Nano LIFE
Vol. 1, Nos. 1 & 2 (2010) 163−173

DOI: 10.1142/S179398441000016X

World Scientific
www.worldscientific.com

LIPID–POLYMER HYBRID NANOPARTICLES: SYNTHESIS, CHARACTERIZATION AND APPLICATIONS

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Received 1 July 2010
Accepted 22 July 2010

Nanotechnology has been extensively explored in the past decade to develop a myriad of functional nanostructures to facilitate the delivery of therapeutic and imaging agents for various medical applications. Liposomes and polymeric nanoparticles represent two primary delivery vehicles that are currently under investigation. While many advantages of these two particle platforms have been disclosed, some intrinsic limitations remain to limit their applications at certain extent. Recently, a new type of nanoparticle platform, named lipid−polymer hybrid nanoparticle, has been developed that combines the positive attributes of both liposomes and polymeric nanoparticles while excluding some of their shortages. This new nanoparticle consists of a hydrophobic polymeric core, a lipid shell surrounding the polymeric core, and a hydrophilic polymer stealth layer outside the lipid shell. In this review, we first introduce the synthesis and surface functionalization techniques of the lipid−polymer hybrid nanoparticle, followed by a review of typical characterization of the particles. We then summarize the current and potential medical applications of this new nanoparticle as a delivery vehicle of therapeutic and imaging agents. Finally we highlight some challenges faced in further developing this robust delivery platform.

Keywords: Hybrid nanoparticle; liposome; polymeric nanoparticle; drug delivery; controlled release; targeted delivery.

1. Introduction

The development of highly selective and effective nanoparticles for drug delivery has brought new hope for the treatment of various notorious diseases such as cancers, cardiovascular diseases, diabetes, bacterial infections, and so on.[1−4] As a delivery vehicle, nanoparticles with a size range of 10−150 nm have demonstrated many advantages compared with the conventional approaches to the uses of drugs.[5−10] For example, therapeutic nanoparticles can improve the solubility of poorly water-soluble drugs, prolong the half-life of drugs in the systemic circulation by reducing immunogenicity, release drugs at a sustained rate and thus lower the frequency of administration, deliver drugs in a targeted manner to minimize systemic side effects, and deliver two or more drugs simultaneously for combination therapy to generate synergistic effects. As a result, a myriad of nanoparticle platforms

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have been designed for drug delivery applications. Some nanoparticle-based drug delivery products have been approved for clinical use. Numerous other ensuing products are currently under clinical testing or entering the pipeline.[11,12] Among these clinical or pre-clinical products, liposomes and biodegradable polymeric nanoparticles represent the two most successful classes of drug delivery nanocarriers.

Liposomes are spherical lipid vesicles with a bilayer membrane structure consisting of amphiphilic lipid molecules.[13] Liposomes have been widely studied and used to deliver both hydrophilic and hydrophobic drugs in the past decades.[7,14] Doxil was the first liposome drug formulation approved by the Food and Drug Administration (FDA) for the treatment of AIDS associated with Kaposi's sarcoma in 1995.[15,16] Other liposomal drug formulations that are also commercially available include DaunoXome (daunorubicin liposomes), DepotDur (morphine liposomes), Visudyne (verteporfin liposomes), DepoCyt (cytarabine in liposomes) and Ambisome (amphotericine B liposomes).[12,17,18] The benefits of liposomal formulations include ability to carry hydrophilic drugs inside the aqueous vesicles and to carry hydrophobic drugs within the lipid bilayer membranes, high biocompatibility that provides perfect shield to protect drugs from external environment, and easy surface modification with other molecules such as polyethylene glycol (PEG) and targeting ligands to achieve prolonged systemic circulation lifetime and targeted drug delivery, respectively. However, the applications of liposomes are typically limited by some unfavorable features such as relatively complicated fabrication steps associated with liposome preparation and purification, low loading efficiency for hydrophobic drugs, burst release kinetics of encapsulated drugs, and instability during storage leading to short shelf-time.

On the other hand, biodegradable polymeric nanoparticles have shown great therapeutic potential as a drug delivery nanocarrier.[19–24] Biodegradable polymers such as poly(D, L-lactic-co-glycolic acid) (PLGA) and poly(ε-caprolactone) (PCL) have been used in several FDA-approved therapeutic products. Their co-polymers with PEG are commonly used to form core–shell structured nanoparticles to encapsulate a variety of therapeutic agents.[25–28] These polymeric nanoparticles can carry hydrophobic drugs with a higher loading capacity than liposomes. Drug release from polymeric nanoparticles is usually dominated by polymer degradation and drug diffusion, which can be controlled by choosing proper polymers with desirable degradation rates and binding affinity with the encapsulated drugs. Moreover, polymeric nanoparticles can be prepared by self-assembly of the block copolymers through a simple nanoprecipitation method, which allows cost-effective large-scale fabrication of the particles. Despite all these appealing features, polymeric nanoparticles have not gained as much success as liposomes, presumably due to their moderate circulation lifetime and potential biocompatibility issues.

