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Review

Lipid-polymer hybrid nanoparticles: Synthesis strategies and biomedical applications

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ABSTRACT

This review article is an updated overview on lipid-polymer hybrid nanoparticles (LPHNs) including the various types of LPHNs polymers used in their preparation, various methods of preparation, their physiochemical, *in-vitro* and *in-vivo* evaluation parameters and their application in various delivery systems. LPHNs show a combined advantage of biodegradable polymeric nanoparticles and liposomes. LPHNs mainly consist of a biodegradable polymeric material core containing drugs or any substances which are to be encapsulated then this core is further enclosed by a phospholipid layer *i.e.* lipid PEG layer. LPHNs show good physical strength and biocompatibility. The hybrid structural design can offer various benefits such as controlled particle size, high drug loading, surface functionality with various ligands (antibody fragments, peptides, monoclonal antibodies, aptamers, and folate molecules), and encapsulation of combinational therapeutic agents, showing prolonged release of drug and drug circulates in the blood for longer duration. Significantly, the LPHNs have recently been confirmed as a better drug delivery route and good cellular delivery efficacy of various drugs as compared to polymeric nanoparticles and liposomes.

1. Introduction

This review will discuss the category of nanoparticles mainly in the size range between 10 and 1000 mm. This system was developed in the last 20 years in the field of medicine for the treatment of disease in more effective ways such as site specificity, receptor targeting in relevant times and doses and removal of the toxic effect of drugs. Nanoparticles can be synthesized from various natural, synthetic and semi-synthetic polymers. Nanoparticles are widely used as delivery vehicles of beneficial therapeutic substances like small drugs, genes, protein-peptides and diagnostic imaging agents encapsulated inside or conjugated on the surface of nanoparticle carriers (Kim et al., 2010; Soppimath et al., 2005; Hans and Lowman, 2002).The comparison between nanometric size drug carriers with microparticles is that they are made up of biodegradable polymers which are only useful for controlled and localized drug release from several weeks to months after administration but they are too large for intravenous administration and also for drug targeting (Müller et al., 2000). For that reason, nanoparticles play an important and successful role in achieving the target of controlled and site-specific drug release. In 1986, Dior introduced liposomes for cosmetic use in the market, but some years after this liposomes were used in pharmaceutical products for the treatment of various diseases. In the area of nanocarriers, micro and multiple emulsions were also invented but they have disadvantages like degradation of active incorporated compounds and instability in the formulation. Commonly used nanocarriers were polymeric nanoparticles, polymeric micelles, dendrimers (polymers), liposomes, solid lipid nanoparticles (lipids) and metal (gold, silver, silica) nanoparticles (Mullera et al., 2002; Hadinoto et al., 2013).

Liposomes are spherical vesicles with a lipoidal bilayer membrane containing amphiphilic lipid molecules which are used to deliver both hydrophobic and hydrophilic drugs that protect the drug from the external environment (Fig. 1A) (Zhang and Zhang, 2010; Albanese et al., 2012; Enlow et al., 2011; Sharma and Sharma, 1997). But the application of liposomes in drug delivery was limited because of low encapsulation efficiency of low water-soluble drugs such that the release of entrapped the drug occurs quickly resulting in the instability of the drug. Some liposomal formulations also available on the market include daunorubicin liposome (DaunoXome), amphotericin B liposomes (Ambisome) and morphine liposome (DepotDur) (Gregoriadis, 1995). On

