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Lipid-enveloped hybrid nanoparticles for drug delivery

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Recent advances in nanotechnology and material sciences have promoted the development of nanomedicine. Among the formulations developed, novel lipid-enveloped hybrid nanoparticles have attracted more attention because of their special structure, properties and clinical applicability. The hybrid nanoparticles are composed of a hydrophilic PEG shell, a nano-sized polymeric or inorganic core and a lipid mono- or bi-layer between the core and PEG shell. This kind of nanoparticle possesses both the characteristics of liposomes and nanoparticles which endows it with many advantages like long circulation, high drug loading efficiency, high stability and biocompatibility, controlled release properties, and drug cocktail delivery. This review describes the recent developments of lipid-enveloped hybrid nanoparticles in cancer treatment, including the fabrication methods, formulations and applications of these hybrid nanoparticles. We expect that the continuing development of lipid-based nanomedicine will greatly improve cancer treatment.

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1 Introduction

Cancer is one of the most dangerous diseases in the world. Recent advances in nanotechnology and materials science have greatly improved cancer treatment. Many nanoformulations have been developed as anti-cancer drug carriers such as liposomes, micelles, polymeric/inorganic nanoparticles, nanogels, polymeric vesicles and so on.[1–3] Among these formulations, liposomes and nanoparticles, especially polymeric nanoparticles, are two typical successful ones.[4–9] Liposomes possess high biocompatibility but they face some obstacles, *e.g.*, complicated multi-step fabrication, storage instability and burst release. Compared to liposomes, polymeric nanoparticles are more stable, easy to prepare and they have a controllable release behavior. However, their circulation half-life is still a concern. For inorganic nanoparticles, the same problem also needs to be solved. To combine the advantages of these formulations, a novel kind of nanoformulation, lipid-enveloped hybrid nanoparticles, have been developed and have shown great potential in nanomedicine.[10,11] The nanoparticles possess both the characteristics of liposomes and nanoparticles with a lipid layer-enveloped on the surface of the polymeric or inorganic core. The hybrid nanoparticles have shown many advantages like long circulation time, high drug loading capability, high stability and controlled release properties, *etc.*

Generally speaking, the lipid-enveloped hybrid nanoparticles are composed of three distinct functional components

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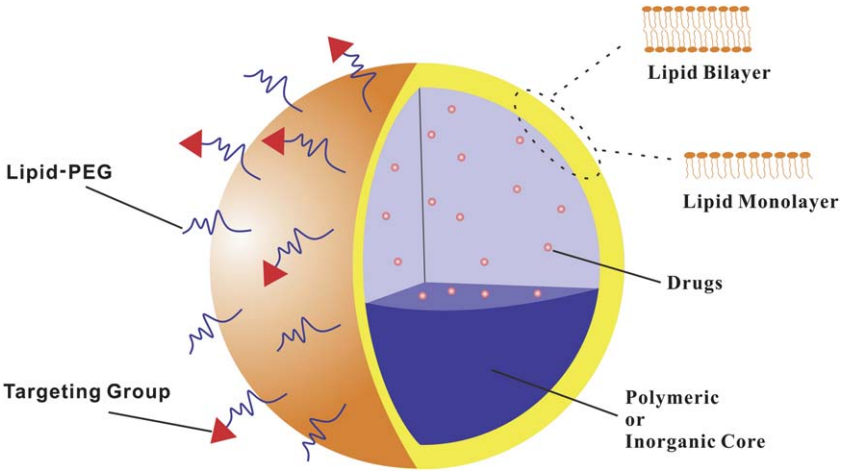

Fig. 1 Scheme of the lipid-enveloped hybrid nanoparticle.

(Fig. 1); (1) the inner core, which can be composed of biodegradable or stimuli-responsive polymeric nanoparticles,[12–21] mesoporous silica nanoparticles (MSN),[22–24] calcium phosphate (CaP) or other inorganic nanoparticles.[25–28] The particle core can be used to entrap various drugs, such as paclitaxel (PTX),[29,30] docetaxel (DOC),[31] doxorubicin (DOX),[12,14] camptothecin (CPT),[14] tumor necrosis factor,[21] cationic vincristine sulfate,[32] (7 alpha-(4′-amino)phenylthio-1,4-androstadiene-3,17-dione (7 alpha-APTADD),[33] antigen,[34–36] genes (DNA, mRNA or siRNA)[37–43] and/or functional small nanoparticles like iron oxide nanocrystals and quantum dots (QDs).[34,44–47] (2) A hydrophilic polymeric shell, especially polyethylene glycol (PEG), which enhances the stability of nanoparticles, prolongs systemic circulation half-life and can be conjugated with targeting ligands, such as folic acid,[20] transferrin,[33] peptide,[41] DNA sequence (AS1411 aptamer),[30] certain antibodies for immunotherapy,[34,35] and fragments of antibody (single-chain Fv);[21] and (3) a middle lipid layer between the core and shell which can serve as a template for surface modification, act as a molecular fence to prevent the leakage of drug inside the core, enhance drug encapsulation efficiency, reduce the water penetration rate and control drug release behavior.[16,17] Besides the usually used lipids, natural cell membranes are also employed as the lipid shell to form a bionic simulation system.[48] Employing lipid-enveloped hybrid nanoparticles as therapeutic carriers gives them great potential in clinical applications.

In this review, we discuss the fabrication methods, formulations, and applications of this kind of hybrid nanoparticle in cancer treatment according to their compositions of particle core and lipid layer shell. We expect that the continuing development of this lipid-based nanomedicine will greatly improve cancer treatment.

2 Lipid-enveloped polymeric nanoparticle for drug delivery

Among hybrid nanoparticles, lipid-enveloped polymeric nanoparticles have received the most attention as the nanoparticle composition can be of FDA approved materials and the formulated nanoparticles can thus be accepted in clinical

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application. Compared with other nano-sized polymeric systems such as polymeric micelles and vesicles,[3,49] the hybrid nanoparticles exhibit well-controlled particle size, improved stability and biocompatibility and controllable biopharmaceutics. Here, we discuss various hybrid nanoparticles according to the materials of polymeric core and lipid shell.

2.1 Lipid-enveloped PLGA hybrid nanoparticles

The polymer PLGA has been approved by the FDA and lipid-enveloped PLGA hybrid nanoparticles are widely investigated. Various methods are reported in the fabrication of these nanoparticles.

2.1.1 Fabrication methods. In general, three fabrication methods are developed to formulate lipid-enveloped PLGA nanoparticles (Fig. 2).

The first one (Fig. 2a) is a two-step method reported by Sengupta *et al.*[12] Typically, drug-loaded PLGA nanoparticles are fabricated and then the nanoparticles suspension is incubated with a formulated aqueous solution of liposomes (it can also be with encapsulated drugs). The mixture is extruded through a porous membrane to form a lipid-coated hybrid structure. Wang *et al.* modified the method by adding the nanoparticles suspension into lipid thin film and following by ultrasonication instead of extrusion.[50] The advantage of the two-step method is that the different drugs can be loaded into both the core and lipid layer of the nanoparticle, respectively. However, the limitation is that the encapsulated drug may be released from the core during lipid-coating process.