Recently, efforts have been made to combine the positive attributes of both liposomes and polymeric nanoparticles into a single delivery system, called lipid–polymer hybrid nanoparticles.[29–35] This type of nanoparticles is typically comprised of three distinct functional components: (i) a hydrophobic polymeric core where poorly water-soluble drugs are incorporated with high loading yields; (ii) a lipid layer surrounding the core that acts as a highly biocompatible shell and as a molecular fence to promote drug retention inside the polymeric core; and (iii) a hydrophilic polymer stealth layer outside the lipid shell to enhance nanoparticle stability and systemic circulation lifetime. The polymeric core and the lipid shell are associated through hydrophobic interactions, van der Waals forces, electrostatic interactions or other non-covalent forces. The hydrophilic polymer stealth layer is often conjugated to the lipid shell through covalent bonds. These lipid–polymer hybrid nanoparticles have been demonstrated to include the unique advantages of both liposomes and polymeric nanoparticles while excluding some of their intrinsic limitations, thereby holding great promise as a delivery vehicle for various medical applications.

In this review article, we will first introduce the synthesis methods of the lipid–polymer hybrid nanoparticles and their surface functionalization, followed by a review of the physicochemical and biomedical characterization of the nanoparticles. We will then summarize the current and potential medical applications of this new class of nanoparticles as a delivery vehicle of therapeutic and imaging agents. Finally we will highlight some challenges faced in further developing this robust delivery platform.

2. Synthesis of Lipid–Polymer Hybrid Nanoparticles

In general, lipid–polymer hybrid nanoparticles can be synthesized through two distinct approaches. As

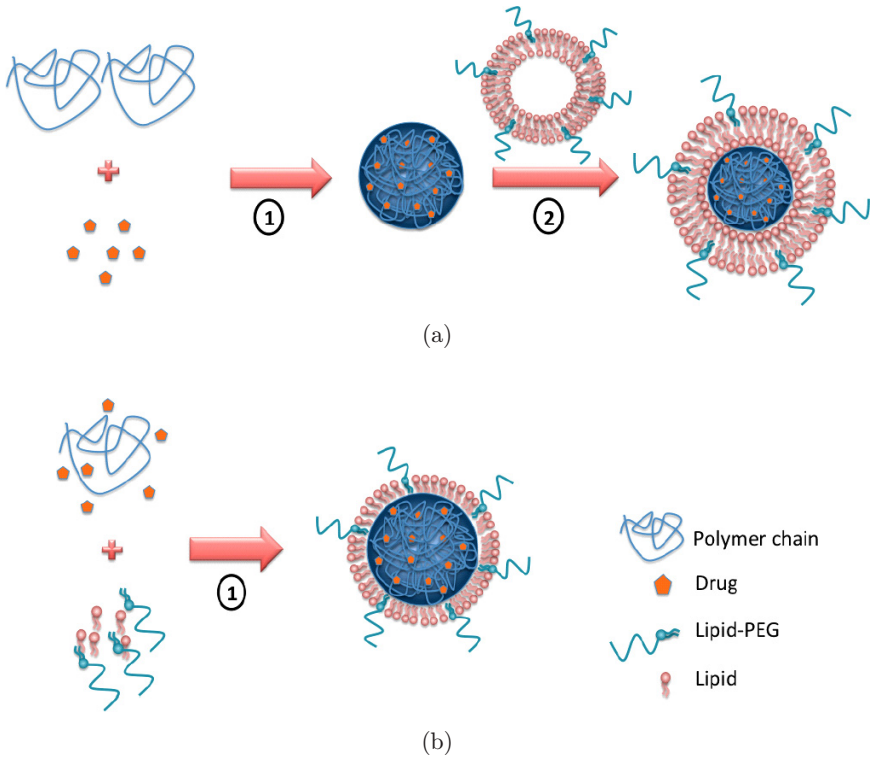

Fig. 1. Schematic illustrations of synthesis approaches of lipid–polymer hybrid nanoparticles. (a) Two-step synthesis approach involving a step of polymer core preparation followed by fusion between polymer cores and preformed lipid films or liposomes. (b) One-step synthesis approach that the hybrid nanoparticles are formed through a nanoprecipitation and self-assembly method in one-pot by mixing drug containing polymer solution with lipid aqueous solution.

illustrated in Fig. 1, one approach involves a two-step process in which the polymer core and lipid shell are prepared separately and then merged together; the other approach involves a single-step process, in which the hybrid nanoparticles are prepared through a one-pot nanoprecipitation and self-assembly method. For biomedical applications, the surface of the resulting lipid–polymer hybrid nanoparticles are usually further functionalized with targeting ligands for cell- or tissue-specific delivery of the payloads.