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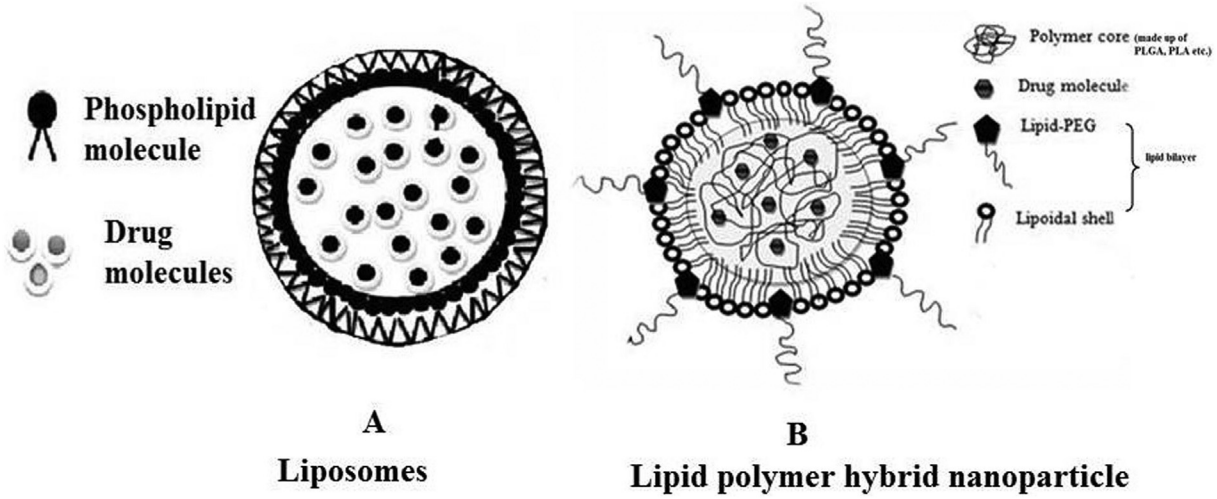

Fig. 1. (A) liposomes bilayer structure, and (B) Lipid polymer hybrid nanoparticles with inner polymer core containing drug molecule surrounded by lipid and lipid PEG layer.

the other hand, polymeric (biodegradable) nanoparticles contain hydrophobic drugs with high loading capacity as compared to liposomes. Drug release from these polymeric nanoparticles with polymer degradation and drug diffusion can be controlled by selecting appropriate polymers, but they also had problems like moderate circulation lifetime and possible biocompatibility issues (Zhang and Zhang, 2010; Pinto et al., 2006.

Due to the various restrictions of liposomes and biodegradable polymeric nanoparticles, a novel drug delivery system known as a lipid-polymer hybrid nanoparticle (LPHNs) was used which has more benefits as compared to liposomes and nanoparticles (Zhang and Zhang, 2010; Zhang et al., 2008). The structure of drug loaded LPHNs is shown in Fig. 1B which shows three distinct functional components. The inner polymer matrix (core) which contains therapeutic drug material (mainly low water soluble/hydrophobic drug) with high loading efficiency and the lipoidal layer which is surrounded by a polymer core that confers biocompatibility and encourages drug retention inside the polymer core. The outer layer is made up of lipid PEG which coats the lipoidal layer and thus enhances the stability, prolongs *in vivo* circulation time and helps in steric stabilization (Chan et al., 2009). The polymeric core and lipid covering are linked through Vander walls forces, hydrophobic interactions, electrostatic interactions, and various noncovalent forces but the water-soluble polymer core layer is frequently conjugated by covalent bonds to the surrounding lipids. Various pharmaceutically bioactive therapeutic molecules like drugs, genes, proteins peptides, vaccines, diagnostic imaging agents and targeting ligands can be encapsulated, adsorbed, or covalently bound in this hybrid polymeric system (Gao et al., 2007; Li et al., 2010; Moon et al., 2012; Mieszawska et al., 2012). LPHNs have been rapidly involved in robust drug delivery platforms due to their many advantages over other nanocarrier systems like high structural reliability, storage stability, controlled release ability attributed to the polymer core high biocompatibility because of the presence of PEG-lipid (Chan et al., 2009; Thevenot et al., 2007).

2. Types of lipid polymer hybrid nanoparticles (LPHNS)

The hybrid nanoparticles were classified into different groups based on the different arrangement of lipids and polymers (Fig. 2).

2.1. Polymer core lipid shell

This is the simplest form of LPHNs. It consists of a polymer partial core which is coated with lipid single layer or bilayer (lipid PEG and