The second one is a modified one-step nanoprecipitation method[13–21] (Fig. 2b). In brief, polymer and drug are first dissolved in a water-miscible organic solvent. After that, the organic solution is added dropwise *via* a syringe to hot water with the lipid at 63–65 °C or lipid aqueous solution with 4% ethanol under sonication or vortex. For this method, lipids and lipid-PEG will simultaneously self-assemble on the surface of the polymeric nanoparticles as a lipid monolayer to lower free energy and improve stability of the system. Combination with microfluidic technology is a recent progress on the nanoprecipitation method. As shown in Fig. 3, a 3D microfluidic flow patterns in the microfluid channel is constructed. Acetonitrile solution of PLGA is in the central inlet and aqueous solution of lecithin and DSPE-PEG with 4% ethanol is in the outer inlet.[51–53] Then rapid mixing and controlled nanoprecipitation can be achieved. The resulting lipid-enveloped nanoparticles are homogeneous with relatively narrow size distribution (~0.1) and well controlled particle size of 30–170 nm.[52,53] More importantly, the productivity of nanoparticles reaches as high as 3 g hour^{-1}. This can enable it to be industrially produced on a large scale. However, both drug and polymer should be soluble in a water-miscible solvent, usually acetone or acetonitrile. This may limit the applications of this method.

The third method is a modified emulsification method.[29,36,54,55] In this method, polymer, hydrophobic drug and lipids are first dissolved in water-immiscible solvent (such as dichloromethane, DCM) and then emulsified in water under sonication as seen in Fig. 2c.[36,55] After solvent evaporation, the lipid-enveloped nanoparticles can be separated from the empty liposomes by density gradient centrifugation.

In the case of hydrophilic molecules, a modified double emulsion technology is used as shown in Fig. 2d.[35,54] An aqueous solution of water-soluble (w1) drug is first emulsified in an organic phase of polymer, lipid and lipid-PEG to form primary w1/o nano-emulsion. Then the emulsion is further emulsified in water (w2) under sonication to fabricate secondary emulsion. When using cationic ethylphosphocholine (EPC) lipids instead of neutral lipids in primary emulsion, lipid-enveloped nanoparticles with a hollow core–shell three-layer structure can be prepared.[56]

The most striking feature of this modified emulsification method is that it can be applied for either hydrophilic or hydrophobic drug entrapment with improved encapsulation efficiency. However, the process can easily promote the formulation of free liposomes and therefore, the density gradient centrifugation method needs to be applied to separate free liposomes from lipid-enveloped polymeric hybrid nanoparticles.

2.1.2 Applications. The lipid-enveloped nanoparticles are mostly applied in encapsulating chemotherapeutic drugs like PTX,[29] DOC,[16] cationic vincristine sulfate[32] and 7 alpha-APTADD.[33] Compared with traditional nanoparticles, the lipid-enveloped nanoparticles have a lipid monolayer which helps to increase encapsulation efficiency, control the drug release, promote the cell uptake and improve the *in vitro* cell cytotoxicity.[16,29] Compared with single-drug therapy, combinational chemotherapy has exhibited much more treatment efficiency, especially in overcoming the widely existing multidrug resistance (MDR). Lipid-enveloped nanoparticles have been applied for combinational therapy. They include PLA-Pt(IV) prodrugs with DOC co-encapsulation,[53] hydrolyzable PTX–gemcitabine conjugates[57] and DOX–PLA and CPT–PLA prodrugs.[14] Combination of chemotherapy and radiotherapy is also achieved when using DOC, indium(111) and yttrium(90) as model drugs.[31]

Besides the core, the lipid layer also enables the encapsulation of drugs. Sengupta *et al.* reported a kind of lipid-enveloped hybrid nanoparticle ('nanocell') to realize the co-delivery of DOX (conjugated with PLGA as the core) and an antiangiogenesis agent, combretastatin-A4 (loaded in the lipid layer) (Fig. 4).[12] These nanoparticles were expected to first release anti-angiogenesis agent in the tumor tissue to block vascularization and then the DOX would be released from the inner core to kill the tumor cells. This strategy greatly improved the therapeutic index with reduced toxicity.

Antigen peptide loaded lipid-enveloped PLGA nanoparticles can also be prepared used emulsification method (Fig. 2c or d) and have been applied in cancer immunotherapy. Cruz *et al.* reported a kind of nanovaccine which significantly decreased the degradation of loaded antigen in lysosomes and increased antigen presentation *in vitro*.[34] Our recent work also showed

