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Liposome-like Nanostructures for Drug Delivery

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Abstract

Liposomes are a class of well-established drug carriers that have found numerous therapeutic applications. The success of liposomes, together with recent advancements in nanotechnology, has motivated the development of various novel liposome-like nanostructures with improved drug delivery performance. These nanostructures can be categorized into five major varieties, namely: (1) polymer-stabilized liposomes, (2) nanoparticle-stabilized liposomes, (3) core-shell lipid-polymer hybrid nanoparticles, (4) natural membrane-derived vesicles, and (5) natural membrane coated nanoparticles. They have received significant attention and have become popular drug delivery platforms. Herein, we discuss the unique strengths of these liposome-like platforms in drug delivery, with a particular emphasis on how liposome-inspired novel designs have led to improved therapeutic efficacy, and review recent progress made by each platform in advancing healthcare.

Keywords

Liposome; nanostructure; nanoparticle; drug delivery; nanomedicine

1. Introduction

Phospholipids in aqueous environments can self-assemble to form closed bilayer structures.[1] This pioneering observation first made a half century ago marked the starting point of liposome research.[2] It also unleashed the potential of liposomes as a class of drug carriers to treat a variety of diseases.[3]

The advantages of liposomes for drug delivery applications are well-known.[3–5] An enormous selection of biocompatible lipids is readily available for formulating liposomes with precisely tailored chemical, biological, and mechanical properties. Owing to their unique self-closed structures, liposomes can entrap hydrophilic agents in their aqueous compartment and hydrophobic ones into their membranes. Liposomes protect the loaded drug molecules from external degradation, and their similarity to biological membranes provides unique opportunities to deliver drug molecules into the cells or their sub-cellular compartments. In addition, various physicochemical properties of liposomes including their size, charge, and surface functional ligands can be altered at different stages of the formulation process, resulting in functionalities favoring specific drug delivery tasks. These advantages, collectively, have made liposomes a leading drug delivery platform with a wide range of uses in the clinic (Table 1).[6, 7]

As the abilities to control and manipulate nanostructures continually advance, engineering strategies to improve on the drug delivery performance of liposomes have also been rapidly

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evolving. Conventional liposomes are liposomal formulations with unaltered surface properties. These liposomes loaded with drug molecules such as amphotericin B represent the first generation of liposomal drugs on market. However, the applications of conventional liposomes face challenges due to their inherit instability. Liposomes, particularly with sub-100 nm size, are prone to fuse with each other to reduce their surface tension, leading to payload loss or undesired mixing.[8–10] One strategy to overcome this problem is to coat the liposome surface with “stealth” polymers such as polyethylene glycol (PEG). The PEG layer not only prevents liposomes from fusion but also enhances their in vivo circulation lifetime by suppressing plasma proteins from adsorbing onto the liposome surface. These stealth liposomes are considered a new generation of liposomal formulations and their success has resulted in a few clinically approved products.[11, 12]

Although liposomes modified with polymers have shown great success for systemic drug delivery, they are less frequently used for topical delivery, particularly to treat bacterial infections.[13] This is because the polymer coating, while effective in stabilizing liposomes against fusion, also prevents them from fusing with bacterial membranes to which the antimicrobial payloads need to be delivered. To address this issue, a new strategy has been reported to stabilize liposomes by adsorbing small nanoparticles onto their outer surfaces.[14] These bound tiny particles provide effective steric hindrance to stabilize liposomes against fusion prior to ‘seeing’ the target bacteria.[15] Once they have arrived at the infection sites, these nanoparticle stabilizers can rapidly detach from the liposomes in response to environmental changes, thereby restoring the fusion activity of the liposomes. Compared to stealth polymer coatings, nanoparticle-based stabilization leaves a large portion of liposome surfaces untouched, which allows for additional mechanisms to trigger cargo release from the liposomes.

Meanwhile, motivated by the great advantages of polymeric nanoparticles in drug delivery, a unique hybrid nanoparticle design that consists of a polymeric core and a lipid shell has emerged.[16, 17] This hybrid design combines the merits of both polymeric nanoparticles and liposomes while excluding some of their limits. Compared to the aqueous cores of the conventional liposomes, the solid polymeric cores provide better control over the mechanical stability, particle morphology, size distribution, and drug release kinetics.[18] Following their initial development, the lipid-polymer hybrid nanoparticles have quickly become a popular drug delivery platform and been intensively explored for a wide range of drug delivery applications. Deeper understandings of the lipid-polymer hybrid nanoparticles, particularly on their immuno-compatibility and self-assembly mechanisms, have also been achieved. Their success has further motivated a number of engineering strategies for large-scale production with highly tunable and uniform structures.[19]

The use of lipid materials, through ‘bottom-up’ engineering processes, has resulted in numerous synthetic liposome-like drug carriers with excellent physicochemical properties. However, it is difficult for these platforms to mimic the complex functionalities found on many natural delivery vehicles.[20] As alternatives, cellular membrane-derived vesicles have emerged as a new class of liposome-like drug carriers. They are typically made through ‘top-down’ approaches that use segments of natural cellular membranes to form individual small vesicles. In this respect, nano-scale vesicles, either directly produced by live organisms or artificially derived from natural cellular membranes, preserve natural functionalities of the source materials that would otherwise be very difficult to replicate.[21] Recently, a unique biomimetic strategy takes this approach one step further to functionalize synthetic polymeric nanoparticles by coating with red blood cell (RBC) membranes.[22, 23] This strategy enables the functionalization of polymeric particles with natural RBC membranes through a ‘top-down’ approach that bypasses the labor-intensive processes of protein identification, purification, and conjugation. The cellular membrane-coated

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nanoparticles are quickly becoming a class of robust nanocarriers for many drug delivery applications.

Following this development path, we choose conventional liposomes, polymer-functionalized liposomes, nanoparticle-stabilized liposomes, core-shell lipid-polymer hybrid nanoparticles, cellular membrane-derived vesicles, and cellular membrane-coated nanoparticles as major liposome-like nanostructures (Figure 1). In this article, we highlight the unique strength of each category and review its recent progress in advancing drug delivery research.

2. Conventional liposomes

Currently, the majority of approved liposomal drugs are based on conventional liposomes (Table 1). Following their initial approval, clinical studies continually expand the use of these validated liposomal formulations into a larger number of clinical indications. For examples, Myocet®, the non-PEGylated liposomal doxorubincin, was investigated in various combination chemotherapies.[24, 25] DaunoXome®, the liposomal daunorubicin, was studied in treating patients with relapsed acute myeloid leukemia.[26, 27] In these trials, the replacement of free drugs with their corresponding liposomal formulations results in superior treatment responses and substantially reduced toxicity. Success achieved by these approved liposomal formulations has encouraged the use of liposomes to carry and deliver an increasing number of drug compounds to treat a variety of diseases. Many of these formulations have entered clinical development.[6, 7, 28] In addition to small molecules, macromolecule drugs such as oligonucleotides and proteins are also formulated with liposomes and are currently under clinical tests.[29–31] Technological advances in liposome engineering have also resulted in formulations tailored for different routes of administration, further reducing the risk of systemic toxicities. For example, aerosolized delivery of Amphotec®, the liposomal amphotericin B, directly to the lungs could be useful as a fungal prophylactic strategy for patients undergoing lung transplantation. The liposomal dry powder of amikacin for aerosol delivery and liposomal lotion of bacteriophage T4 endonuclease V for topical delivery are emerging formulations in clinical trials for respiratory infection and xeroderma pigmentosum, respectively.[32–34]

Another unique feature of conventional liposomes is that those made with diameters of 100 nm or smaller can readily fuse with bacterial or fungal membranes, thereby causing cell damage. Therefore, these sub-100 nm conventional liposomes are particularly useful for antimicrobial treatment.[35] For example, liposomal amphotericin B, including Ambisome®, Abelcet®, and Amphotec®, intercalate drug molecules within their lipid bilayer membranes. It has been shown that the resulting liposomes preferentially fuse with fungi and facilitate the cellular membrane permeation of amphotercin B for enhanced anti-fungal activity.[36–38] Recently, a similar strategy was applied to the delivery of free fatty acids (FFAs), a class of naturally derived molecules that have attracted much attention because of their selective antimicrobial activity.[39] The amphiphilic nature of FFAs makes them especially suitable for liposome encapsulation; loading of FFAs into the lipid bilayers can overcome their poor water solubility, protect them from degradation, and help transport them into bacterial membranes for bioactivity. A variety of FFAs, including lauric acid, oleic acid, and linolenic acid, have been successfully loaded into liposomes and the resulting formulations have demonstrated potent antibacterial activities against *Propionibacterium acnes*,[40] methicillin-resistant *Staphylococcus aureus* (MRSA),[41] and *Helicobacter pylori* (*H. pylori*),[42] respectively (Figure 2). Traditional antibiotics largely interfere with the chemical-biological pathways of bacteria. In contrast, liposomal FFAs fuse with bacterial membranes through a ‘physical’ process and thus kill the bacteria by disrupting their membranes, thereby lowering the probability of the induction of drug resistance. For example, liposomal linolenic acid

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was shown to be effective against both viable and dormant forms of *H. pylori*, as well as multiple clinical isolates of *H. pylori* bacteria resistant to standard treatment.[42] More importantly, compared to standard antibiotics such as metronidazole, liposomal linolenic acid showed a significantly lower rate of resistance development. These findings suggest that liposomal FFAs hold a strong potential to become a class of effective antimicrobial agents against microbial infections.