2.1. *Two-step synthesis approach*

The two-step synthesis approach is typically used to prepare lipid–polymer hybrid nanoparticles with a lipid bilayer or multilayer shell. In this approach, the polymer core is formed through an emulsion method,[34] high-pressure homogenization method,[30] or nanoprecipitation method.[32] Liposome is prepared through a sonication method or extrusion method.[13] Then the polymer cores are mixed with the preformed liposomes at desirable molar ratios to prepare lipid–polymer hybrid nanoparticles by needle extrusion, high-pressure homogenization, or simply vortexing.

Several methods can be used to synthesize the polymer core of the hybrid nanoparticles depending on the hydrophobicity of the payloads and the needed size of the core. If the payload is miscible with the polymer in an organic solvent or the payload is covalently conjugated to the polymer chain, a single emulsion method is usually applied to prepare the payload-encapsulated polymer core.[36,37] Briefly, the payloads and the polymers are dissolved in a water immiscible organic solvent such as chloroform. Emulsion will be formed by adding the polymer solution into an aqueous solution that contains proper emulsifier, followed by high-speed homogenization. If the payloads are hydrophilic and cannot be dissolved into the organic solvent, a water-in-oil-in-water (w/o/w) double emulsion method is needed to prepare the polymer core.[38,39] Briefly, a water-in-oil (w/o) emulsion is formed by adding a payload-containing aqueous solution into a polymer-containing organic solvent. The resulting w/o emulsion is subsequently added into a second aqueous solution to form w/o/w double emulsion. The double emulsion is

then hardened in an aqueous solution by evaporating the organic solvent. Generally, the emulsion method involves multiple steps in preparation and high-energy homogenization and results in large polymer particles with high polydispersity index.

High-pressure homogenization represents another method to prepare polymer core by using very high pressure to break polymer solutions or melted polymers into small droplets as they pass through a very narrow nozzle.[40] The obtained sub-micron-sized droplets are subsequently hardened by spray drying or simply cooling down to room temperature. This method is simple and easy to control, however it has high demands on equipment and the resulting polymer particles are typically hundreds of nm in diameter.

In contrast, nanoprecipitation method is widely used to prepare sub-100 nm polymer particles.[19,41,42] This method involves the use of two miscible solvents; one is a good solvent of the polymer and the other one is a poor solvent of the polymer. The polymer is first dissolved in the good solvent and then added to the poor solvent. As the good solvent diffuses into the poor solvent, the polymer will spontaneously precipitate out to form tiny particles. The mixing of the two solvents can happen through dropwise addition, stirring or sonication. Polymer concentration, volume ratio of the two solvents, and mixing rate can be tuned to control the size and polydispersity of the particles.

Once the polymer particles are prepared, they can be mixed with preformed lipid films or liposomes to form lipid−polymer hybrid nanoparticles. The lipid components are usually dissolved in an organic solvent such as chloroform.[43] A thin lipid film is formed by evaporating the organic solvent, to which an aqueous solution is added to rehydrate the lipids. The polymer particles can be added together with the aqueous solution to rehydrate the lipid films or mixed with liposomes after they are prepared. Nevertheless, upon mixing through high-pressure homogenization, high-speed vortexing, or extrusion, the lipid films or liposomes fuse on the surface of polymer core resulting in the formation of lipid−polymer hybrid nanoparticles. When the concentrations of both lipids and polymer core are properly controlled, a lipid bilayer will form on the surface of the polymer core due to noncovalent interactions. For example, cationic lipids can form a bilayer on carboxylic-acid-terminated PLGA polymer core through electrostatic attractions. When extra lipids are present in the mixture, a multilayer of lipids will form on the polymer core, resulting in an irregular lipid shell.[35]