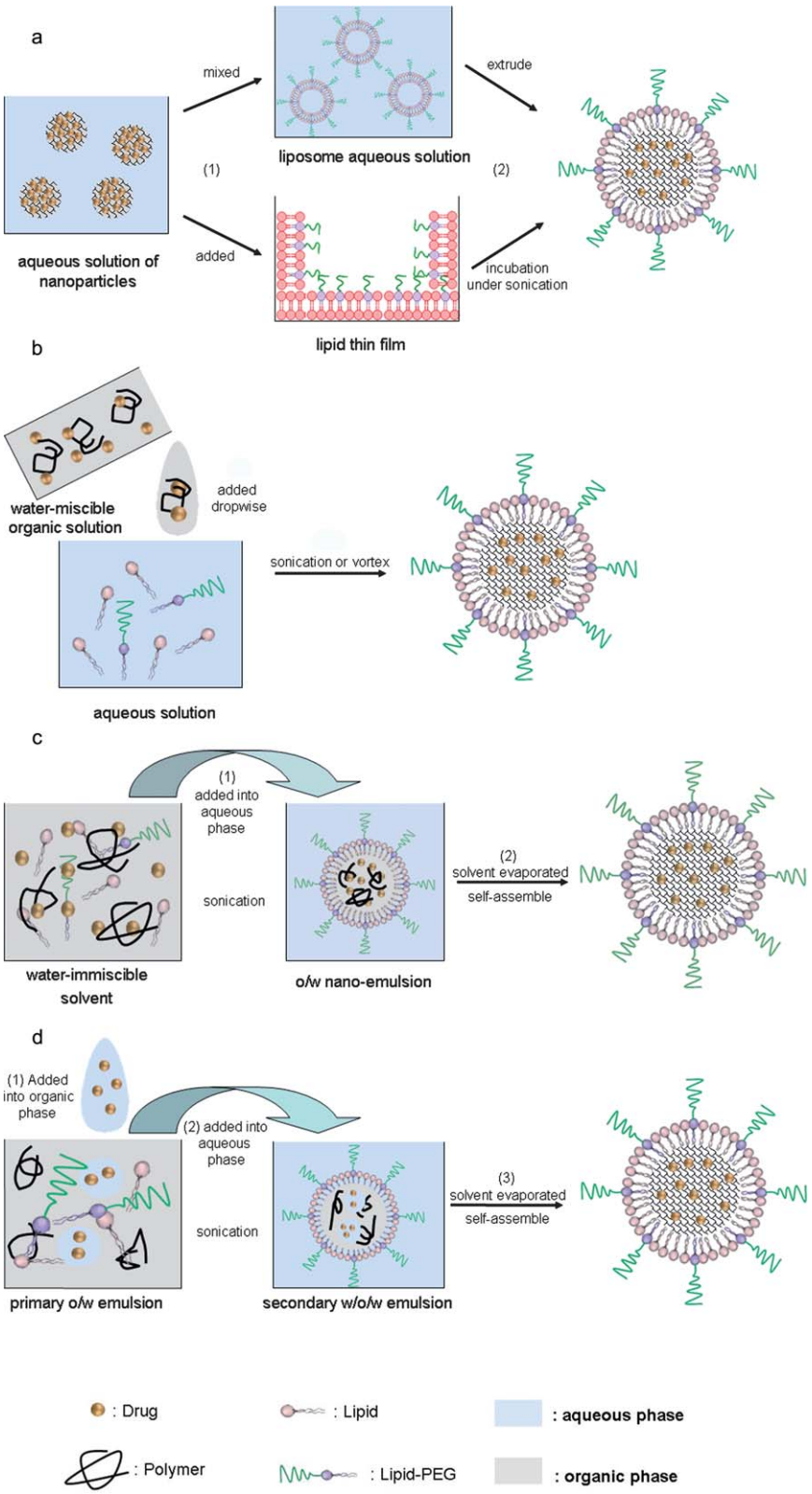

Fig. 2 Schematic diagram of the fabrication of lipid-enveloped hybrid nanoparticles by: (a) two-step method, nanoparticle cores are prepared first then mixed with liposome or lipid film, (b) one-step nanoprecipitation method, (c) single emulsification method, and (d) double emulsification method.

that the lipid-enveloped structure was able to encapsulate hydrophobic and/or hydrophilic peptides efficiently. The nanoparticles could submit the antigen peptide to dendritic cells (DCs) and successfully induce antigen-specific T cell responses.[58] As a result, the growth of B16-F10 tumor was significantly delayed.

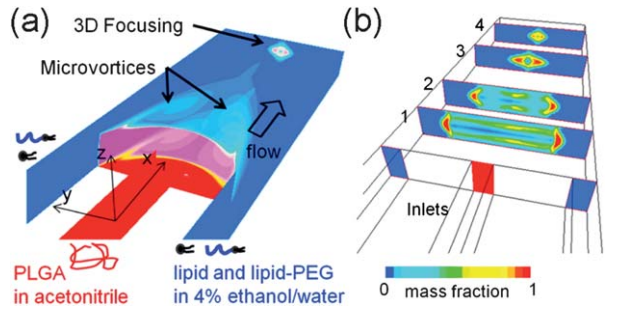

Fig. 3 Mass production and size control of lipid–polymer hybrid (LPH) nanoparticles through controlled microvortices. Schematic (a) and cross section views (b) of a simple, single-layer, and three-inlet microfluidic platform generating two symmetric microvortices and a 3D focusing pattern.[51] Reproduced with permission.

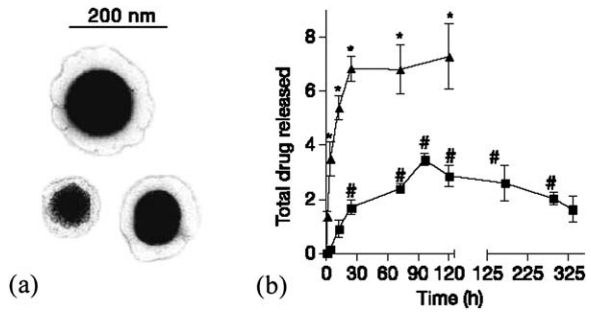

Fig. 4 (a) Transmission electron micrograph of the cross-section of three nanocells shows the dark nuclear nanoparticle within the phospholipid block copolymer envelope. (b) Physicochemical release kinetics shows the temporal release of combretastatin (triangles, scale in 10^2 μg) and doxorubicin (squares, scale in μg).[12] Reproduced with permission.

2.2 Stimuli-responsive lipid-enveloped nanoparticles

Compared with traditional materials (*e.g.* PLGA), stimuli-responsive polymers have shown many advantages and are widely used in drug delivery systems.[1,59] These smart nanocarriers can realize tumor specific delivery and superior distribution, enhanced cellular uptake and well-controlled drug release and thus result in improved therapeutic efficiency. Two kinds of stimuli-responsive lipid-enveloped nanoparticles were investigated, pH-sensitive core and pH-responsive shell layer.

The first one (pH sensitive core) employs lipid bilayer-enveloped pH-sensitive poly(β-amino ester) (PBAE) nanoparticles for mRNA delivery.[40] These nanoparticles can be prepared by either a modified solvent emulsification method (Fig. 2c) or a nanoprecipitation method (Fig. 2b) using PBAE instead of PLGA. mRNA is bound on the surface of these positively charged nanoparticles through electrostatic interaction and the lipid layer can minimize the toxicity of the cationic core and prevent the degradation of mRNA. The PBAE core was chosen to promote endosome escape of mRNA after nanoparticles uptake by cells. As a result, the mRNA-loaded hybrid nanoparticles exhibited fast and stable expression of encoded protein in DCs both *in vitro* and *in vivo*, which makes this system a potential candidate for noninvasive delivery of mRNA-based vaccines.

The other kind (pH-responsive shell layer) was fabricated by one-step nanoprecipitation method using a pH-triggered hydrolyzable lipid–(succinate)–mPEG conjugate with a diester succinate linker between the lipid and PEG moieties to replace the usually used lipid DSPE-PEG.[15] The hybrid nanoparticles were stable at pH 7.4. When the pH value was changed to 5.0, aggregations of nanoparticles appeared due to the hydrolyzation of the linking ester bond and the loss of PEG shell. Furthermore, the pH-sensitive behavior of nanoparticles could be controlled by adjusting the amount of lipid–(succinate)–mPEG on the surface. The higher the lipid–(succinate)–mPEG content in the lipid shell, the more stable the particles was. As a result, the nanoparticles can be chosen to aggregate in the cancer intercellular matrix or in the endosome by controlling the lipid–(succinate)–mPEG content in the lipid shell and then fusing with cellular or endosomal membranes to achieve controlled drug delivery.