To further enhance the therapeutic efficacy of liposomal drugs, numerous approaches have been developed to bestow conventional liposomes with stimuli-responsive cargo release ability.[43] A number of environmental cues are applied for responsive liposome design, including thermal energy, pH gradient and shear stress. For example, ThermoDox® is a thermally sensitive formulation encapsulating doxorubicin, which is now under a pivotal Phase III clinical trial.[44] In this formulation, lysolipids are incorporated into the lipid bilayers, which undergo temperature-dependent phase transition when heated above 39°C, thereby creating defects on the liposome membranes and thus allowing doxorubicin to be released at the target sites. While ThermoDox® uses thermally responsive phospholipids for heat-controlled drug release, other systems achieve thermo sensitivity with the cargo enclosed in the aqueous core of the liposomes. For example, a unique liposome system containing NH_4HCO_3, a thermally decomposable compound, was recently designed (Figure 3).[45] When taken up by cancer cells and intracellularly trafficked to lysosomes, the liposomes were triggered to explode by mild external heating, generating powerful disruptive forces inside the cells and eventually inducing cell death. This innovative approach exploits liposomes to effectively convert chemical energy to mechanical forces, which subsequently destroy cancer cells by physical disruption. Overall, thermally sensitive liposomes bridge the widely applied liposomal chemotherapy with heat-based treatment regimes such as radiofrequency thermal ablation, microwave hyperthermia and high intensity focused ultrasound, thereby holding great clinical application potential.[46]

The acidic pH present in the extracellular environment of solid tumors or intracellular compartments such as endosomes and lysosomes has been widely used to improve liposomal drug delivery for cancer treatment.[43] In particular, zwitterionic lipids that can switch charge and molecular conformation in response to a pH gradient have attracted much attention.[47] For example, liposomes made with zwitterionic lipids possess high stability and long circulation following injection. However, when they reach tumor sites, these liposomes are better retained and can quickly release their therapeutic payloads.[48] At the sub-cellular level, zwitterionic lipid-based liposomes are also shown to escape endosomes and release cargo in the cytoplasm, resulting in effective small interfering RNA (siRNA)-mediated gene knockdown or specific cell organelle-targeted drug delivery.[49, 50]

Mechanical stress is also used to trigger drug release from liposomes. Obstruction of blood vessels due to cardiovascular diseases such as atherosclerosis, thrombosis, and embolism induces significant changes in the endogenous shear stress between healthy and constricted arteries. To harness mechanical stress for responsive drug delivery, recently an artificial phospholipid was synthesized that contained two amide bonds. When forming liposomes, these lipids allowed for a better steric alignment for directionally dependent hydrogen-bond formation in the polar hydrated region (Figure 4).[51] The resulting liposomes exhibit a lenticular morphology with two spherical segments joined by a discontinuity around the diameter, creating preferential breaking points along the equator. This unique design makes the liposomes sensitive to shear stress, allowing for in situ drug release at the sites of narrowed blood vessels.

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3. Polymer-stabilized liposomes

By adopting lipids with novel molecular structure and precisely tailoring liposome compositions, conventional liposomes have become increasingly functional. However, their applications are still limited by their intrinsic instability. Conventional liposomes, particularly with sub-100 nm size, have high surface curvature and are prone to fuse with each other when they collide to minimize their surface tension, and the fusion process leads to payload loss or undesired mixing.[8, 9] In addition, without proper surface modifications, these liposomes also interact strongly with serum proteins, cells, and tissues, causing fast immune clearance and undesired off-target toxicity. [52, 53]

To overcome these challenges and improve on the use of liposomes as potent delivery nanocarriers, various strategies have explored the use of polymers to reinforce the liposome structure and provide liposomes with steric hindrance. These designs prevent liposome fusion and inhibit protein opsonization (Figure 5). For example, through electrostatic interactions, polyelectrolytes can be adsorbed onto the liposome surfaces with opposite charges.[54, 55] Alternatively, hydrophilic polymer chains can be covalently conjugated to the head group of the lipids followed by the liposome formation.[56, 57] Stabilization can be also achieved within liposome bilayers by cross-linking the hydrophobic tails of the lipids.[58, 59] Furthermore, polymerizable monomers linked to the lipid or cholesterol molecules can be first anchored onto the liposome surface and then polymerized to form a polymer cage surrounding and stabilizing the liposomes.[6061]

To stabilize liposomes and achieve stealth ability, a wide range of polymers has been used to modify liposome surface properties. Among them, polyethylene glycol (PEG) has been intensively studied and has become the polymer of choice to produce stealth liposomes.[62] Internally, the grafted PEG layer reinforces the structural integrity even when the liposomes undergo environment-induced stress.[63] Externally, the PEG layer provides steric hindrance and prevents liposomes from fusing with one another. Enhancement on the in vivo circulation lifetime brought by the PEG layer is also well known.[64] As a result, PEG has become the gold standard for liposome coating and the success of PEGylated liposomes has led to a group of clinically approved therapeutic products that exploit passive targeting for systemic cancer therapy (Table 1). In addition to PEG, various polymers including poloxamers, poloxamines, dextran, sialic acid derivatives, polyacrylic and polyvinyl polymers, and polyglycerols have also been attempted for use as liposome stabilizers with mixed outcomes.[53] More recently, super-hydrophilic zwitterionic polymers have been developed to create stealth liposomes with promising results.[56] Compared to PEG, this class of molecules achieves stronger hydration, interacts weakly with lipid bilayers and provides better liposome stabilization.

Together with the development of liposome engineering, polymer-stabilized liposomes have been applied to advance various drug delivery applications. Among them, intracellular delivery of siRNA is a fast developing field.[65] Successful RNA interference requires delivery vehicles to escort siRNA through the bloodstream and extracellular matrix, mediate siRNA transport across the cellular membrane of the target cell, and facilitate endosomal escape before lysosomal digestion.[66] Liposomes that use cationic lipids for siRNA condensation and PEG coating for stealth represent the most advanced examples of systemic siRNA delivery. Among these materials, lipidoids, a class of lipid-like cationic molecules, have attracted much attention. Compared to traditional lipids, lipidoids require fewer steps to synthesize and purify, thereby allowing for high throughput combinatorial synthesis and rapid in vitro screening.[67] Lipidoid-based liposome-like complexes have been demonstrated to facilitate sequence-specific knockdown in a variety of cellular targets and animal species including mice, rats, and nonhuman primates.[68–70] To further improve on efficacy, high

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throughput material discovery is also applied to create a diverse molecule library by incorporating side chains featuring systematic variation on their capacities for hydrogen bonding, hydrophobic interactions, and protonation states, hence providing new possibilities to optimize the chemical makeup of lipid-like siRNA delivery materials.[71] Aimed at increasing the material space available for potential siRNA therapeutics, lipidoids, which are ineffective if used alone, have been combined and found to induce near-complete gene knockdown. The observed synergy resulted from the combination of individual lipidoids that respectively mediate cellular uptake and endosomal escape.[72] Recently, high throughput molecule discovery has also been applied to synthesize and screen new classes of lipid-like molecules that facilitate gene silencing with orders-of-magnitude increase in potency.[73] Besides material discovery, a novel formulation method using microfluidics has enabled rapid preparation of high-quality liposome-like complexes and significantly improved the efficiency and accuracy of screening candidate molecules.[74] Successful siRNA delivery achieved by using these materials has also advanced the basic understandings on how biological systems respond to RNA inference ranging from cellular to systemic levels.[75–77]

