPC: MPOx chimeric advanced drug delivery nano systems (chi-aDDnSs): physicochemical and structural characterization, stability and drug release studies. Int J Pharm 2013;450(1–2): 1–10. https://doi.org/10.1016/j.ijpharm.2013.03.052.
[179] Papagiannopoulos A, Pippa N, Demetzos C, Pispas S, Radulescu A. Lamellarity and size distributions in mixed DPPC/amphiphilic poly(2-oxazoline) gradient copolymer vesicles and their temperature response. Chem Phys Lipids 2021;234: 105008. https://doi.org/10.1016/j.chemphyslip.2020.105008.
[180] Papagiannopoulos A, Pippa N, Demetzos C, Pispas S, Radulescu A. Formation of Uni-lamellar vesicles in mixtures of DPPC with PEO-b-PCL amphiphilic diblock copolymers. Polymers (Basel) 2020;13(1):4. https://doi.org/10.3390/polym13010004.
[181] Pippa N, Chountoulesi M, Kyrili A, Meristoudi A, Pispas S, Demetzos C. Calorimetric study on pH-responsive block copolymer grafted lipid bilayers: rational design and development of liposomes. J Liposome Res 2016;26(3): 211–20. https://doi.org/10.3109/08982104.2015.1076464.
[182] Naziris N, Pippa N, Skandalis A, Miłowska K, Balcerzak Ł, Pispas S, et al. Thermoresponsive chimeric nanocarriers as drug delivery systems. Colloids Surf B: Biointerfaces 2021;208:112141. https://doi.org/10.1016/j.colsurfb.2021.112141.
[183] Naziris N, Pippa N, Sereti E, Chrysostomou V, Kędzierska M, Kajdanek J, et al. Chimeric stimuli-responsive liposomes as nanocarriers for the delivery of the anti-glioma agent TRAM-34. Int J Mol Sci 2021;22(12):6271. https://doi.org/10.3390/ijms22126271.
[184] Chountoulesi M, Pippa N, Forys A, Trzebicka B, Pispas S. Structure-based evaluation of hybrid lipid-polymer nanoparticles: the role of the polymeric guest. Polymers (Basel) 2024;16(2):290. https://doi.org/10.3390/polym16020290.
[185] Triantafyllopoulou E, Selianitis D, Pippa N, Gazouli M, Valsami G, Pispas S. Development of hybrid DSPC: DOPC: P(OEGMA950-DIPAEMA) nanostructures: the random architecture of polymeric guest as a key design parameter. Polymers (Basel) 2023;15(9):1989. https://doi.org/10.3390/polym15091989.
[186] Triantafyllopoulou E, Selianitis D, Balafouti A, Lagopati N, Gazouli M, Valsami G, et al. Fabricating hybrid DSPC: DOPC: P(OEGMA-co-LMA) structures: self-assembly as the milestone of their performance. Colloids Surf A Physicochem Eng Asp 2024;684:133015. https://doi.org/10.1016/j.colsurfa.2023.133015.
[187] Triantafyllopoulou E, Forys A, Perinelli DR, Balafouti A, Karayianni M, Trzebicka B, et al. Deciphering the lipid-random copolymer interactions and encoding their properties to design a hybrid system. Langmuir 2024;40(23): 11936–46. https://doi.org/10.1021/acs.langmuir.4c00278.
[188] Triantafyllopoulou E, Perinelli DR, Forys A, Pantelis P, Gorgoulis VG, Lagopati N, et al. Unveiling the performance of co-assembled hybrid nanocarriers: moving towards the formation of a multifunctional lipid/random copolymer nanoplatform. Pharmaceutics 2024;16(9):1204. https://doi.org/10.3390/pharmaceutics16091204.
[189] Colombo S, Beck-Broichsitter M, Bøtker JP, Malmsten M, Rantanen J, Bohr A. Transforming nanomedicine manufacturing toward quality by design and microfluidics. Adv Drug Deliv Rev 2018;128:115–31. https://doi.org/10.1016/j.addr.2018.04.004.
[190] Matthews BA, Rhodes CT. Use of the derjaguin, landau, verwey, and overbeek theory to interpret pharmaceutical suspension stability. J Pharm Sci 1970;59(4): 521–5. https://doi.org/10.1002/jps.2600590417.
[191] Dukhin AS, Xu R. Chapter 3.2.5: Zeta-potential measurements. In: Hodoroaba Vasile-Dan, Unger Wolfgang ES, Shard Alexander G, editors. Micro and Nano Technologies, Characterization of Nanoparticles. Elsevier; 2020. p. 213–24. https://doi.org/10.1016/B978-0-12-814182-3.00014-6.
[192] Schulz M, Olubummo A, Binder WH. Beyond the lipid-bilayer: interaction of polymers and nanoparticles with membranes. Soft Matter 2012;8:4849–64. https://doi.org/10.1039/C2SM06999G.
