LIPID-POLYMER HYBRID NANOPARTICLES
FOR DRUG DELIVERY APPLICATIONS

Introduction

Since the last decade, there has been a burgeoning interest in the use of nanoparticle-based platforms for drug delivery applications. Nanoparticle-based delivery offers a number of advantages over traditional drug delivery platforms, including the ability to load multiple drugs, attach targeting ligands, enhance drug circulation time, and reduce non-specific drug toxicity. Nanoparticle formulations such as polymeric nanoparticles, liposomes, dendrimers, gold nanoparticles, carbon nanotubes and quantum dots have been widely researched, but only a handful of them have ever reached clinical use.1

The inherent advantages of liposomes and polymeric nanoparticles make them the most commonly studied among available drug delivery platforms. For example, liposomes offer excellent biocompatibility,2 while polymeric nanoparticles possess excellent stability and drug loading capacity.3 Although the majority of polymeric nanoparticles are still years away from clinical application, researchers have sought to combine the advantages of the two platforms—biocompatibility and high drug loading—by designing hybrids, known as lipid-polymer hybrid nanoparticles (LPNs).4

A typical LPN has a core-shell structure, consisting of a polymeric core for loading the cargo, such as small molecule drugs and/or diagnostic molecules, surrounded by a lipid shell for enhanced biocompatibility. The most widely used polymer in the core is poly(lactic-co-glycolic acid) (PLGA) due to its biocompatibility, biodegradability and general drug loading versatility.1,6 Several lipids, including phosphatidylcholine (PC); 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC); 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE); cholesterol; myristic acid; stearic acid; and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) have been used in the shell, in addition to poly(ethylene glycol) (PEG) lipid conjugates.5,7

One-step Synthesis of LPNs

Previously, researchers used various two-step synthesis methods (Figure 1), that require the lipid vesicles and polymeric nanoparticle to be separately synthesized before being fused together.8 This approach gives rise to LPNs with bilayer or multilayer lipid shells. Techniques used to fuse the liposomal shell and the polymeric nanoparticle core together include extrusion, sonication, direct hydration, vortexing, and high-pressure homogenization.

As first demonstrated by Zhang et al., a more convenient one-step synthesis method uses nanoprecipitation and the spontaneous self-assembly of lipid and polymer components (Figure 1A), yielding LPNs coated with a lipid monolayer shell.9 In this method, the polymer and cargo are dissolved in the organic phase (water-immiscible organic solvent) and the lipids are dissolved in the aqueous phase. The organic phase is added dropwise to the aqueous phase under continuous stirring, followed by self-assembly at room temperature. To the best of our knowledge, this is the simplest method of synthesizing LPNs currently available.

Alternatively, LPNs can be synthesized using an emulsification technique where the polymer is dissolved in the organic phase (water-immiscible organic solvent) and the lipids are dissolved in the aqueous phase. The solutions are mixed and sonicated to disperse the polymer into droplets and coat the polymers with a layer of lipid. The organic solvent is slowly evaporated under gentle stirring, and the LPNs are then purified for further use.

Figure 1: Schematic showing one- and two-step LPN synthesis. A) One-step synthesis method. B) Two-step synthesis method.
The basic LPN formulation can be modified using targeting moieties to enable site-specific cargo delivery. In many cases, the lipid shell is functionalized using simple conjugation chemistry such as EDC-NHS or thio-maleimide chemistry. For example, LPNs developed by Aravind et al. include AS1411 anti-nucleolin aptamers conjugated to the lipid shell to specifically target cancer cells over-expressing nucleolin receptors. In another example, Clawson et al. developed stimuli-responsive LPNs using a pH-sensitive, lipid-succinate-mPEG coating. In a low-pH tumor microenvironment, disassembly of the PEG layer is triggered causing the internalization of LPNs by cell membrane fusion.

While it is assumed the polymeric core can hold a variety of cargo (sometimes more than one type at once), the lipid shell can also be used to load cargo. Sengupta et al. loaded an anti-angiogenic agent combretastatin-A4 in a lipid shell containing DSPE-PEG, phosphatidylcholine, and cholesterol. At the tumor site, the angiogenic agent combrestatin-A4 is first released, which shuts down the tumor microenvironment, disassembly of the PEG layer is triggered causing the internalization of LPNs by cell membrane fusion.

Although LPNs have been previously formulated using two-step synthesis methods, here we describe a one-step synthesis method that makes them ideal candidates for drug delivery applications, including the ability to load multiple drugs, precisely control drug loading and drug release, and functionalize with targeting moieties.

Although LPNs have been previously formulated using two-step synthesis methods, here we describe a one-step synthesis method that is convenient and reproducible. This method gives rise to LPNs that are less polydisperse in size and whose physicochemical properties can be precisely controlled.

### Method: One-step Synthesis of LPNs

The following procedure describes a one-step synthesis method as performed by Prof. Juliana Chan’s research group at Nanyang Technological University.

LPNs are synthesized from soybean lecithin, DSPE-PEG, and PLGA using a one-step nanoprecipitation method combined with self-assembly.

1. **The aqueous solution is prepared by adding the following to a 4% ethanol aqueous solution in a glass vial (ethanol, Sigma-Aldrich Prod. No. E7023):**
   - Soybean lecithin consisting of 90–95% phosphatidylcholine (MP Biomedicals, Solon, OH)

The soybean lecithin/DSPE–PEG molar ratio can range from 7:3 to 8.5:1.5.

2. The organic solution is prepared by adding the following to a water-miscible organic solvent such as acetone:
   - Poly(ε-caprolactone-co-glycolide) (PLGA) with a 50:50 monomer ratio, ester-terminated, and viscosity of 0.72–0.92 dl/g
   - Small molecule drug such as docetaxel.

   The initial drug weight must not exceed 10–30% of the polymer weight for the drugs to be properly encapsulated by the polymer. The lipid/polymer weight ratio can range from 15%–20%.

3. The aqueous solution is heated to 65 °C on a hotplate stirrer under gentle stirring conditions for 3–5 min.

4. Once the reaction temperature is reached, the organic solution is added dropwise to the aqueous solution under gentle stirring conditions, followed immediately by vigorous vortexing for 3 min.

5. The mixture is returned to gentle stirring conditions and the LPNs are allowed to self-assemble for 2 h at room temperature.

6. The LPNs are washed three times using a Amicon Ultra-4 (Millipore, Billerica, MA) with a molecular weight cut-off of 10 kDa. The washed LPNs are re-suspended in water or buffer at a final desired concentration.

7. The LPNs are used immediately, stored at 4 °C overnight, or lyophilized for extended storage at ~80 °C.

### References