Using nanomaterials to modulate the immune system as a means of better disease treatment is another rapidly evolving field, where liposomes have been increasingly exploited to solve problems in translational immunology.[78,79] Lipid vesicles with varying physicochemical properties, including method of antigen attachment, lipid composition, bilayer fluidity, lamellarity, and surface charge have been tested as vaccines, resulting in effective antimicrobial or antitumor immune responses in small-animal models.[79] In early studies, conventional liposomes were popular platforms; however, stabilized liposomes with predictable stability have been increasingly used for precise immune modulation with the hope of achieving a more durable, broader, and more potent immune response. For example, compared to conventional liposomes, those reinforced with polymerized lipids were shown to have superior adjuvantic activities, which lead to enhanced antibody production.[58] In addition to efficient antigen delivery, stabilized liposomes also effectively target adjuvant molecules to antigen presenting cells. For instance, the hydrophobic TLR4 agonist monophosphoryl lipid A was loaded into the hydrophobic leaflets of multilamellar liposomes (Figure 6).[80] The liposomes were then stabilized by first cross-linking the head groups of lipids followed by grafting PEG onto the surfaces. Such delicate liposome architecture and stabilization provided sustained antigen presentation and immune activation. Compared to soluble adjuvants, the liposomes showed dramatically increased potency, broader humoral responses, and a more balanced Th1/Th2 cytokine profile from antigen-specific T cells.[81]

Despite the broad applications of PEGylated liposomes, stabilization by PEG has also been found to generate undesirable impact in drug delivery.[82] On one hand, polymer grafting enhances liposomal stability for longer in vivo survival; on the other hand, the steric hindrance limits liposome tissue penetration at disease sites and its endosomal escape after endocytic uptake.[83] To overcome this ‘PEG dilemma’, sheddable PEG coating responsive to environmental cues has been developed. For example, pH-sensitive linkers, such as esters,[84–86] vinyl ether,[87] and hydrazone,[88] have been embedded within PEG-lipid conjugates to enable pH-triggered PEG shedding. In addition, PEG-lipid conjugates joined by a disulfide bond have been applied to make liposomes sensitive to reducing environments such as endolysosomal compartments in cells for PEG shedding.[89] In particular, dithiobenzyl urethane linkage responds to mild thiolytic conditions, which are more characteristic of in vivo environments, and its cleavage generates amine-terminated rather than thiolated lipid byproducts, which may be safer for in vivo uses.[90] Furthermore, using enzyme-sensitive cross-linkers for PEG shedding provides higher disease or site specificity. In this respect, peptide linkers responsive to a variety of enzymes, including cathepsin B, papain, pronase E, and cancer-associated proteases, have been developed.[91–94]

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4. Nanoparticle-stabilized liposomes

Despite the wide applications of polymer-stabilized liposomes in systemic drug delivery, they are less frequently used for antimicrobial delivery to treat infections. This is mainly because the polymer coating, while stabilizing liposomes against fusion with each other, also prevents them from fusing with bacterial membranes, to which the antimicrobial payloads need to be delivered.[35] Ideally, liposomes should be stabilized against fusion prior to 'seeing' the target bacteria, while their fusion activity resumes once they arrive at the infection sites. Therefore, an alternative stabilization strategy is needed for liposomes to achieve this goal.

In this perspective, an emerging strategy to stabilize liposomes is to bind tiny nanoparticles to liposome surfaces (Figure 7).[14] Particularly, the non-specific adsorption of charged nanoparticles onto phospholipid bilayers provides steric and electrostatic repulsions. In a density-dependent manner, these adsorbed nanoparticles prevent liposomes from fusing with each other to form larger vesicles.[95] In addition, the nanoparticle stabilizers are found to cause lipid surface reconstruction at sites where nanoparticles adsorb. Such surface reconstruction reduces liposome surface tension and further enhances their stability.[15] This stabilization strategy, together with various choices of nanoparticle stabilizers including polystyrene, gold, and silica nanoparticles, opens exciting opportunities for engineering advanced liposome formulations for improved drug delivery.[96]

Using nanoparticles to stabilize liposomes, the charge and charge density of both the nanoparticle stabilizers and the liposomes can be precisely tailored to enable stimuli-responsive binding and detaching of the nanoparticles, thereby offering an on-demand control over liposome fusion activity for smart cargo delivery. For example, it has been shown that carboxyl-modified gold nanoparticles adsorbed onto the outer surface of phospholipid liposomes effectively prevented liposomes from fusing with each other at neutral pH value.[97] At acidic environments (e.g., pH<5), the gold particle stabilizers detached from the liposomes and the liposome fusion activity was resumed. Human skin is typically acidic (pH = 3.9~6.0),[98] especially at areas such as infectious lesions on the skin[99]. For example, the pH value is about 4.0 at acne lesions[100] and 4.5–6.3 at comedones[101]. Therefore, this class of acid-responsive liposomes with tunable fusion ability is attractive for dermal drug delivery.

In addition to pH responsiveness, nanoparticle-stabilized liposomes offer other possibilities to target infections. For example, nanoparticles attached to the liposome surfaces can serve as spots for manipulating liposome properties with high spatial resolution. Particularly, gold nanoparticles adsorbed onto liposomes allow for heat induction and nano-scale reversible gel-fluid phase transitions in the phospholipid membrane, a useful property for on-site drug delivery.[102] On the other hand, stabilization by small nanoparticles also leaves a substantial fraction of the liposome surfaces untouched, making it possible to incorporate additional functionalities to the liposomes and allowing for site-selective cargo release. For instance, the adsorption of positively charged chitosan-modified gold nanoparticles onto the surface of anionic liposomes prevented liposome fusion and undesirable payload leakage under both storage and physiological environments. However, once these protected liposomes reached bacterial infection sites, the uncovered portion of the liposome surfaces could be accessed by the bacteria's pore-forming toxins. Toxin insertion into the liposome membrane subsequently triggered drug release against the toxin secreting bacteria.[103]

5. Core–shell lipid–polymer hybrid nanoparticles

Recently, novel nanoparticle designs that combine a lipid shell and an enclosed solid core into a single particle architecture have emerged.[104] Inorganic materials such as silica, metal

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oxides, or organic materials such as polysaccharides, polystyrene, polyelectrolyte capsules, or hydrogels have been explored as cores.[105] Depending upon the formulation process and the specific core-shell combinations, single or multiple layers of lipids can constitute the shell.[106, 107]

Among various hybrid formulations, nanoparticles composed of lipid monolayers and poly(lactic acid) (PLA) or poly(lactic-co-glycolic acid) (PLGA) cores have gained significant attention (Figure 8).[16, 17] This hybrid design promises to take advantage of the unique strengths from both polymeric nanoparticles and liposomes. In this design, solid cores act as a support that provides mechanical stability, controlled morphology, biodegradability, increased surface area-to-volume ratio and narrow size distribution. In addition, the core of these particles is composed of PLA or PLGA polymer, which is an established biopolymer for drug delivery and imparts controlled drug release. At the same time, the lipid shell enveloping the core mimics biological membranes in interacting with the environment. It also acts as a barrier to reduce drug diffusion and water penetration across the core-shell interface, thereby facilitating drug encapsulation and altering drug release rates. The shell is commonly anchored with a lipid-PEG moiety that allows the particles to evade uptake by the immune system and confers long-circulating characteristics. The shell can also be further modified with functional ligands for targeted drug delivery.

Following their initial development, hybrid nanoparticles have been intensively studied for the delivery of hydrophobic drugs to improve cancer treatment. Docetaxel,[16, 17] paclitaxel,[108, 109] and doxorubicin[110–112] were readily encapsulated and the resulting drug-loaded hybrid nanoparticles showed improved efficacy in various tumor models. The platform has also been applied for combinatorial cancer treatment. For example, doxorubicin-combretastatin[113], doxorubicin-Elacridar,[114] and paclitaxel–cisplatin[115] combinations were shown to improve tumor inhibition in various cancer models. In addition, docetaxel was combined with ^{111} indium and ^{90} yttrium into the hybrid nanoparticle system, resulting in a class of ‘ChemoRad’ nanoparticles for the targeted delivery of concurrent chemoradiation.[116]

In addition to cancer treatment, lipid-polymer hybrid nanoparticles can also be used for drug delivery to treat other diseases. For example, 60 nm hybrid nanoparticles have been reported for spatiotemporal controlled delivery of drugs to injured vasculature. Specifically, paclitaxel was linked to the polymeric core through an ester bond for slow-eluting release over approximately 12 days (Figure 9).[117] The particle surface was conjugated with a small peptide ligand that specifically targets the basement membrane of injured vasculature. The nanoparticles inhibited human aortic smooth muscle cell proliferation in vitro and showed greater in vivo vascular retention during percutaneous angioplasty than non-targeted controls. Using the same delivery system, an antiproliferative agent was also delivered to injured vasculature.[118] The results showed that drug-loaded nanoparticles had ≥3.5-fold improved maximum tolerated dose compared to free drug. In efficacy studies using a rat carotid injury model, the targeted hybrid nanoparticle group resulted in lower neointima-to-media scores at 2 wk versus control groups. Compared with sham-injury groups, a ~50% reduction in arterial stenosis was observed with targeted nanoparticle treatment. Lipid-polymer hybrid nanoparticles with the capabilities of improved tolerability, sustained release, and vascular targeting could potentially provide a safe and efficacious option in the management of coronary artery disease.