2.3 Lipid-enveloped cationic hybrid nanoparticles

Efficient and safe DNA/RNA transfer to mammalian cells is the key to successful gene therapy.[37,60] Lipid-enveloped cationic hybrid nanoparticles have been reported as a new kind of efficient nonviral gene transfer vector which can protect the DNA/RNA from degradation by plasma nucleases, extend its circulation time in the body, increase its efficiency to target cells with high specificity and promote endosomal escape and cytosolic dispersion.[38,39] The cationic hybrid nanoparticles can be fabricated with either a cationic core or cationic lipid layer for DNA/RNA delivery. Cationic macromolecules such as polyethylenimine (PEI) and protamine have both been reported as the core to bind DNA/RNA.[37,38,41–43] PEI is a widely used gene delivery carrier and the lipid-enveloped PEI-based nanoparticles exhibited higher transfection efficiency and less cytotoxicity *in vitro* compared to an ordinary DNA loaded PEI nanoparticle.[38] A similar PEI system was also used in siRNA delivery, which was found to be able to induce specific knock-down of an endogenous genes with minimum liver toxicity and immune response.[41] However, PEI had some toxic effects, which limits its further application. So another cationic macromolecule, protamine was employed to fabricate the core of nanoparticles for siRNA delivery and siRNA/DNA co-delivery.[37,42,43] The protamine-based hybrid nanoparticles were prepared by a two step method through electrostatic interaction and they demonstrated an enhanced siRNA encapsulation efficiency of 90%, reduced nonspecific *in vivo* drug distribution and realized up to 32.5% of the injected siRNA distribution into the tumor.[42,43]

The second kind of cationic lipid enveloped hybrid nanoparticles is the one with a cationic lipid layer.[50,61] Yang *et al.* fabricated the hybrid nanoparticles with PLA core and a cationic (*N*,*N*-bis(2-hydroxyethyl)-*N*-methyl-*N*-(2-cholesteryloxycarbonyl aminoethyl) ammonium bromide, BHEM-Chol) lipid shell.[61] The siRNA-bound nanoparticles were capable of decreasing expression of the oncogene, inducing cancer cell apoptosis and significantly suppressing tumor growth. Another attractive example is the work of Wang *et al.*, which realized the co-delivering drugs and genes by cationic lipid-enveloped

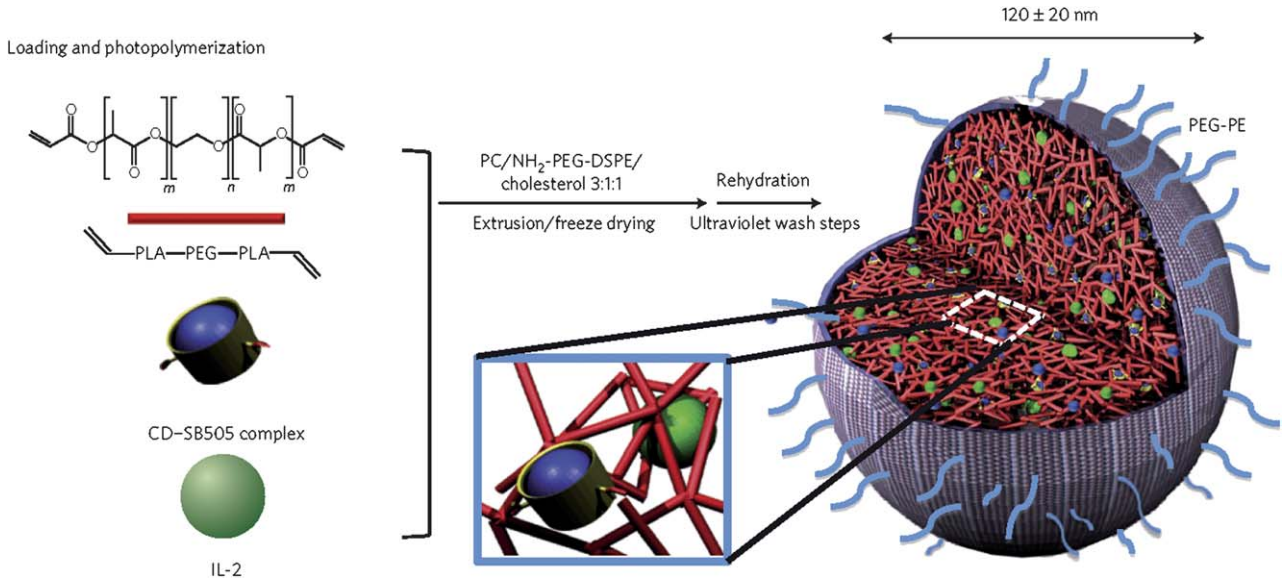

Fig. 5 The formulation of lipid-enveloped nanogel nLGs from biodegradable crosslinking polymer, acrylated-CD–SB505 complex, and IL-2 cytokine. This core–shell structure facilitated entrapment of the drug-loaded CD (blue) and the IL-2 (green) in a biodegradable polymer matrix (red) with a PEGylated liposomal coating (grey). After loading, photoinduced polymerization of the polymer and acrylated-CD results in gel formation.[68] Reproduced with permission.

nanoparticles.[50] Cationic PEGlated amphiphilic octadecyl-quaternized lysine modified chitosan and cationic folate acid coated amphiphilic octadecyl-quaternized lysine modified chitosan were chosen as the lipid shell to bind DNA while PLGA core was used to load DOX. These nanoparticles could simultaneously deliver drugs and genes to one cancer cell and exhibited a high treatment efficacy due to their synergistic effect.

A special lipid-enveloped cationic nanoparticle with a differently charged hollow core–shell three layer structure was prepared by Shi *et al.* through a modified w/o/w double-emulsion technique, as mentioned in Section 2.1.[56] The inner surface of the hollow core was covered by positively charged lipid layer. Thus siRNA can be entrapped in the hollow core with high encapsulation efficiency of 78–82%. These siRNA-loaded nanoparticles demonstrated strong ability of silencing the targeting gene both *in vitro* and *in vivo*.

2.4 Lipid-enveloped nanogels

Aqueous colloidal nanogels are a novel nanofabrication with high biocompatibility and desirable mechanical properties.[62] They can be prepared *in situ* in aqueous media and the resulting interior network is an excellent candidate for incorporating biomolecules without reducing their activities. But the stability of nanogels in the blood stream and the sustained drug release ability may be a challenge. So lipid-enveloped nanogels based on electrostatic interaction or chemical crosslink were developed to solve these problems.[11,63–68] Wu's group reported a series of lipid-enveloped nanogels based on electrostatic interaction. They were prepared through a complex-dilute method and the encapsulation efficiency was up to 70%.[63–67] However, only positively charged drugs like DOX and verapamil·HCl could be loaded by this method. Recently reported lipid-enveloped nanogels prepared by a modified two-step method based on photocrosslinking technique have shown some improved properties. In this method, a solution containing the macromolecular and/or small molecular monomer, model drugs, other functional materials is mixed with lipids though a thin lipid film hydration method or co-extruding method (similar to Fig. 2a). Then polymerization was induced by UV to get the final lipid-enveloped nanogel. Murphy *et al.* prepared a protein-based lipid-coated nanogel using human serum albumin, albumin or α1-acid glycoprotein to deliver some chemotypes. PTX, DOC, bortezomib, 17-AAG, sorafenib, sunitinib, bosutinib, and dasatinib could be loaded in these nanoparticles.[11] This nanogel could be further modified by RGD and showed a 15-fold improvement on antitumor activity than Abraxane® in orthotopic breast and pancreas tumours in mice.