2.2. *One-step synthesis approach*

The one-step synthesis approach is typically used to prepare lipid−polymer hybrid nanoparticles with a lipid monolayer shell. In this approach,[32] free polymers and hydrophobic drugs are dissolved in a water miscible organic solvent such as acetonitrile, while lipids and lipid−PEG conjugates are dissolved in an aqueous solution. In order to facilitate the solubilization of phospholipids in the aqueous solutions, a small amount of water miscible organic solvent can be added in the aqueous solution. The polymer solution is then added into the lipid aqueous solution dropwise. The organic solvent diffuses into the aqueous solution quickly, leaving polymer to precipitate into nanoparticles. The lipids and lipid−PEG will self-assemble on the surface of polymer nanoparticles through hydrophobic interactions to reduce the system's free energy. The hydrophobic tail of lipids will stick to the hydrophobic polymer core and the hydrophilic head group of lipids will extend into the external aqueous environment. The lipid−PEG conjugate will also participate into the self-assembly process with its lipid moiety inserting into the lipid monolayer and its PEG moiety facing outside of the lipid monolayer to form a stabilizing and stealth corona of the nanoparticles. An elevated temperature above lipid phase transition temperature may help the self-assembly of lipids and lipid−PEG conjugates. As the self-assembled lipid monolayer forms due to hydrophobic interactions, a hydrophobic polymer such as PLGA and PCL should be used. This one-step self-assembly approach represents a cost-effective, scalable, and predictable formulation strategy of lipid−polymer hybrid nanoparticles.

2.3. *Surface functionalization of lipid−polymer hybrid nanoparticles*

The PEG layer is essential to maintain the stability of the hybrid nanoparticles both *in vitro* by reducing nanoparticle aggregation and *in vivo* by allowing the particles to evade recognition by the reticulo-endothelial system (RES) and other immune cells. Moreover, the PEG molecules also provide functional

groups for further modification of the hybrid nanoparticles with targeting ligands for cell- or tissue-specific drug delivery. Many targeting ligands have been disclosed by researchers including monoclonal antibodies, antibody fragments, aptamers, peptides, and small molecules such as folic acid.[44] In principle all these targeting ligands can be conjugated with the lipid–polymer hybrid nanoparticles to enhance delivery efficiency (see Fig. 2). For example, Chan *et al.* have demonstrated the conjugation of a peptide (sequence: KLWVLPK) to the surface of lipid–polymer hybrid nanoparticles for spatio-temporal delivery of nanoparticles to injured vasculature.[33] The peptide was covalently conjugated to PEG molecules through maleimide-thiol coupling. It was found that the targeted hybrid nanoparticles inhibited human aortic smooth muscle cell proliferation *in vitro* and showed greater *in vivo* vascular retention during percutaneous angioplasty over nontargeted controls.

In addition, Hu *et al.* have recently reported the use of half-antibody functionalized lipid–polymer hybrid nanoparticles for targeted drug delivery to carcinoembryonic antigen presenting pancreatic cancer cells.[45] Wang *et al.* have conjugated A10 RNA aptamer, a ligand with high specificity and affinity to prostate-specific membrane antigen (PSMA), to the hybrid nanoparticles through 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) coupling reaction.[46] The resulting targeted hybrid nanoparticles achieved high selectivity to prostate cancer cells.

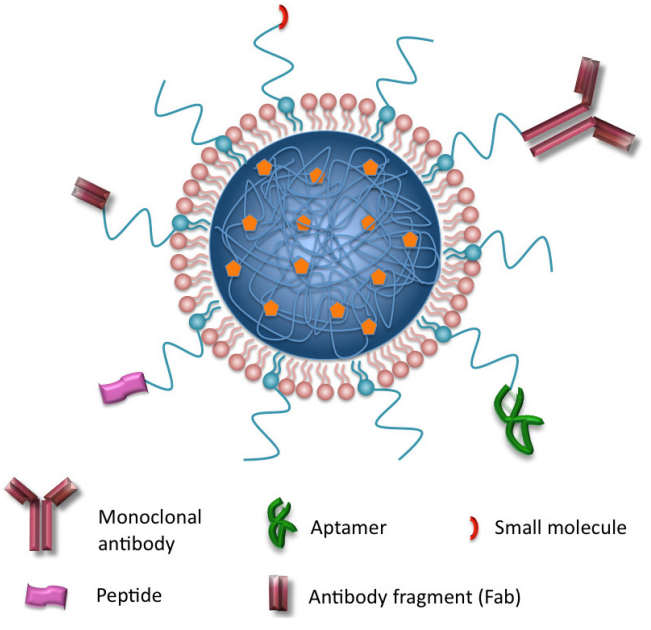

Fig. 2. Schematic illustration of possible targeting ligands that can be utilized to functionalize the surface of lipid–polymer hybrid nanoparticles for targeted drug delivery. Targeting ligands are conjugated to the nanoparticles through covalent bonds with the functional groups at the end of the PEG chains.