Lipid-polymer hybrid nanoparticles comprised of cationic lipids have also been applied for siRNA delivery. For example, the hybrid nanoparticles carrying polo-like kinase 1 (Plk1)-specific siRNA (siPlk1) down regulated the expression of the oncogene Plk1 and suppressed tumor growth following systemic administration.[119] Based on the lipid-polymer hybrid

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platform, a differentially charged hollow nanostructure was also proposed for siRNA delivery (Figure 10).[120] This derivative system, engineered through a modified double-emulsion solvent evaporation technique and self-assembly method, comprises a positively charged lipid layer as the inner hollow core, a middle hydrophobic PLGA layer, and a neutral lipid layer containing lipid-PEG as the outer shell. Using a xenograft tumor model, the hybrid nanoparticles were shown to effectively deliver siRNA to the target and inhibit luciferase expression in vivo.

Meanwhile, various engineering strategies that modify different components of the hybrid nanoparticles have been developed to further improve on their drug delivery ability. For example, an increasing number of disease biomarkers and associated ligands have been conjugated to the hybrid nanoparticles for targeted delivery. In this respect, antibody fragments retain the high antigen-binding specificity of antibodies, whereas their smaller molecular size results in enhanced tissue penetration and ease of engineering.[111] With the rapid development of ligand screening technologies, small peptides[117, 118] and aptamers[121] are becoming appealing choices of targeting ligands and are applied to improve the delivery of hybrid nanoparticles. In addition, PEG on the particle surface can be conjugated to the lipid through a pH labile cross-linker, leading to responsive PEG shedding for improved drug delivery.[122] Similarly, drug molecules can be also covalently conjugated to the polymer chains through an acid responsive cross-linker, leading to significantly reduced drug leakage in circulation but enhanced release at the target sites.[123] Responsive release mechanisms are also implemented to design hybrid nanoparticles for on-demand drug release. As one example, iron oxide nanoparticles were loaded together with drug molecules into the PLGA cores and the resulting hybrid nanoparticles could be triggered by a remote radio frequency magnetic field for controlled drug release.[124]

Lipid-polymer hybrid nanoparticles also offer a good platform for researchers to readily study and thus gain deep understandings on the surface properties of self-assembled nanostructures. For example, the hybrid nanoparticles were used to investigate the immunocompatibility properties common surface functional groups such as methoxyl, carboxyl, and amine groups.[125] All possible combinations of these groups were found to activate the complement system to a certain extent, and the nanoparticles with amine surface groups induced the highest activation. The activation was primarily via the alternative pathway as opposed to the lectin pathway. Studies of both complement activation and coagulation activation suggested that nanoparticles with methoxyl surface groups may be advantageous for certain drug delivery applications because they were not likely to cause adverse immunological reactions in the human body. Intriguingly, these functional groups were also found to spontaneously form heterogeneous patches on the surface of hybrid nanoparticles, most likely due to the segregation of two different functional groups.[126] Patch formation is observed when tracing the functional groups with quantum dots, gold nanoparticles, and fluorescent dyes. This discovery may lead to the design of patchy nanoparticles with novel functionalities.

The therapeutic potential of hybrid nanoparticles further motivates the development of formulation processes aimed at producing hybrid nanoparticles with precisely engineered architecture and large-scale production. The conventional approach to synthesizing polymeric nanoparticles relies on ‘bulk’ nanoprecipitation by solvent exchange. It remains difficult to engineer nanoparticles with ‘batch-to-batch’ consistency and quantities sufficient for large-scale clinical research.[127] In this respect, microfluidic technologies offer a solution that may overcome these challenges and accelerate the clinical translation of nanoparticle drug delivery.[128] For example, 2D focusing microfluidic designs can rapidly mix water and organic phases with a characteristic mixing time faster than that in the conventional assembly process, resulting in highly homogeneous nanoparticles.[129, 130] Recently, a 3D

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focusing microfluidic technology took the development one step further.[131] This breakthrough allows for the isolation of precipitating polymers that would otherwise clog the fluidic channel, hence making possible the production of highly uniform hybrid nanoparticles with polymers of high molecular weight and high concentration. Additionally, to scale up the production yield, strategies for the rapid and large scale synthesis of lipid-polymer hybrid nanoparticles with the production of gram quantities have been recently developed, bringing the hybrid nanoparticles closer to clinical translation.[132, 133] Traditional formulation of hybrid nanoparticles requires laborious operations including heating, mechanical vortexing, and prolonged evaporation. Instead, a quick, one-step method was recently developed using the sonication of a cocktail mixture of the precursor polymer and lipid solutions. This scaling up method led to a twenty-fold reduction in the time required for particle preparation and also allowed for tunable particle sizes with enhanced monodispersity.

6. Cellular membrane-derived vesicles

Liposomal nanostructures made of synthetic materials represent a bottom-up process in which specific functionalities are built rationally into each platform. While this approach allows for incredible control over final particle characteristics, it has proven difficult in mimicking or replicating complex functionalities found on natural systems for drug delivery applications.[20] As alternatives, increasing effort has been put on leveraging natural cellular membrane-derived vesicles for use as drug carriers among other related applications. The benefit of natural as opposed to synthetic membranes is that the resulting vesicles retain many of the natural functionalities of the source material, providing each system with unique characteristics that would otherwise be very difficult to achieve.[134] In addition, using genetic modification to alter the antigen profile of natural membranes has also proven effective in enhancing the functionality of the derived vesicles for drug delivery.[135, 136]

Nanoscale vesicles can be directly found in nature. These natural vesicles serve different biological purposes, for example, exosomes released by mammalian cells are for extracellular signaling[137, 138] and bacterial outer membrane vesicles (OMVs) are for self-defense[139]. Alternatively, the vesicles can also be artificially derived from purified cellular membranes. In this case, membrane ghosts are first prepared by removing the intracellular contents followed by preparing liposomal vesicles of desired sizes.[140, 141]

Exosomes are vesicles approximately 40~100 nm in size. Various types of mammalian cells produce exosomes by inward budding of plasma membranes followed by their release into the extracellular space.[142, 143] In addition to their endogenous origin and close resemblance to the source cells' plasma membranes, exosomes have attracted attention for drug delivery applications because of their diverse biological roles in immunity, angiogenesis, hemostasis, and tumor pathogenesis.[144–146] For example, $CD11b^{+}Gr\text{-}1^{+}$ cells circulating in the peripheral blood scavenge tumor cell-produced exosomes. As sequestered $CD11b^{+}Gr\text{-}1^{+}$ cells contribute to the acute lung inflammation in lipopolysaccharide (LPS)-induced sepsis, exosomes loaded with anti-inflammatory drugs led to higher apoptosis of $CD11b^{+}Gr\text{-}1^{+}$ cells compared to formulations using synthetic liposomes.[147] Intriguingly, exosomes can also be used to deliver therapeutic agents to the brain. Crossing of the blood brain barrier by exosomes results from their inherent targeting ability to brain cells. For example, intranasal administration of drug-loaded exosomes was selectively taken up by microglial cells in the brain, resulting in higher cell apoptosis and better efficacy for treating brain inflammatory-related diseases.[148] Additionally, exosomes can be engineered to present brain-specific ligands for targeted delivery. For example, dendritic cells engineered to express Lamp2b, an exosomal membrane protein, fused with the neuron-specific RVG peptide showed efficient targeting to brain.[149] When injected intravenously, these exosomes delivered siRNA

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specifically to neurons, microglia, and oligodendrocytes in the brain, resulting in specific gene knockdown. Moreover, the regulatory roles played by exosomes in tumor pathogenesis inspire the use of these vesicles for cancer immunotherapy. For example, exosome-bound tumor antigens induced a more potent antigen-specific antitumor immune response than the corresponding soluble antigens.[150] The translational potential of exosome-based vaccines has also been verified recently for MV-BN-PRO, a candidate immunotherapy product currently in clinical phase I/II for treating prostate cancer.[151]