Park *et al.* developed another kind of lipid-enveloped nanogel from β-cyclodextrin (β-CD) and PLA–PEG–PLA to realize the co-delivery of hydrophobic drugs (transforming growth factor-β inhibitor, SB05124) and hydrophilic cytokines (interleukin-2, IL-2, a conventional cytokine for metastatic melanoma) (Fig. 5).[68] SB05124 was solubilized in β-CDs and IL-2 was encapsulated in the polymer-hydrogel space outside of the β-CD. This kind of hybrid nanogel enabled the sustained and simultaneous release of both SB05124 and IL-2 and thus showed significant inhibition of tumour growth, extended survival of tumour-bearing mice, enhanced activity of natural killer cells and intratumoral-activated $CD8^+$ T-cell infiltration.

3 Erythrocyte membrane-enveloped PLGA nanoparticles

Living cells have been exploited as drug carriers due to their natural properties (high biocompatibility and little

immunogenicity).[69–71] Red blood cells (RBC) have a life span of 121 days and have been widely used in long-circulating drug or antigen delivery systems.[72,73] Nanosized RBC membrane vesicles with a diameter of about 100 nm could be prepared by extruding RBCs and used as drug carriers. However, these RBC vesicles have two problems.[70] One is that it is difficult to load drugs into the vesicle. Conjugating the drug onto the membrane surface would result in easy clearance from bloodstream.[74,75] The other one is the instability of the vesicles. The RBC membrane vesicles can form large aggregates in the lung after injection.[74] Recently, Hu *et al.* greatly improved this drug delivery system.[48] They developed a novel RBC membrane enveloped PLGA nanoparticle (Fig. 6) by a two-step method (similar to Fig. 2a). The resulted RBC membrane-enveloped PLGA nanoparticles were around 80 nm in diameter with an outer lipid bilayer shell thickness of 7–8 nm. This RBC membrane on the nanoparticles had almost the same protein content as natural RBC. The nanoparticles were stable in PBS and fetal bovine serum and they also showed a long circulation property *in vivo*. Their circulation half-life was 39.6 h in a mouse model, which was much higher than that of PEG-based nanoparticles (only 15.8 h). Obviously, these well engineered biomimetic nanoparticles provide a novel way of bridging natural and artificial materials. The polymeric core can encapsulate many therapeutic drugs and/or other functional agents like QD and iron oxide nanocrystals, while the RBC surface could be further modified with some targeting groups. These may expand applications of the nanoparticles in drug delivery system. However, this approach still faces some challenges. The cell membrane structure may get broken during extruding process though the total protein content remains unchanged. The circulation half-life of this RBC coated nanoparticle may be further improved by integrating it with other technology. A mechanobiological mimicry of RBC has been reported with long-circulation half-life of 93.3 h due to the deformability of hydrogel microparticles.[73] Decreasing the modulus of the core may be a possible way of prolonging the circulation time of RBC coated nanoparticles.

4 Lipid-enveloped silica nanoparticles

Lipid-enveloped silica nanoparticles were first described by Mornet and colleagues in 2005.[76] The following studies systematically investigated such hybrid material with interests both in basic and applied fields. Recently, mesoporous silica

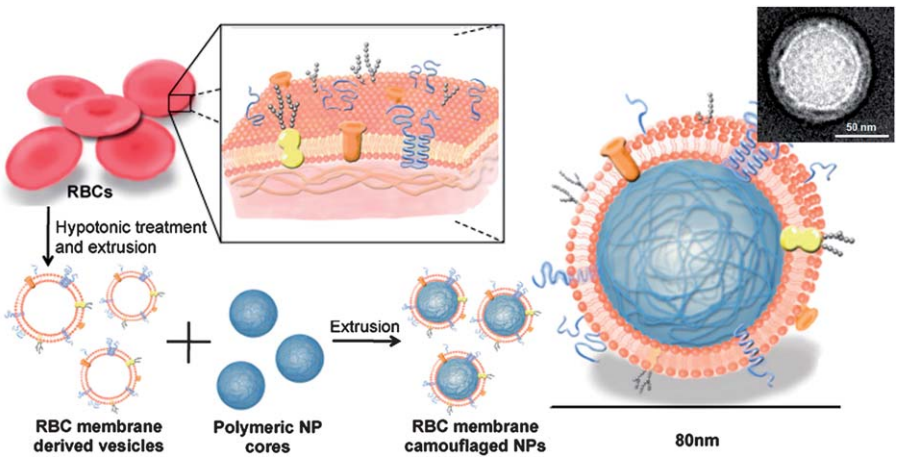

Fig. 6 Schematics of the preparation process of the RBC-membrane-coated PLGA nanoparticles and the TEM image.[48] Reproduced with permission.

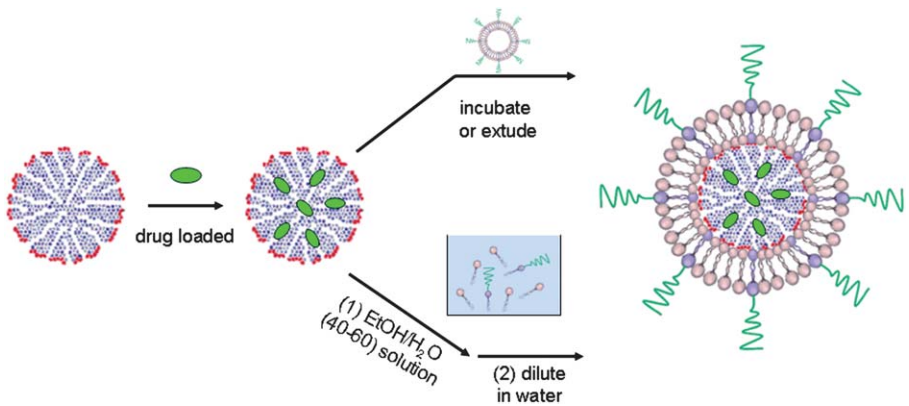

Fig. 7 Schematic diagram of the fabrication process of lipid bilayer-enveloped nanoparticles. (a) Drug-loaded silica nanoparticles were incubated with lipid thin film or liposome solution, (b) silica nanoparticles dispersed in lipid solution and then diluted in water.

nanoparticles (MSNs), with unique material features such as large surface area and high pore volume, were proposed as one of the most appealing candidates for drug delivery.[77] However, the particles will accumulate in physiological condition, which may cause long-term toxicity *in vivo*. Consequently, the exploration of lipid-enveloped MSNs has attracted great attention to tackle this problem.