[193] Kang SH, Shin YS, Lee DH, Park IS, Kim SK, Ryu D, et al. Interactions of nanoparticles with macrophages and feasibility of drug delivery for asthma. Int J Mol Sci 2022;23(3):1622. https://doi.org/10.3390/ijms23031622.
[194] Carton F, Malatesta M. Assessing the interactions between nanoparticles and biological barriers in vitro: a new challenge for microscopy techniques in nanomedicine. Eur J Histochem 2022;66(4):3603. https://doi.org/10.4081/ejh.2022.3603.
[195] Pippa N, Forys A, Katifelis H, Chrysostomou V, Trzebicka B, Gazouli M, et al. Design and development of DSPC: DAP: PDMAEMA-b-PLMA nanostructures: from the adumbration of their morphological characteristics to in vitro evaluation. Colloids Surf A Physicochem Eng Asp 2022;632:127768.
[196] Huang Q, Cai T, Li Q, et al. Preparation of psoralen polymer-lipid hybrid nanoparticles and their reversal of multidrug resistance in MCF-7/ADR cells. Drug Deliv 2018;25(1):1056–66. https://doi.org/10.1080/10717544.2018.1464084.
[197] Liu Y, Yang G, Jin S, Xu L, Zhao CX. Development of high-drug-loading nanoparticles. Chempluschem 2020;85(9):2143–57. https://doi.org/10.1002/cplu.202000496.
[198] Christoforou I, Kalatzis A, Siamidi A, Vlachou M, Pispas S, Pippa N. The ubiquitous use of polyethylene glycol in pharmaceutical design and development: technological aspects and future perspectives. Nanomaterials (Basel) 2025;15 (23):1762. https://doi.org/10.3390/nano15231762.
[199] Ottonelli I, Duskey JT, Rinaldi A, Grazioli MV, Parmeggiani I, Vandelli MA, et al. Microfluidic technology for the production of hybrid nanomedicines. Pharmaceutics 2021;13(9):1495. https://doi.org/10.3390/pharmaceutics13091495.
[200] Streck S, Hong L, Boyd BJ, McDowell A. Microfluidics for the production of nanomedicines: considerations for polymer and lipid-based systems. Pharm Nanotechnol 2019;7(6):423–43. https://doi.org/10.2174/2211738507666191019154815 [PMID: 31629401].
[201] Barros C, Aranha N, Severino P, et al. Quality by design approach for the development of liposome carrying ghrelin for intranasal administration. Pharmaceutics 2021;13(5):686. https://doi.org/10.3390/pharmaceutics13050686.
[202] Soares S, Sousa J, Pais A, Vitorino C. Nanomedicine: principles, properties, and regulatory issues. Front Chem 2018;6:360. https://doi.org/10.3389/fchem.2018.00360.
[203] Marques MRC, Choo Q, Ashtikar M, Rocha TC, Bremer-Hoffmann S, Wacker MG. Nanomedicines - tiny particles and big challenges. Adv Drug Deliv Rev 2019;151-152:23–43. https://doi.org/10.1016/j.addr.2019.06.003.
[204] Mukherjee A, Waters AK, Kalyan P, Achrol AS, Kesari S, Yenugonda VM. Lipid-polymer hybrid nanoparticles as a next-generation drug delivery platform: state of the art, emerging technologies, and perspectives. Int J Nanomedicine 2019;14: 1937–52. https://doi.org/10.2147/IJN.S198353.
[205] Colby AH, Liu R, Doyle RP, et al. Pilot-scale production of expansile nanoparticles: practical methods for clinical scale-up. J Control Release 2021;337: 144–54. https://doi.org/10.1016/j.jconrel.2021.07.012.
[206] Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. Engineering precision nanoparticles for drug delivery. Nat Rev Drug Discov 2021; 20(2):101–24. https://doi.org/10.1038/s41573-020-0090-8.
[207] Osouli-Bostanabad K, Puliga S, Serrano DR, Bucchi A, Halbert G, Lalatsa A. Microfluidic manufacture of lipid-based nanomedicines. Pharmaceutics 2022;14 (9):1940. https://doi.org/10.3390/pharmaceutics14091940.
[208] Karati D, Mukherjee S, Prajapati B, Bose A, Paul S, Elossaily GM, et al. A review on lipid-polymer hybrid nanocarriers in cancer. J Drug Deliv Technol 2024;97: 105827. https://doi.org/10.1016/j.jddst.2024.105827.
[209] Sainz V, Conniot J, Matos AI, Peres C, Zupancic E, Moura L, et al. Regulatory aspects on nanomedicines. Biochem Biophys Res Commun 2015;468(3):504–10. https://doi.org/10.1016/j.bbrc.2015.08.023.