In contrast to endogenous vesicles, OMVs are secreted by bacteria.[139, 152] These vesicles, approximately 20–250 nm in size, have been intensively explored as adjuvanted antigen carriers for immunotherapy against infections.[153, 154] In particular, OMV vaccines have been successfully applied to control outbreaks of serogroup B meningococcal disease in Norway, New Zealand, and Cuba.[155, 156] Genetic manipulation of bacteria to modify the membrane composition and protein expression of the resulting OMVs offers a compelling strategy to further improve on OMV-based vaccines.[157] For example, green fluorescent protein (GFP) used as a model antigen was fused with ClyA of *Escherichia coli* and subsequently expressed on OMV surfaces. These genetically modified OMVs induced strong GFP-specific titers without the need of an additional adjuvant.[158]

Cellular membrane-derived vesicles can also be artificially made from natural membranes. Compared to vesicles produced by natural processes, artificial enrichment of cellular membranes may be advantageous for larger scale vesicle production, making them promising for downstream translation as seen with liposomal nanostructures made of synthetic materials. In this respect, RBC membrane has received the most attention, mostly due to the relative simplicity of RBCs, the well-established protocols of membrane collection, and the significant biological functions that RBCs play.[140] RBC membrane-derived vesicles, also termed “nanoerythrosomes” were first prepared by physical extrusion of RBC ghosts through membranes with a defined pore size.[159] This approach maintains the material biocompatibility and preserves the complex protein makeup of RBCs essential for their bio-functionalities. Nanoerythrosomes have an average diameter of 100~200 nm, a size range allowing for passive targeting to tissues with leaky vasculature. In addition, these vesicles are biodegradable without toxicity and have no immunogenicity in autologous applications. In a mouse model bearing P388 D1 leukemia, the daunorubicin-conjugated nanoerythrosomes showed higher antineoplastic activity compared to free daunorubicin, suggesting that nanoerythrosomes could better deliver the drug to cancer cells.[160] To understand the mechanism of improved efficacy, further study found that phagocytosis of the drug-conjugated nanoerythrosomes was not involved in their mechanism of action.[161] Closer examination of the interactions between nanoerythrosomes and targeted cells revealed that the daunorubicin-nanoerythrosome complex did not diffuse across the cell membranes, nor did they enter the cell by endocytosis. Instead, according to fluorescence microscopy studies, they were rapidly adsorbed onto the cell surfaces, slowly released free drugs by hydrolysis of the cross-linker, and produced a high concentration of free daunorubicin in the cell’s vicinity over a long period of time. In addition, pharmacokinetic studies showed that different sample purification procedures and routes of parenteral administration caused different biodistribution of nanoerythrosomes in mice. Similar to conventional liposomes, the lack of stability is a concern that limits the applications of nanoerythrosomes.[162] Accordingly, strategies such as covalent conjugation of PEG to the amino acid residues on the membrane surface were attempted to further stabilize the nanoerythrosomes.[163]

Efforts have also been made to derive vesicles by using membranes collected from other types of mammalian cells, which have more complex structure and function compared to RBCs. In this respect, mesenchymal stem cells (MSCs) are often chosen as an efficient

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source to produce cancer-targeting vesicles, as MSCs are capable of homing to many types of cancers at different developmental stages (Figure 11).[164–166] MSCs themselves have also been used as cellular delivery vehicles to target anticancer agents to malignant tumors.[167, 168] Therefore, using MSC membrane-derived vesicles, a variety of tumors requiring MSC support may be targeted through a tumor-tropic mechanism. For example, systemic administration of drug-loaded MSC membrane-derived vesicles significantly reduced tumor burden by 80% in a human prostate tumor model.[166] Vesicles were also derived from the membranes of cells expressing CCR5, the human receptor for gp120, an antigen found on the surface of virions and HIV-infected cells.[169] These membrane-derived vesicles preferentially targeted HIV-infected gp120-expressing cells and induced a higher level of cell death compared to control cells that did not express gp120.

7. Cellular membrane-functionalized nanoparticles

More recently, using cellular membrane to functionalize synthetic nanoparticles has emerged as a new and promising strategy to create biomimetic hybrid nanocarriers with great potential for a broad range of drug delivery applications. The encapsulation of synthetic nanoparticles within cellular membranes can be achieved by taking advantage of natural cellular processes where nondegradable particles are first internalized by cells through endocytosis and subsequently released from the cells in a vesicle-enclosed form.[170] Using this approach, various membrane-coated hybrid nanoparticles were produced including magnetic, magnetic-metallic and magnetic-fluorescent particles. These hybrid nanoparticles combine the advantageous properties of each integrant component and represent useful platforms for nanotheronostics.[171]

Another intriguing approach to functionalizing nanoparticles with cellular membranes is to first collect intact cellular membranes and then to coat them on the particles. Since this approach is independent of cellular processes, the membrane coating can be applied not only to inert inorganic particles but also to biodegradable particles. The separate preparation of cellular membranes and particle cores prior to coating offers a new level of engineering flexibility.[22] Centered in this technological advancement is RBC membranes used as coating materials. RBCs are nature's long-circulating carriers, and have long inspired researchers to develop man-made systems with extraordinary delivery capabilities.[21] For example, polymeric particles mimicking the shape and deformability of natural RBCs have been shown to pass through vessels more easily than their stiffer counterparts.[172–175] Similarly, the biochemical features of RBC membranes such as the CD47 'mark-of-self' protein were mimicked and used to functionalize nanoparticles, which allows for reduced uptake of the particles by macrophages.[176–179] In contrast to this type of 'bottom-up' approach for mimicking the complex functionalities of RBCs, translocating the entire cellular membrane to the surface of synthetic nanoparticles presents a robust 'top-down' approach for preparing cellular membrane-functionalized nanoparticles.

For example, to formulate RBC membrane-coated polymeric nanoparticles, polymeric cores and RBC membrane-derived vesicles were first prepared by nanoprecipitation and mechanical extrusion, respectively.[22] The two components were then mixed and extruded together, forcing the adsorption of RBC membranes onto the surface of polymeric cores. The resulting particles were sub-100 nm in size with excellent stability in biological buffer solutions and serum. Transmission electron microscope imaging demonstrated a core-shell structure, and gel electrophoresis measurements confirmed that the bulk of the RBC membrane proteins were retained on the RBC membrane-coated nanoparticles. The co-localization of RBC membranes and polymeric cores upon cellular uptake of the hybrid nanoparticles further verified their structural integrity and stability during particle formulation and drug delivery. Further study showed that the coating process preserves the

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right-side-out orientation of the cell membrane and that CD47 is present at a density consistent with that of native RBCs.[180] Additionally, these biomimetic nanoparticles had an in vivo circulation half-life of nearly 40 hr in a mouse model, a significant improvement over the 16 hr of the corresponding PEGylated nanoparticles.[22] Comparison of the RBC membrane-coated nanoparticles with the aforementioned nanoerythrosomes also showed a significant improvement in particle stability in vivo, implying that the polymeric cores played a significant role in maintaining the integrity and structural stability of RBC membranes. Moreover, chemotherapy drugs such as doxorubicin could be readily loaded into the polymeric cores by either physical encapsulation or chemical conjugation for controlled and sustained drug delivery. The membrane coating surrounding the particles provided an additional diffusion barrier that reduced drug release rate.[181]

Meanwhile, the unique biological roles played by RBCs in host-pathogen interactions has inspired the development of RBC membrane-coated nanoparticles beyond cargo delivery applications. For example, in bacterial infections, attack by pore-forming toxins is a major virulence mechanism.[182] These toxins are secreted by pathogenic bacteria to disrupt host cells by forming pores in cellular membranes and altering their permeability.[183] Polymeric nanoparticles wrapped within RBC membranes can act as a toxin ‘nanosponge’ that mimics natural RBCs to absorb membrane-damaging toxins and divert them away from their cellular targets (Figure 12).[184] In a mouse model, the nanosponge markedly reduced the toxicity of staphylococcal alpha-hemolysin (α-toxin) and offered a significant survival advantage for the toxin-challenged mice. The most innovative feature of the toxin nanosponge lies in its capability for targeting the membrane-disrupting mechanism of pore-forming toxins; it can therefore function as an all-purpose toxin decoy to adsorb various types of pore forming toxins regardless of their molecular structures. In contrast, existing detoxification techniques such as antisera,[185] monoclonal antibodies,[186] small-molecule inhibitors[187, 188] and molecularly imprinted polymers[189] target the molecular structures of toxins and thereby require toxin-specific custom synthesis for different disease treatments.