4.1 Fabrication of lipid bilayer fused silica nanoparticles

A two-step approach is usually applied for preparation of lipid-enveloped MSNs: molecular cargos are encapsulated within the mesopores of MSNs first, and then intact supported lipid bilayers (SLB) are inducted onto the surface of MSNs (Fig. 7). One strategy of achieving SLB coating is adding MSNs into the aqueous solution of liposomes or a container with lipid thin film to mediate a fusion process, which is similar to that of fabricating lipid-enveloped PLGA nanoparticles as mentioned above (Fig. 2a).[78] Another method is applying a solvent-exchange method, which also enables the generation of SLB. MSNs and lipids were both suspended in an ethanol–water solution (with ratio of 2/3, v/v), followed by the dilution with abundant water to obtain lipid-enveloped MSNs.[79] Very recently, a reducible lipid bilayer-enveloped MSNs was reported by

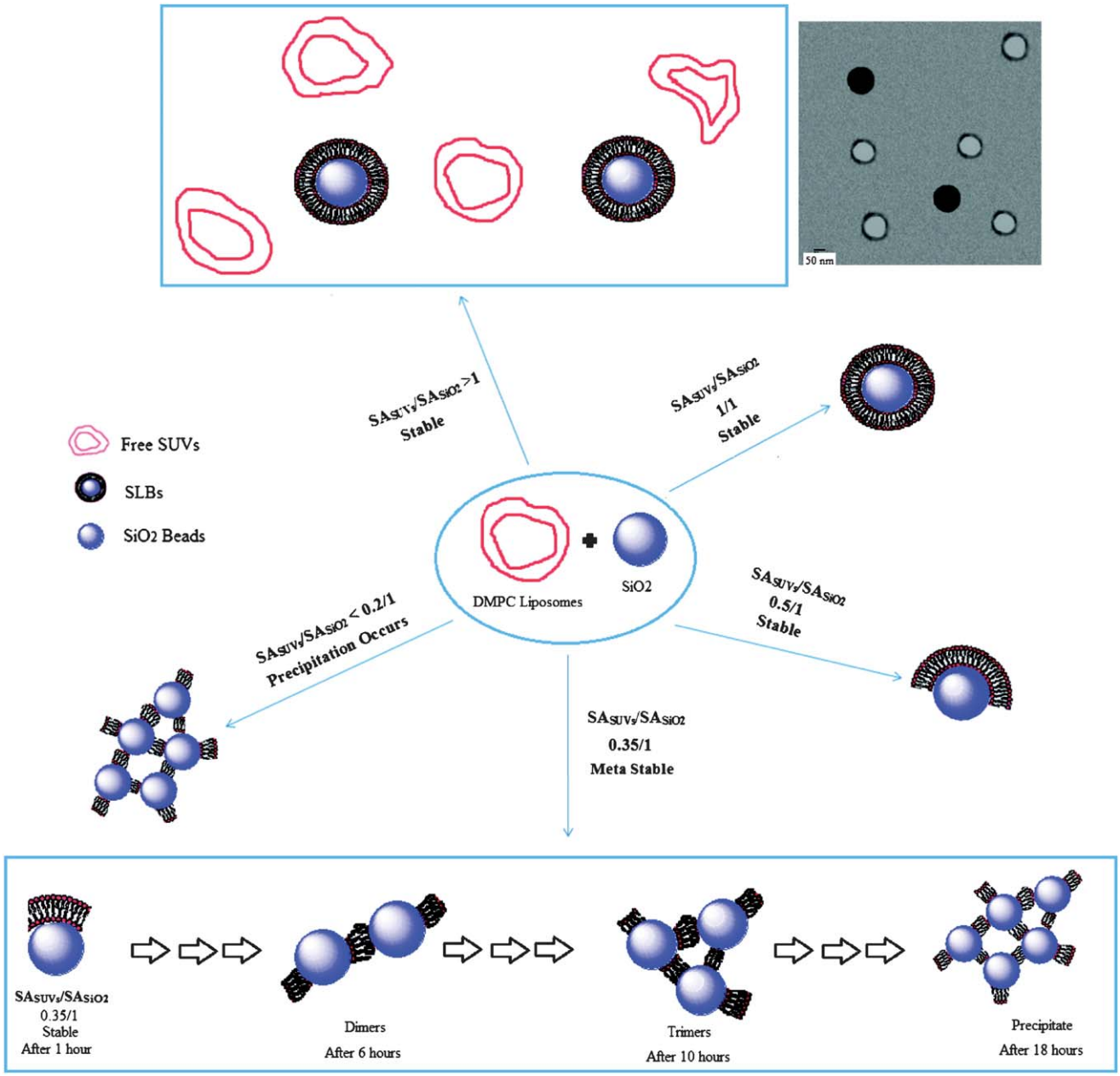

Fig. 8 Schematic of structures formed at 0.75 mM NaCl, pH = 7.6, $\varphi = 0.21\%$, for $0.005 < SA_{SUV}/SA_{SiO_2} < 3$. Illustration is for nominal 100 nm SiO_2 and 100 nm DMPC SUVs that have been incubated at 40 °C for 1 h. The TEM image is shown for $SA_{SUV}/SA_{SiO_2} = 3/1$, where SLBs are separated by the SUVs. The black particles are SLBs and the black circles are the SUVs.[83] Reproduced with permission.

Roggers *et al.*[80] The inner layer was chemically conjugated with silica surface through disulfide bonds, and the outer lipid layer (cell interaction layer, CIL) was induced to cover the inner layer. Lipid bilayers prepared in this manner would dissociate in the reducing conditions of cytoplasm, which resulted a burst release of encapsulated drugs.

The formation of lipid-enveloped silica nanoparticles was supposed to be a spontaneous process driven by the co-action of entropy and enthalpy.[81] This process could be affected by several factors. The most important one is the surface charge of MSNs and lipids. As reported by Mornet *et al.*, the negatively charged silica surface could only be entirely covered by lipid bilayers with positive, neutral, or low net negative charge density.[76] Similarly, Liu and co-workers pointed out that the fusion between MSNs and liposomes only occurred spontaneously when they possessed opposite surface charge.[82] Besides the surface charge, the ratio of surface area of lipid–silica also takes an essential role in the formulation process.[83] When the ratio is higher than 1, an intact SLB can form on the silica surface. If the ratio becomes lower than 1, bilayer bridges appear among the nanoparticles and the resulting structures depend on the ratio (Fig. 8). The amounts of –OH groups on the silica surface can also affect the SLB formation process. Ahmed and colleagues have found that higher silanol density resulted in larger hydration repulsion against lipid that slows down the formation of SLB.[84] Last but not least, the size of the inner nanoparticle, the composition of phospholipid and the ionic strength in solution are also related to the formation and stability of this silica–lipid hybrid.[55,85–88]

4.2 Applications of mesoporous silica nanoparticles supported lipid bilayer

'Protocell' is a current delivery platform that developed on the basis of lipid-enveloped MSNs (Fig. 9).[22–24] The inner MSNs endow protocells with capabilities of loading large amounts of cargo molecules and controlling the release behavior. The external lipid bilayers of protocells not only improve the biocompatibility and stability of MSNs, but also serve as gatekeepers to prevent the leakage of encapsulated drugs from the mesopores. Moreover, the lipid shell can be modified with specific molecules to realize different functions such as avoiding