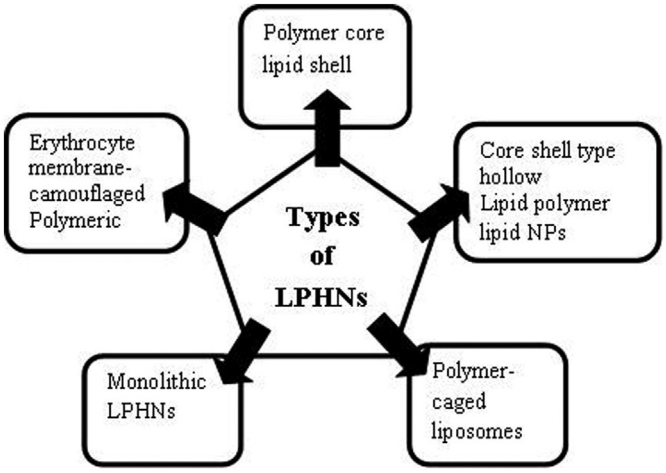

Fig. 2. Different types of lipid-polymer hybrid nanoparticles.

lipiodial shell) (Fig. 1B), the biomimetic quality of lipids and structural advantage of biodegradable polymer core jointly give an advanced and beneficial delivery system which is useful for the treatment of various systemic and topical disorders. (Thevenot et al., 2008).

2.2. Core-shell type lipid polymer-lipid hybrid nanoparticle (CSLPHNs)

This type of nanoparticle has an empty inner core which is bordered by a concentrated lipid covering, followed by a polymeric coating on the inner core and surrounded by lipid coating with lipid PEG as shown in Fig. 3A. This polymeric core is surrounded by single or several layers of lipid. They are usually arranged by mixing the properties of both liposomes and polymeric nanoparticles to form the complexes of polymer and lipid in which a bilayer of lipid or lipid multilayer surrounds the polymeric core surface. The gap between the core of the polymers and the layer of lipid is generally filled with aqueous buffer or water. For promoting electrostatic interactions with oppositely charged polymers mainly cationic or zwitterion phospholipids were used to prepare the covering of the lipoparticles (Mandal et al., 2013; Shi et al., 2011).