The technique of using natural cellular membrane to coat synthetic nanoparticles was recently extended from polymeric nanoparticles to inorganic gold nanoparticles (AuNPs).[190] AuNPs have found widespread applications as imaging agents and drug carriers in biology and medicine.[191, 192] Their modification using the entirety of a cell membrane provides improved functions and advanced biomimetic features. Facilitated by external mechanical forces, RBC membranes spontaneously fuse onto solid AuNPs to form RBC membrane-coated AuNPs (RBC-AuNPs). The resulting RBC-AuNPs possess right side-out RBC membranes and the associated membrane proteins, which provide the AuNPs with immunosuppressive functionalities for evading macrophage uptake. In addition, the membrane coating effectively shields the particles from interacting with thiolated compounds. When synthetic AuNPs are integrated with natural cellular membranes, the particles can be bestowed with a wide range of functionalities responsible for cells’ diverse antigenic, transport, and mechanical characteristics. The RBC-AuNPs embody a new materials design strategy and present an intriguing class of advanced materials for a broad range of biomedical applications.

Cellular membrane-coated nanoparticles promise to usher in a new class of biomimetic delivery platforms. The technique can be extended to membranes of other cell types such as leukocytes on different solid cores such as silica.[193] This strategy allows for the use of validated biomaterials that have been extensively studied, and the membrane coating process does not demand major chemical modification. The use of natural membranes also bypasses the labor-intensive processes of protein identification, purification, and conjugation. Looking forward, the integration of natural and synthetic biomaterials to form functional

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nanostructures offers a new paradigm of thinking about the designs and uses of nanomedicine.

8. Conclusion

Liposomes have come a long way to become a class of validated drug carriers. As they are continually advancing to improve healthcare, an increasing variety of liposome-like nanostructures are under development, each with unique strengths suitable for specific drug delivery tasks. Meanwhile, knowledge in understanding the interactions between these nanostructures and biological systems is rapidly progressing. A substantial amount of information on their circulation time, tissue accumulation, and potential toxicity has been obtained. It is certain that liposome-like nanocarriers will play a larger role for drug delivery in the foreseeable future.

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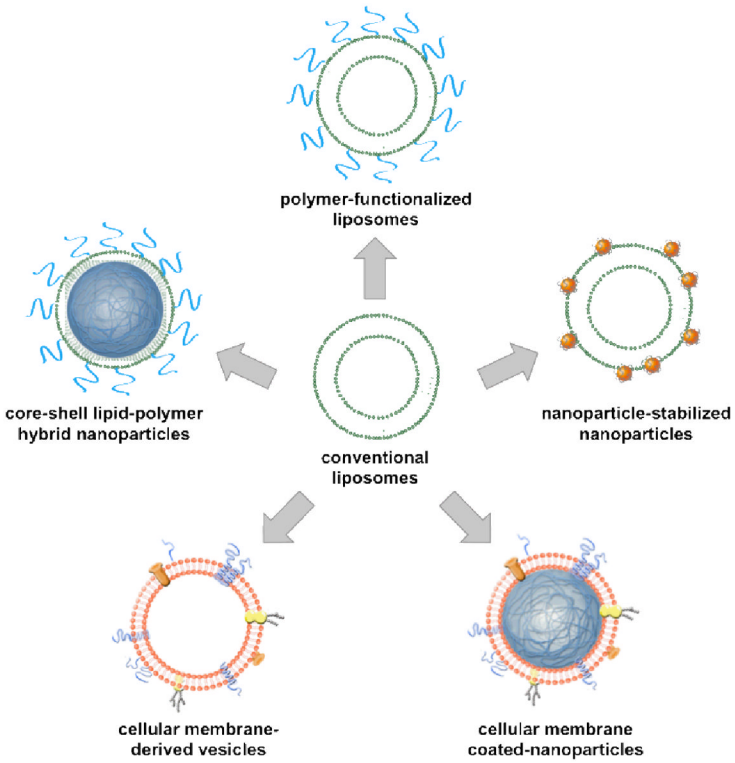

Figure 1.
Major liposome-like nanostructures reviewed in this article, including conventional liposomes, polymer-functionalized liposomes, nanoparticle-stabilized liposomes, core-shell lipid-polymer hybrid nanoparticles, cellular membrane-derived vesicles, and cellular membrane-coated nanoparticles.

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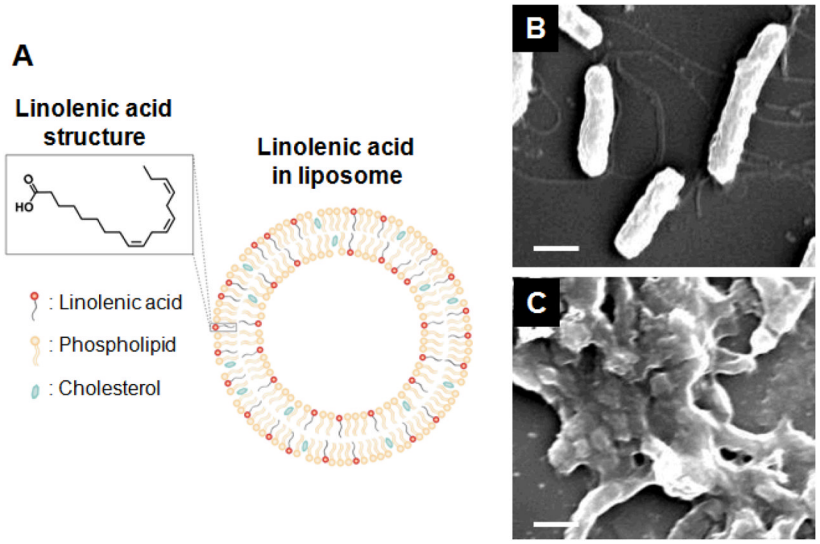

Figure 2.
(A) A schematic drawing showing the molecular structure of linolenic acid and a liposome composed of linolenic acid, phospholipid and cholesterol. (B)–(C): Morphology of *H. pylori* ss1 bacteria in their spiral form treated with (B) PBS and (C) liposomal linolenic acid. The scale bar in the image represents 1 μm. Reproduced with permission from reference [42].

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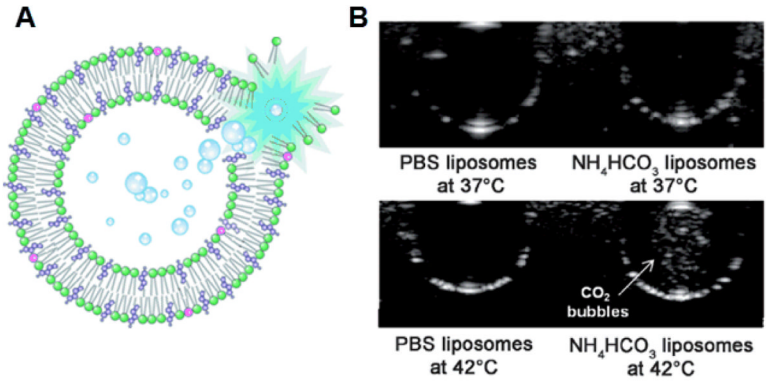

Figure 3.
(A) A schematic illustration of the liposomes that generate bubbles upon heating, thus killing cancer cells by the generated mechanical forces. (B) Ultrasound images of PBS and NH_4HCO_3 liposomes suspended in aqueous media and heated to 37 and 42°C. Reproduced with permission from reference [45].

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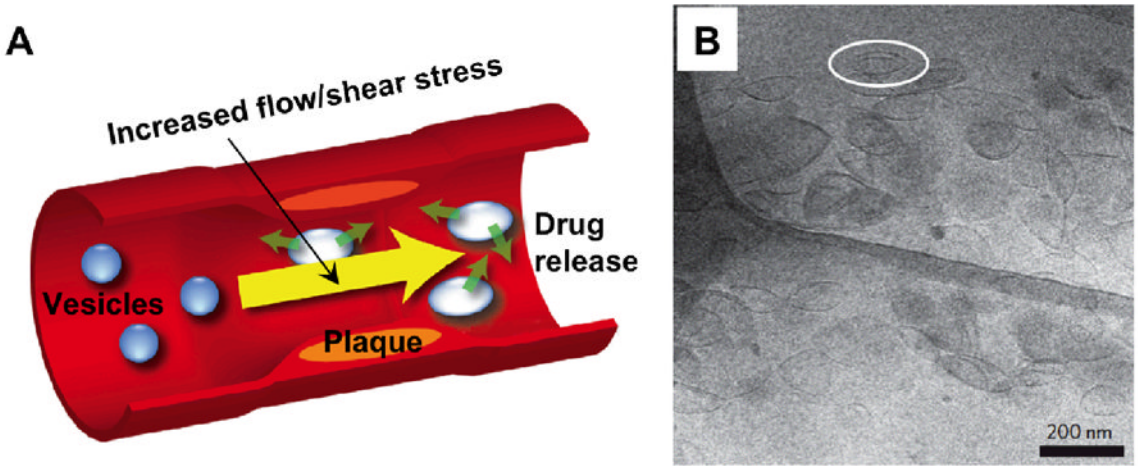

Figure 4.