Another recent effort made by Liu *et al.* has demonstrated that with folate incorporated on the shell, the hybrid nanoparticles had improved targeting ability and cellular toxicity against breast cancer cells but not fibroblast cells.[31] Although further *in vivo* tests in animal models are required to validate the therapeutic potential of these targeted hybrid nanoparticles, the functional PEG layer provides tremendous flexibility for formulation design and modification of the hybrid nanoparticles.

3. Characterization of Lipid–Polymer Hybrid Nanoparticles

3.1. *Physicochemical properties*

Nanoparticle size, surface zeta potential and morphology are key physicochemical properties that determine the *in vivo* profiles of the hybrid nanoparticles.

Particle size is one of the most critical factors to determine systemic circulation lifetime of the nanoparticles and their ability to passively accumulate in tumor tissues. It has been well-documented that nanoparticles with a size range of 10–150 nm are highly beneficial and favorable for systemic drug delivery.[47–49] The hydrodynamic size and size distribution of nanoparticles can be measured by dynamic light scattering (DLS). This method is fast and straightforward without involving additional sample treatments prior to the measurement. However, this technique does not always provide the actual physical size of the particles, especially when the particles are not uniformly spherical and have large polydispersity. Scanning electron microscopy (SEM) or transmission electron microscopy (TEM) will then be used to measure the physical dimension and structure of the particles.

Valencia *et al.* have studied several factors that affect particle size and polydispersity of the lipid–polymer hybrid nanoparticles.[50] Rapid mixing of lipid and polymer solutions results in more homogeneous nanoparticles compared to slow mixing. In addition, polymer concentration and polymer

inherent viscosity also affect particle size. Higher polymer concentration leads to larger particle size, while polymer with higher inherent viscosity produces smaller particles.

Surface zeta potential is a measure of electrokinetic potential between particle surface and the bulk solution.[47] It represents the surface electrical charges of the nanoparticles and is a critical factor to determine both *in vitro* and *in vivo* stability of the particles. The zeta potential of nanoparticles can be measured using DLS by applying an oscillating electric field and monitoring the movement of the particles as they are attracted or repulsed by the electric field. For lipid−polymer hybrid nanoparticles, their surface zeta potential can be tuned by changing the end functional groups of the PEG molecules. For example, by utilizing −COOH, $-CH_3$, and $-NH_2$ end groups, the zeta potential of the hybrid nanoparticles showed negative, nearly neutral and positive surface charges, respectively.[51] Usually higher absolute zeta potential values lead to more stable nanoparticles *in vitro* as the surface charges repel particles from contacting one another. However, immunocompatibility study has shown that the hybrid nanoparticles with methoxyl surface groups induced the lowest complement activation, while the nanoparticles with amine surface groups induced the highest activation.[51] Therefore an optimal surface charge needs to be selected to balance *in vitro* stability and *in vivo* immunocompatibility of the hybrid nanoparticles.

The morphology and the core−shell structure of the hybrid nanoparticles can be measured by electron microscopy. The nanoparticles are typically dried or fixed on a silicon wafer substrate for scanning electron microscopy (SEM) imaging, from which an actual physical size and size distribution can be obtained. With high resolution SEM, surface morphology of hybrid nanoparticles may also be observed.[31] The internal core−shell structure is typically measured by transmission electron microscopy (TEM), in which negative stains are often used to increase electron contrast in order to highlight specific particle components. For instance, Thevenot *et al.* was able to see a lipid multilayer absorbing outside a polymer core using TEM with sodium silico tungstate for negative staining.[35,52] Uranyl acetate is another common negative stain that enhances the electron density of lipids and lipid−PEG conjugates. Zhang *et al.* observed a dim ring with less than 5 nm thickness surrounding the polymer core in their lipid−polymer hybrid nanoparticles prepared through a one-step self-assembly process.[32] Cryo-electron microscopy technique (CEM) is another powerful tool to study the core−shell structure of the hybrid nanoparticles, in which the samples are imaged under extremely cold temperature (usually liquid nitrogen temperature). Using this technique, Bershteyn *et al.* was able to observe the striking effects of lipid quantity and composition on the structure of lipid shell.[53]

3.2. *In vitro stability*

Nanoparticle stability in PBS solution and serum is critical for their utility as a drug delivery vehicle *in vivo*. High surface area to volume ratio associated with nanoparticles makes them prone to aggregate in solution. Nanoparticle stability can be evaluated by monitoring their changes of size, polydispersity, and surface zeta potential at different *in vitro* conditions. Several factors will determine the *in vitro* stability of the lipid−polymer hybrid nanoparticles, including nanoparticle concentration, surface charge density, and surface repulsive layer.