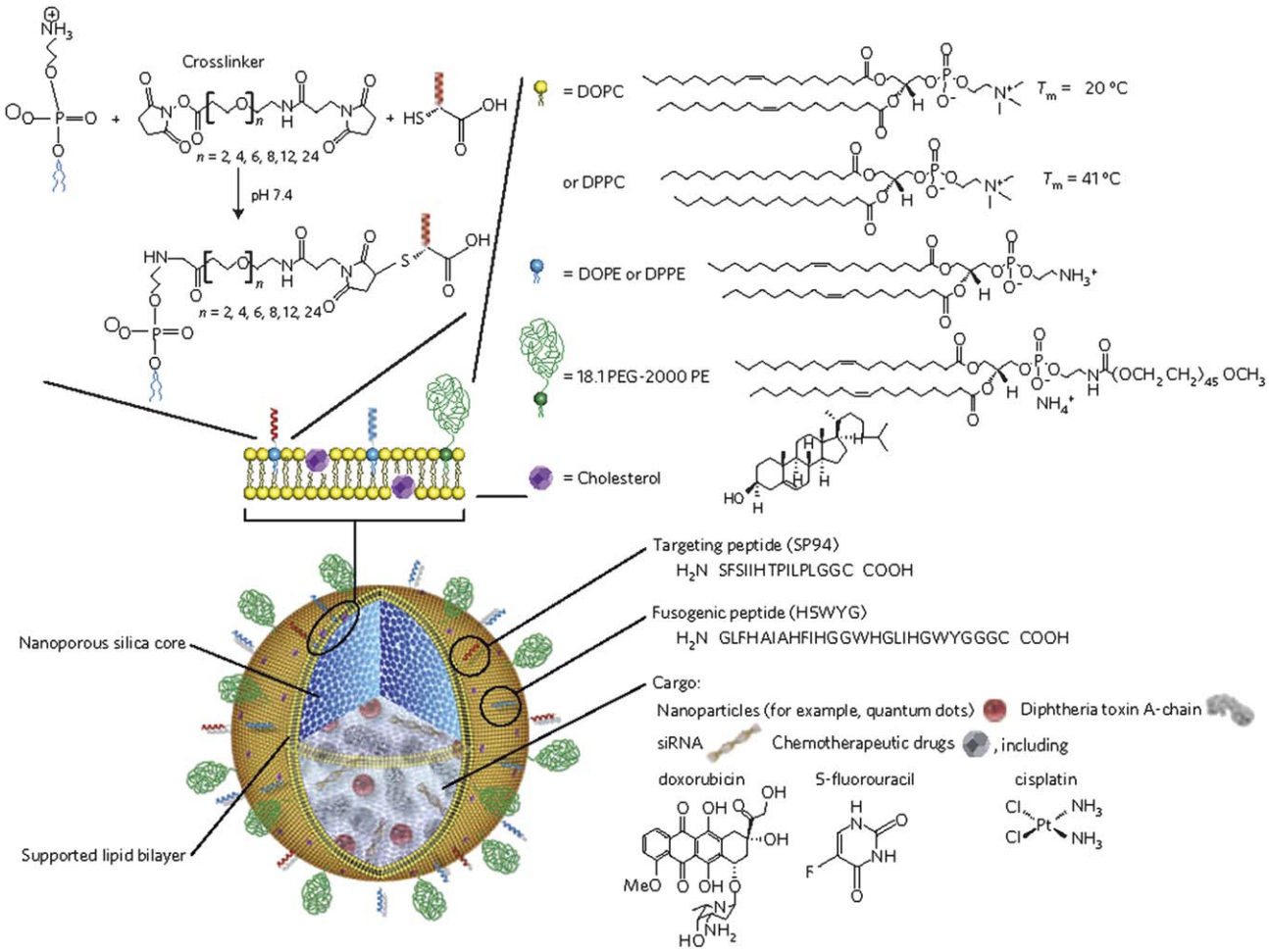

Fig. 9 Schematic diagram of mesoporous silica nanoparticles-supported lipid bilayer. Various types of therapeutic and diagnostic agents can be entrapped within the nanopores and liposome structure can be fused on the surface of nanopores with targeting and fusogenic peptides conjugated to the surface *via* a heterobifunctional crosslinker.[23] Reproduced with permission.

RES uptake, mediating endosomal escape and targeting specific cells, *etc.* SP94, a human hepatocellular carcinoma (HCC) targeting peptide, was conjugated onto the surface of protocells and the resulting affinity for HCC cells was 10 000-fold greater than that for hepatocytes, endothelial cells or immune cells. Such remarkable targeting efficiency was achieved by utilizing quite a few targeting ligands. This should be attributed to a 'mobile targeting' effect that the fluidity of lipid bilayer enables the target-ligands moving on the protocell surface to meet and bind with the receptors on cell surface.[23,89] The protocells were further utilized to package a variety of molecular cargos for combinational therapy. In the case of delivering a drug cocktail (DOX, 5-fluorouracil and cisplatin) into drug-resistant HCC cells, the therapeutic efficiency of protocells was 10^6 times higher than that of comparable liposomes.

Similarly, multiple siRNA molecules could also be packaged within a single protocell, and the encapsulating capability was 10–100-fold higher than corresponding liposomes.[24] After conjugation with targeting (SP94 and Hep3B) and endosomolytic (H5WYG) peptides onto the lipid bilayers, the obtained protocells intensively down-regulated the expression of specific genes in targeted HCC cells, resulting in a potent induction of growth arrest and apoptosis. Notably, the IC90 values (the concentrations of siRNA required to repress protein expression by 90%) decreased significantly from 331.5, 223.9, 543.6, and 1883.7 pM for cyclin A2, B1, D1, and E-loaded DOTAP liposomes to 125.3, 92.1, 149.0, 370.4 pM for protocells, respectively. Besides small molecular drug or siRNA, optical agents such as QDs could also be encapsulated within protocell core at meantime, which enabled protocells to possess both diagnostic and therapeutic functions. These highlighted progresses of protocells indicate that the lipid-enveloped MSNs have great potential in cancer treatment.[90]

5 Lipid-enveloped calcium phosphate particles

Calcium phosphate (CaP) has been extensively investigated and developed as a non-viral gene delivery system due to its excellent biocompatibility and physiological acceptability.[91] This material undergoes a rapid degradation under acidic pH, which probably promotes the escape from the endolysosomes/lysosomes and the release of genes into the cytoplasm of cells. Although the CaP-based delivery system exhibits several advantages *in vitro*, this formulation has not hitherto been tested after systemic administration.