(A) Schematic of employing the changes in endogenous shear stresses as a physical trigger for liposomal drug delivery. (B) The lenticular morphology of the 100 nm liposomes is visualized by cryo-TEM. An example of this lenticular shape, with two spherical segments joined by a discontinuity around the diameter, is highlighted. The straight edges contrast with the round morphology typically found in liposomes formulated from natural phospholipids. Reproduced with permission from reference [51].

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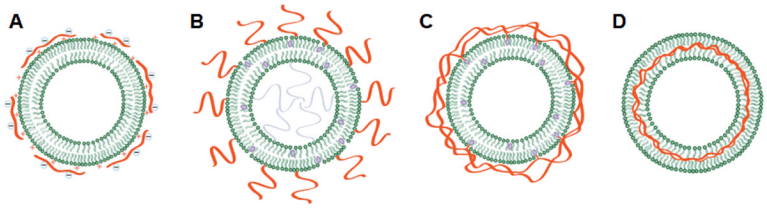

Figure 5.
Strategies of using polymers to stabilize liposomes. (A) Polymers are physically adsorbed onto the outer surface of liposomes for stabilization. (B) Polymers are covalently linked to the head groups of lipids to stabilize liposomes. (C) Polymerizable monomers are pre-conjugated to the head groups of lipids and then polymerized to form a polymer cage surrounding and stabilizing the liposomes. (D) The hydrophobic tail groups of lipids are cross-linked within lipid bilayers to stabilize liposomes.

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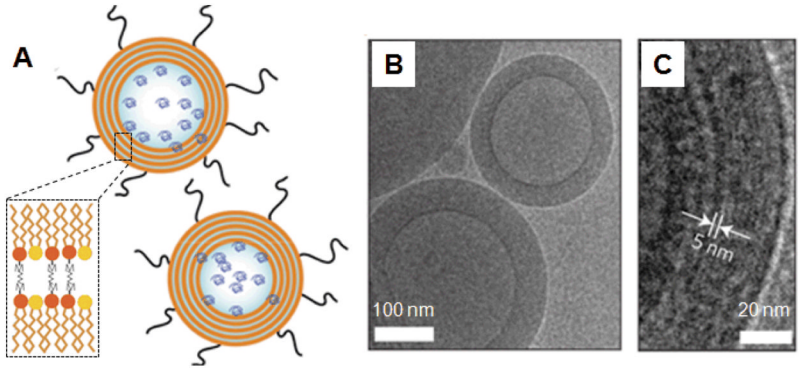

Figure 6.
(A) Schematic illustration of interbilayer-crosslinked multilamellar vesicles (ICMVs). Dithiols are added to crosslink maleimide lipids on apposed lipid bilayers in the multilamellar vesicles and the resulting lipid particles are PEGylated with thiol-terminated PEG. (B) Cryo-electron-microscope images of the ICMVs with thick lipid walls. (C) A zoomed-in image of an ICMV wall. Reproduced with permission from reference [80].

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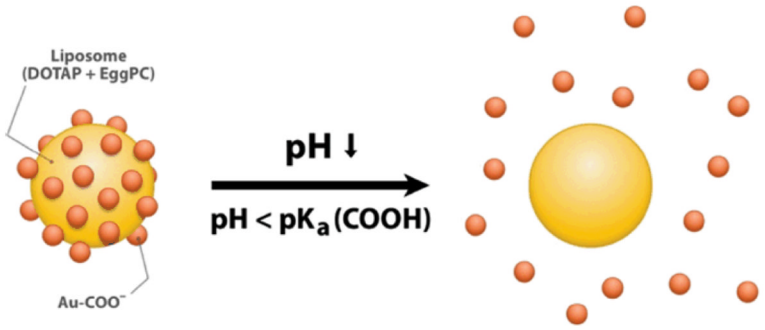

Figure 7.

Schematic illustration of a carboxyl-modified gold nanoparticle (AuC)-stabilized liposome and its destabilization at acidic pH. The liposome is stabilized by deprotonated AuC (Au-COO^-) at neutral pH. When pH drops below the pKa value of the carboxylic groups (pKa~5), Au-COO^- nanoparticles are protonated to form Au-COOH, which subsequently detach from the liposome, resulting in the formation of a bare liposome with restored fusion activity. Reproduced with permission from reference [14].

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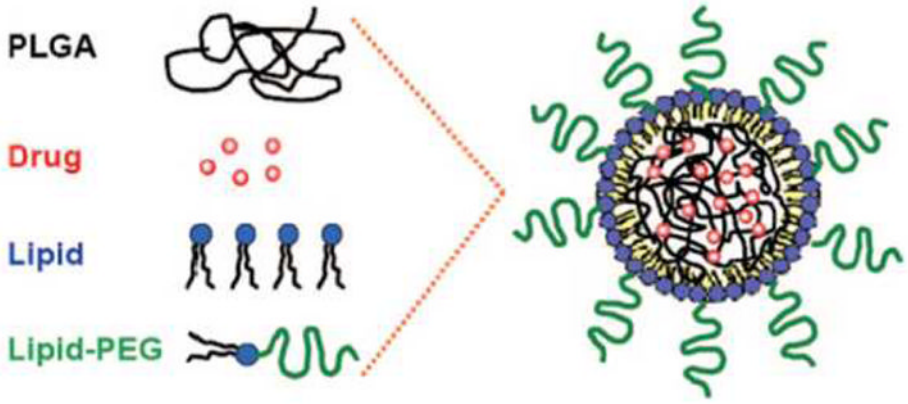

Figure 8.
Schematic illustration showing the formulation of lipid-polymer hybrid nanoparticles. The nanoparticles comprise a hydrophobic PLGA core, a hydrophilic PEG shell, and a lipid (lecithin) monolayer at the interface of the hydrophobic core and the hydrophilic shell. Reproduced with permission from reference [16].

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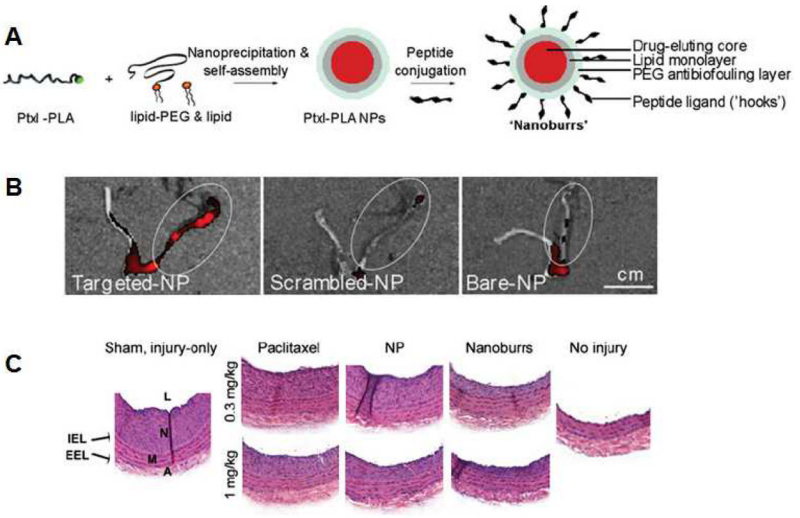

Figure 9.

(A) Schematic of nanoburr synthesis by nanoprecipitation and self-assembly. Nanoburrs have a drug-eluting polymeric core, a lipid monolayer, a PEG antibiofouling layer, and peptide ligands (hooks) that adhere to the exposed basement membrane during vascular injury. (B) Nanoburrs targeting angioplasty model of injured vasculature. Fluorescence images overlayed on photographs of carotid arteries incubated with nanoburrs, compared with scrambled-peptide and non-targeted nanoparticles. For imaging, Alexa Fluor 647–PLGA dye conjugates were encapsulated in place of Ptxl–PLA drug conjugates. (Scale bar, 1 cm.) (C) Representative H&E-stained sections from different treatment groups, where the nanoburrs were used as a treatment for cellular proliferation after arterial injury. Note the difference in lumen patency between different doses of the same treatment group and also compared with sham, injury-only groups. L, lumen; N, neointima; M, media; A, adventitia; IEL, internal elastic lamina; EEL, external elastic lamina. Reproduced with permission from references [117,118].