When nanoparticle concentration is too high, the chance of particles running to each other significantly increased. This will increase the possibility of particle aggregation due to particle−particle interactions through van de Waals forces.[54] Hence, the total volume fraction of nanoparticles in the solution needs to be controlled.

Surface charge density is directly related to the *in vitro* stability of drug delivery nanoparticles. For the lipid−polymer hybrid nanoparticles, their surface charge density is determined by the lipid surface coverage and lipid/lipid−PEG ratio. It has been shown that nearly complete surface coverage by lipids is essential to avoid nanoparticle aggregation in PBS buffer. In addition, the lipid/lipid−PEG molar ratio also plays a significant role in stabilizing the hybrid nanoparticles, especially when the PEG molecules are terminated with charged carboxylic acid or amine groups.[50] As mentioned earlier, surface charge density is measured as surface zeta potential. Typically nanoparticles with a surface zeta potential value larger than 30 mV or smaller than −30 mV are considered as a stable formulation for *in vitro* storage.

A surface repulsive layer that provides steric repulsive forces to keep particles away from one another is needed when concentration and surface

charge density are not sufficient to stabilize the nanoparticles. Steric repulsive force in hybrid nanoparticles is provided by PEG molecules on the nanoparticle surface. Both PEG chain length and lipid/lipid−PEG molar ratio have significant impact on nanoparticle stability. Thevenot *et al.* studied the effect of PEG chain lengths ($n = 16, 45, 113$) on the stability of their hybrid nanoparticles with a lipid bilayer shell, called LipoParticle, in PBS solution.[35] It was found that 10 mol% of lipid−PEG ($n = 16$) formed a lipid/lipid−PEG shell with a thickness of about 2.4 nm, which was not sufficient to provide any improvement in particle stability. In contrast, by replacing the PEG ($n = 16$) with PEG ($n = 131$), the shell thickness increased to 9.8 nm and the nanoparticles remained stable with small size and narrow size distribution. As another example, Chan *et al.* found the most stable formulation of their hybrid nanoparticles with a lipid monolayer shell was to be 15 wt% lipid/polymer mass ratio and 7.5:2.5 lipid/lipid−PEG molar ratio.[29]

Besides testing nanoparticle *in vitro* stability in water and PBS solution, some studies were performed in serum or plasma, which would provide valuable prediction of particle behavior *in vivo*. Unstable nanoparticles, once mixed with whole serum, quickly adsorb serum proteins and form aggregates resulting in large particle size and broad size distribution. While some researchers have performed 100% serum stability studies for other types of nanoparticles,[55,56] most studies are carried out using diluted serum, 10% serum in particular. This is simply because whole serum contains many proteins and protein aggregates, which interfere with the DLS measurement of nanoparticle size. Other techniques are needed to evaluate particle stability in 100% serum or whole blood.

3.3. *Drug loading and release*

The lipid−polymer hybrid nanoparticles represent a robust drug delivery platform to carry hydrophobic drugs with high loading yields and to control their release rates. The hydrophobic polymer core can contain large amount of hydrophobic drugs, which can be either physically encapsulated inside the polymer core or chemically linked to the polymer chains. The lipid shell is expected to (a) prevent small drug molecules from freely diffusing out of the polymer core, thereby improving drug loading yield; and (b) reduce water penetration rate into the polymer core, thereby decreasing the rate of polymer degradation and slowing down drug release from the particles. Zhang *et al.* have demonstrated that the addition of a lipid monolayer shell significantly improved the encapsulation and loading yields of docetaxel, a hydrophobic anticancer drug, as compared to the corresponding polymeric nanoparticles without a lipid shell.[32]

There are several factors that affect drug release profile of the hybrid nanoparticles, including drug−polymer interaction, drug solubility, polymer degradation rate, and particle size. For physically encapsulated drugs, they are released from the hybrid nanoparticles through drug diffusion and polymer erosion. For chemically conjugated drugs, their release is determined by the hydrolysis of the linkers between the drugs and polymer chains and subsequent drug diffusion.[57] Drug release study is usually performed through a dialysis method. Briefly, a dialysis cassette containing drug-loaded nanoparticles is placed in a large volume release medium at 37°C with moderate agitation. The drug molecules will continuously diffuse out of the nanoparticles and leach into the release medium. The released drugs or the drugs remained inside the nanoparticles are collected at a series of time points for quantification using analytical tools such as high performance liquid chromatography (HPLC) and mass spectrometer.[58,59]