Recently, Huang's group developed a novel kind of lipid-enveloped calcium phosphate (LCP) nanoparticles for efficient delivery of siRNA.[25–27] The CaP core was prepared in water-in-oil microemulsions and then CaP nanoparticles were co-extruded

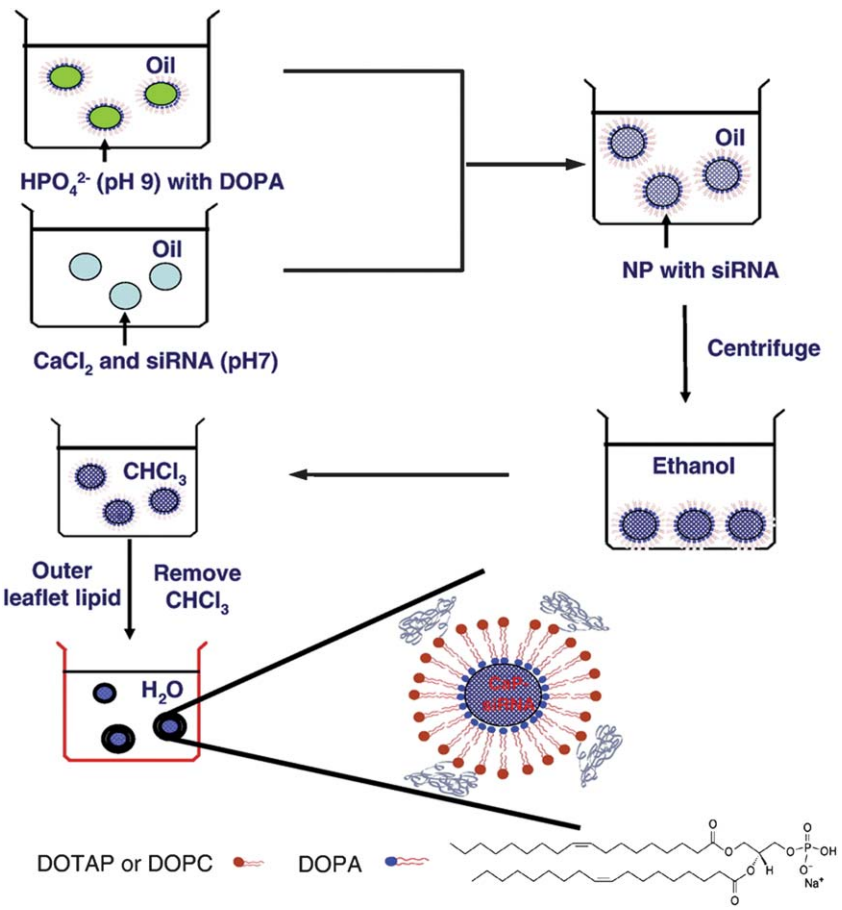

Fig. 10 The formulation process of liposome–calcium–phosphate (LCP) nanoparticles.[26] Reproduced with permission.

with DOTAP/cholesterol liposome to form the lipid-enveloped CaP nanoparticle (coded as LCP-I). The combination of CaP and lipids endowed LCP-I with an enhanced endosomal release capacity and a prolonged circulation time *in vivo* with little immunotoxicity.[25] However, these nanoparticles had a wide size distribution and the coated lipid bilayer was heterogeneous. Therefore, a modified fabrication approach was reported in their subsequent work (Fig. 10).[26] An anionic phospholipid, dioleoylphosphatidic acid (DOPA), was employed as a pre-coating reagent during the formation of siRNA-packaged CaP cores. Then the obtained small and hollow CaP cores (25–30 nm) were suspended in a chloroform solutions of different types of lipids to generate lipid-enveloped CaP hybrid nanoparticles (coded as LCP-II). Such LCP-II possessed a homogeneous and asymmetric lipid bilayer and their surface characteristics were determined by the outer leaflet phospholipids. In subsequent gene silencing experiments, it was found that the RNAi activity of LCP-II was improved 10-fold as compared to LCP-I and 40-fold to lipid–protamine–siRNA formulation. Based on the improved LCP-II platform, a siRNA cocktail (MDM2, c-myc, and VEGF) was encapsulated in the CaP core and then PEG and anisamide were conjugated on the outer lipid layer. Intravenous injection of this siRNA formulation resulted in simultaneous silencing of the respective oncogenes in metastatic nodules, thus significantly inhibiting lung metastases with efficiencies of 70–80% and substantially prolonged the mean survival time of the injected animals by 27.8% as compared to the control groups.[27]

6 Other lipid-enveloped inorganic nanoparticles

Besides the aformentioned MSNs and CaP, other inorganic nanoparticles such as single-walled carbon nanohorn (SWHN), iron oxide nanocrystals and QDs can also serve as the cores of lipid-enveloped hybrid nanoparticles.[28,44–47,92,93]

SWHN nanoparticle is a type of nontoxic aggregate of single graphene tubules with a spherical dahlia-like shape. To prepare lipid-enveloped SWNH nanoparticles, SWHN was first functionalized with carboxyl groups and followed by ultrasonication to obtain nanosized aggregates. Then, the SWHN cores were incubated with an aqueous solution of DOTAP liposomes to realize the coating of lipid bilayers.[28] The resulting lipid-enveloped SWNH nanoparticles were capable of loading drugs or magnetic resonance imaging (MRI) contrast agents, thus serving as drug delivery vectors or diagnostic reagents.

Small functional nanoparticles, such as iron oxide nanocrystals and QDs, can be also coated with lipid layers. Three fabrication methods are reported to form such lipid-enveloped structure. One is the thin-film hydration method.[44,45,92] It is nearly the same as the fabrication of liposomes except, it involves the addition of nanoparticles into a thin-film of the lipids. The second one is dual solvent exchange method.[45] The chloroform solution of the lipid and nanoparticles is first mixed with DMSO, followed by evaporating the chloroform. Then DMSO was replaced with water to form the lipid-enveloped structure with improved coating efficiency and quality. The third method is a modified emulsion method, in which the fat soluble nanoparticles are used instead of the hydrophobic drugs during the fabrication process as discussed in Section 2.1.3.[46] The stability and biocompatibility of inorganic nanoparticles, as well as *in vivo* circulation time, were significantly improved due to the lipid bilayer-enveloped structure.[47,93]

7 Summary and prospective

In summary, lipid-enveloped hybrid nanoparticles have been developed in theranostics and exhibited many advantages: (1) improved biocompatibility and stability; (2) extended circulation time; (3) specific targeting function; (4) enhanced drug encapsulation efficiency and reduced drug leakage (5) great potential in multimodal therapeutics. All of these properties give them great potential in cancer treatment.

In the future, carriers with structure identical to lipid-enveloped structure, such as protocells and nanocells, may promote the development of combinational therapy. Elucidating the mechanism of combinational therapy and achieving a precisely controlled release of therapeutic agent may be a challenge in this area. Bionic structure may be another important lipid-enveloped system. RBC membrane-enveloped structures have exhibited great clinical potential for their ability to be disguised as RBC. In order to realize the potential, some obstacles may still need to be overcome, such as retaining RBC properties and fluidity, prolonging the circulation time and simplifying the separation and preparation process of the hybrid nanoparticle. Taking MSNs as the core may improve the fluidity of RBC membrane, just like the 'protocell',[23,89] while fabricating the core with nanogel will increase the deformability of nanoparticles,[73] both of which may be helpful to improve the RBC-coated system. Finally, the reproducibility and productivity of nanoparticles are also important in order to get the clinical applications. Microfluidics has exhibited a promising progress in fabricating lipid-enveloped nanoparticles due to the controllable, reproducible and industrially producible properties. Anyway, how to realize clinical applications of these lipid-enveloped hybrid nanoparticles is still the main challenge. The progression of this system for cancer therapy may promote its translation from the laboratory to the clinic, because some of these hybrid nanoparticles have been prepared by FDA approved materials.

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