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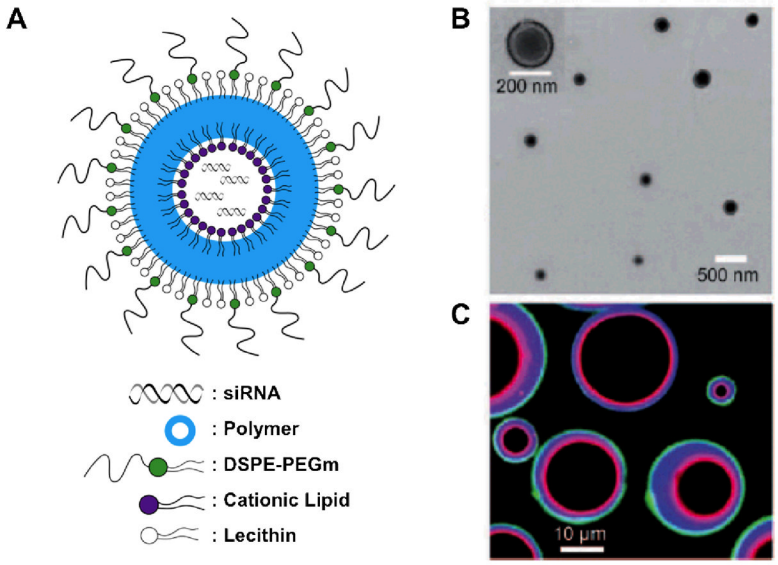

Figure 10.

(A) Schematic representation of a lipid–polymer–lipid hybrid nanostructure developed for siRNA delivery. The particle is composed of an outer lipid–PEG surface, a middle polymer layer, and an inner cationic lipid hollow core entrapping aqueous siRNA. (B) Representative TEM image of the hybrid nanoparticles and (C) confocal laser scanning fluorescence image of the hybrid microparticles demonstrated the existence of outer lipid–PEG layer (green) and inner lipid layer (red), separated by a PLGA layer (blue). Reproduced with permission from reference [120].

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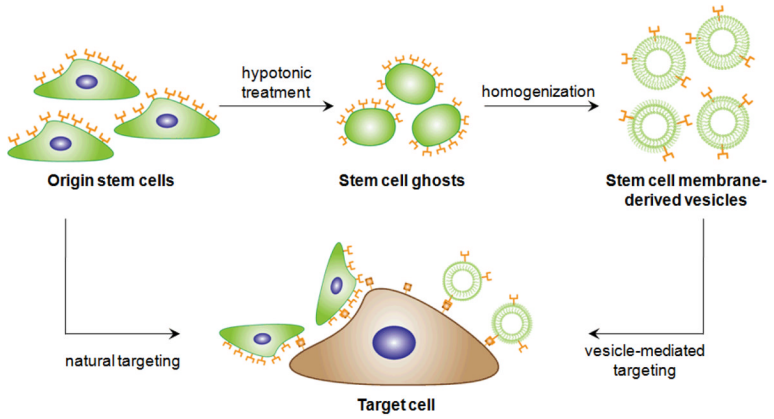

Figure 11.

Schematic showing vesicle derivation from mesenchymal stem cells (MSCs). Both original stem cells and the MSC membrane-derived vesicles are used as targeted delivery platforms. The MSC-derived vesicles retain MSC-specific tumor targeting capabilities both in vitro and in vivo. These vesicles are biocompatible and can be cleared from circulation by blood-filtering organs. Reproduced with permission from reference [116].

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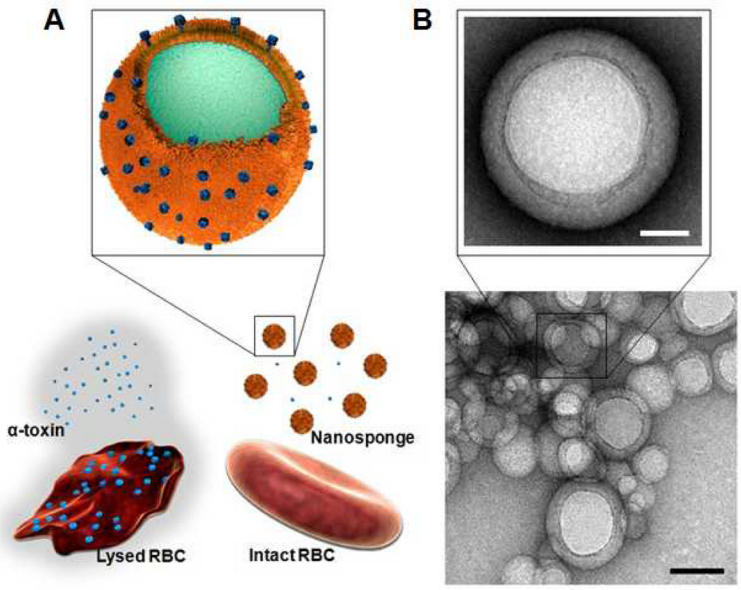

Figure 12.
The schematic and actual structure of the nanosponge. (A) Schematic structure of toxin nanosponges and their mechanism of neutralizing pore-forming toxins (PFTs). The nanosponges consist of substrate-supported RBC bilayer membranes into which PFTs can incorporate. After being absorbed and arrested by the nanosponges, the PFTs are diverted away from their cellular targets, thereby avoiding target cells and preventing toxin-mediated haemolysis. (B) TEM visualization of nanosponges mixed with a-toxin (scale bar, 80 nm) and the zoomed-in view of a single toxin-absorbed nanosponge (scale bar, 20 nm). Reproduced with permission from reference [184].

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Table 1

Liposomal drugs on market (adapted from references [6,7]).

Name	Drug	Injection Route	Storage Form	Lipid Composition	Approved Indication
Ambisome	Amphotericin B	Intravenous	Powder	HSPC, DSPG, cholesterol, and amphoteracin B (2:0.8:1:0.4 molar ratio)	Fungal infections
Abelcet	Amphotericin B	Intravenous	Suspension	DMPC and DMPG (7:3 molar ratio)	Fungal infections
Amphotec	Amphotericin B	Intravenous	Powder	Cholesteryl sulfate	Fungal infections
DaunoXome	Daunorubicin	Intravenous	Suspension	DSPC and cholesterol	Leukemia
Myocet	Doxorubicin	Intravenous	Powder	EPC and cholesterol (55:45 molar ratio)	Combination therapy with cyclophosphamide in metastatic breast cancer
Visudyne	Verteporfin	Intravenous	Powder	EPG and DMPC (3:5 molar ratio)	Age-related molecular degeneration, pathological myopia, ocular histoplasmosis
Depocyt	Cytarabine	Spinal	Suspension	Cholesterol, Triolein, DOPC, and DPPG (11:1:7:1 ratio)	Neoplastic meningitis and lymphomatous meningitis
DepoDur	Morphine sulfate	Epidural	Suspension	Cholesterol, Triolein, DOPC, and DPPG (11:1:7:1 ratio)	Pain management
Epaxal	Inactivated hepatitis A virus (strain RG-SB)	Intramuscular	Suspension	DOPC and DOPE	Hepatitis A
Inflexal V	Inactivated hemaglutinine of influenza virus strain A and B	Intramuscular	Suspension	DOPC and DOPE	Influenza
Doxil	Doxorubicin	Intravenous	Suspension	HSPC, cholesterol, and PEG2000-DSPE (56:39:5 molar ratio)	Kaposi’s sarcoma, ovarian and breast cancer
Lipo-dox	Doxorubicin	Intravenous	Suspension	HSPC, cholesterol, and PEG2000-DSPE (55:39:5 molar ratio)	Kaposi’s sarcoma, ovarian and breast cancer

Abbreviations: DOPE, dioleoylphosphatidylethanolamine; DOPC, dioleoylphosphatidylcholine; DPPG, dipalmitoylphosphatidylglycerol; HSPG, hydrogenated soy phosphatidylcholine; DSPG, distearoylphosphatidylglycerol; EPC, egg phosphatidylcholine; DSPC, distearoylphosphatidylcholine; DMPC, 1-α-dimyristoylphosphatidylcholine; DMPG, 1-α dimyristoylphosphatidylglycerol; EPG, egg phosphatidylglycerol; PEG 2000-DSPE, polyethylene glycol 2000-distearoylphosphatidylethanolamine

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