3.4. *Cellular uptake and cytotoxicity*

Cellular uptake and cytotoxicity are typical *in vitro* assays to assess specificity and effectiveness of drug-loaded nanoparticles against target cells prior to *in vivo* evaluations. Cellular uptake of nanoparticles is examined by tagging the nanoparticles with proper fluorescent probes such as fluorescence isothiocyanate (FITC) followed by incubating these fluorescence-tagged nanoparticles with cells. After removing the excess particles, the cells will be imaged to visualize particle internalization and distribution using fluorescence microscopy such as a confocal laser scanning microscope (CLSM).[60] The mechanisms of *in vitro* cellular uptake of nanoparticles are believed to be endocytosis or nonspecific engulfment. To facilitate cellular uptake of the hybrid nanoparticles, targeting ligands are typically conjugated to the surface of the nanoparticles to activate the receptor-mediated endocytosis. As described in Sec. 2.3, a variety of targeting

ligands have been used to promote binding specificity and cellular uptake of the hybrid nanoparticles. As one example, by conjugating a monoclonal half-antibody onto the hybrid nanoparticle surface, Hu *et al.* have demonstrated that specific and intensive cellular uptake of the particles by pancreatic cancer cells occurred within only 30-minute incubation.[45]

Cellular cytotoxicity analysis is usually performed by incubating drug-loaded nanoparticles with cells for a period of time. Then the cells will be washed and supplemented with fresh media. Following 72 hours of additional culture, cell viability will be assessed with appropriate assays such as the well-known MTT assay and ATP assay. It has been well-documented that the cytotoxicity of the drugs encapsulated inside the hybrid nanoparticles was well-preserved. In fact, as compared to free drugs, the nanoparticles significantly enhanced the toxicity of the drugs by delivering a bolus dose of drugs to individual diseased cells after the particles are internalized by the cells.[61,62]

3.5. *In vivo evaluation*

One of the major issues with drug delivery nanoparticles is their limited circulation lifetime in the blood stream. Once the nanoparticles enter the circulation system, plasma proteins can quickly adsorb onto the surface of the particles and then promote opsonization, resulting in rapid clearance of the particles from blood by the mononuclear phagocyte system (MPS) in the liver and spleen.[63] It has been disclosed that particle size, surface charge, PEG modification and targeting functionality are all important factors to determine the *in vivo* behavior of the drug delivery nanoparticles.[25,64,65] It is commonly accepted that pegylated nanoparticles with a size range of 10−150 nm and slightly negative surface charge are able to stay in the systemic circulation systems for hours and preferentially extravasate into the tumor tissues through passive diffusion and active targeting effects.[47−49] Because of the unique core−shell structure and flexibility in controlling their size, surface charge, and surface functionalization, lipid−polymer hybrid nanoparticles hold great promise to achieve desirable *in vivo* pharmacokinetic properties. Sengupta *et al.* have successfully demonstrated the excellent pharmacokinetics and therapeutic efficacy of their lipid−polymer hybrid nanoparticles, called nanocell, to cure melanoma and Lewis lung carcinoma.[34] Overall, as a relatively new drug delivery platform, limited amount of *in vivo* data is available for the hybrid nanoparticles, although extensive *in vivo* studies are currently ongoing in different research laboratories.

Targeted delivery represents another major interest of nanoparticle drug delivery research. A common method is to conjugate cell- or tissue-specific ligands onto the surface of the nanoparticles to actively target the particles to sites of action. While some very promising results have been observed in cell culture experiments for the lipid−polymer hybrid nanoparticles, further *in vivo* studies are needed to evaluate their tissue targeting ability.

4. Applications of Lipid–Polymer Hybrid Nanoparticles

4.1. *Therapeutics delivery*

Lipid−polymer hybrid nanoparticles can be formulated to efficiently encapsulate and deliver a wide variety of therapeutic agents. These drugs can be loaded inside the nanoparticles alone or in a combination with two or more different types of drugs. Hydrophobic drugs can be directly and physically entrapped in the polymer core during the nanoprecipitation process and lipophilic drugs can be incorporated into the lipid shell. To further control the release kinetics of the drugs, they can be covalently linked to the polymer chains. For single-drug delivery, a successful example was recently reported by Chan *et al.*, in which a "nanoburr" system was designed to deliver paclitaxel for the treatment of injured vasculature.[33] The nanoburr contains a paclitaxel-conjugated PLA core and a lecithin/DSPE-PEG shell, which is further modified by a basement membrane targeting peptide. The sub-100 nm lipid−polymer hybrid nanoparticles specifically accumulated in injured vasculature in a rat model and continuously released drugs over two weeks.

Moreover, the hybrid nanoparticles have shown great potential for combinatorial drug delivery. For example, Wang *et al.* have reported a targeted hybrid nanoparticle system to concurrently deliver chemotherapy and radiotherapy agents for the treatment of prostate cancer.[46] In their system, an anticancer drug, docetaxel, was first encapsulated inside the polymer core during the nanoprecipitation process, and then a radioisotope, ^{111}In, was

chelated onto the particle surface. The resulting dual-drug-loaded nanoparticles showed distinct release profiles for both drugs and enhanced killing of tumor cells. As another example, Sengupta *et al.* have designed a "nanocell" system to fight against tumor step by step.[34] The nanocell consists of a PLGA polymer core containing a chemotherapy drug (doxorubicin) and a lipid multilayer shell containing an anti-angiogenic agent (combretastatin). The resulting nanocell significantly improved tumor reduction and increased mouse survival rates as compared to the treatment with single drug or a mixture of the two drugs. The synergistic effect was achieved by temporal release of the two anti-cancer agents: the outer lipid shell first released the anti-angiogenesis agent, causing a rapid vascular shutdown; the inner polymer core that has already been entrapped inside the tumor tissue then released the chemotherapy agent to further destroy the tumor cells. Very recently, a new approach has been reported by Aryal *et al.* to concurrently load both hydrophobic and hydrophilic drugs to the lipid–polymer hybrid nanoparticles based on a combinatorial drug conjugation method.[61] These nanoparticle-assisted combination therapies may provide a new paradigm for effective cancer treatment.

4.2. *Imaging agent delivery*

Besides delivering therapeutic agents, the lipid–polymer hybrid nanoparicle can also be used to deliver a variety of imaging agents such as iron oxide, fluorescent dyes, and quantum dots (QDs) by encapsulating them inside the polymer core. Moreover, Valencia *et al.* used QDs to replace the hydrophobic polymer to fabricate lipid–QD hybrid nanoparticles by a fast mixing method within a microfluidic device.[50] TEM images showed monodispersed lipid–QD hybrid particles with an average size of 60 nm. The encapsulated QDs remained their fluorescence properties and exhibited high stability in solutions.

5. Conclusion and Future Prospective

Lipid–polymer hybrid nanoparticles offer numerous advantages as a drug delivery platform including simple fabrication process, tunable size and surface charge, high loading capacity of poorly water-soluble drugs, sustained and controllable release profile of the drugs, high *in vitro* stability, and excellent *in vivo* properties. All of these features make the lipid–polymer hybrid nanoparticles an ideal drug delivery platform. Despite the great progress made on synthesis, characterization and applications of the hybrid nanoparticles, we call attention to a few key unmet challenges in further developing this new nanoparticle platform as a robust drug delivery system for medical applications.

First, optimizing the targeting ligand density on the nanoparticle surface is critical to achieve optimal therapeutic efficacy. It is well-documented that the physicochemical properties of the hybrid nanoparticles such as particle size, surface charge, PEG chain density will affect their *in vivo* pharmacokinetics. Therefore the conjugation of targeting ligands on the one hand will improve the cell- or tissue-specific targeting ability, but on the other hand may compromise the surface properties of the hybrid particles and thus negatively affect their pharmacokinetic properties. Many types of targeting ligands have been conjugated to improve the accumulation of the hybrid nanoparticles to the sites of action. It would be desirable to screen and optimize the ligand density through *in vivo* experiments.

Secondly, precise control of multiple drugs with different hydrophobicity inside the same hybrid nanoparticles remains challenging. Although several attempts have been demonstrated to concurrently load dual drugs to the hybrid nanoparticles, the molar ratio of the two drugs and their loading yields are difficult to be precisely controlled. This may potentially limit the synergistic effects among the drug combinations.

Lastly, large-scale fabrication of these hybrid nanoparticles has received little attention, which could become a key factor that determines the bench-to-bedside translation of these drug delivery vehicles. The simplicity of the synthesis process, especially the one-step self-assembly process, dramatically increases the likelihood of producing the lipid–polymer hybrid nanoparticles in a scalable and economical manner.

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