2.3. Erythrocyte membrane-camouflaged polymeric nanoparticles

These nanoparticles are also called biomimetic nanoparticles. They

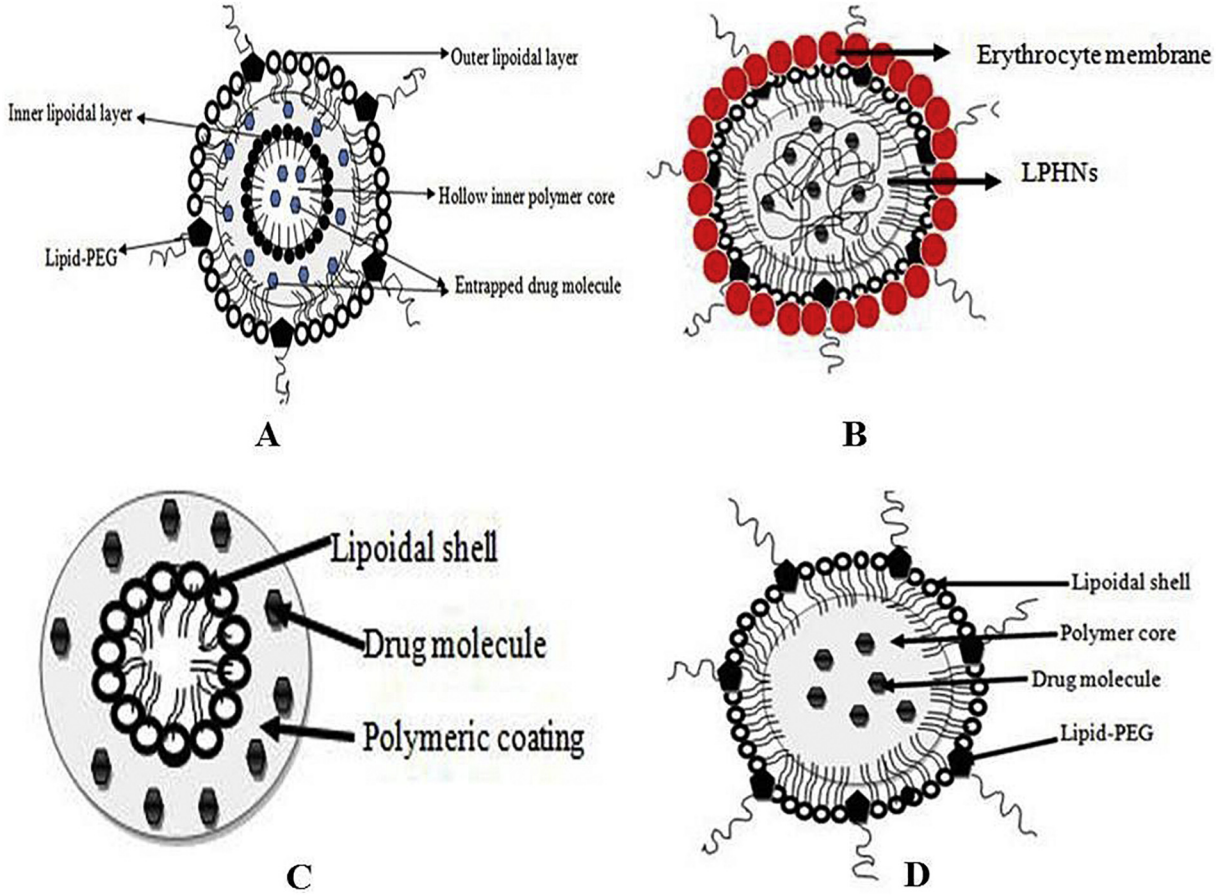

Fig. 3. Different types of lipid-polymer hybrid nanoparticles.

are in the sub-100 nm size range coated with an erythrocyte cell membrane (RBC) polymer as shown in Fig. 3B to form vesicles that mimic or copy the difficult surface chemistry of the erythrocyte membrane (Hu et al., 2011). These types of nanoparticles easily cross the membrane barrier and easily deliver drugs for long periods of time by circulating in the bloodstream.

2.4. Monolithic lipid-polymer hybrid nanoparticles

Monolithic LPHNs are also known as mixed lipid-polymer hybrid nanoparticles. In this type of nanoparticle lipid or lipid PEG molecules are distributed in a polymeric core matrix which contains drug molecules as shown in Fig. 3C (Shi et al., 2011).

2.5. Polymer caged liposomes

This method involves the self-possession of polymers coated at the liposome surface to provide stability of the system as shown in Fig. 3D (Enlow et al., 2011; Lee et al., 2007).

3. Various methods of preparation of lipid polymer hybrid nanoparticles

Generally, LPHNs can be synthesized mainly by two broad methods; one step processes and two-step processes which can be further subdivided shown in Fig. 4. All the methods of preparation are also described below.

3.1. Two-step method

The two-step process is the frequently adopted technique used in the starting phase of LPHNs formulation; it is commonly used to prepare monolayer, bilayer or multilayer shells. In this method, with the help of electrostatic interactions, cationic lipid vesicles are combined with anionic polymeric nanoparticles. Firstly lipid nanoparticles were prepared by various methods like micro-emulsification, ultrasonication, high-pressure homogenization (hot and cold), melt emulsification, solvent emulsification, and solvent injection methods. Then the polymer cores were prepared by dissolving suitable biodegradable polymers in water-immiscible organic solvents such as chloroform containing drugs, then the polymeric solution was mixed with the preformed lipid nanoparticles to make lipid-polymer hybrid nanoparticles with the help of simply vortexing, needle extrusion, or ultrasonication high-pressure homogenization. (Hadinoto et al., 2013; Zhang and Zhang, 2010; Mandal et al., 2013; Troutier et al., 2005b) Fig. 5 shows the main steps for the preparation of LPHNs by the two-step method. Two-step methods are mainly of two types conventional and non-conventional two-step methods, which are described below.

3.1.1. Conventional two-step methods

Conventional methods are used for mainly small scale preparations of hybrid nanoparticles. Polymeric nanoparticles were prepared with the help of Emulsification solvent evaporation, nanoprecipitation, or high-pressure homogenization. Then these nanoparticles are complexed with the preformed lipid vesicles with the help of electrostatically bonded lipids or PEG which adhere to the surface of the polymeric nanoparticles. The polymeric nanoparticles prepared earlier were added to a dried thin film of lipid formed by dissolving the lipid in an organic solvent followed by vaporization in a rotary evaporator. Then the solution mixed above was subjected to ultra-sonication or vortexing at temperatures greater than the transition temperature of lipids required to form LPHNs. The LPHNs were centrifuged to separate them

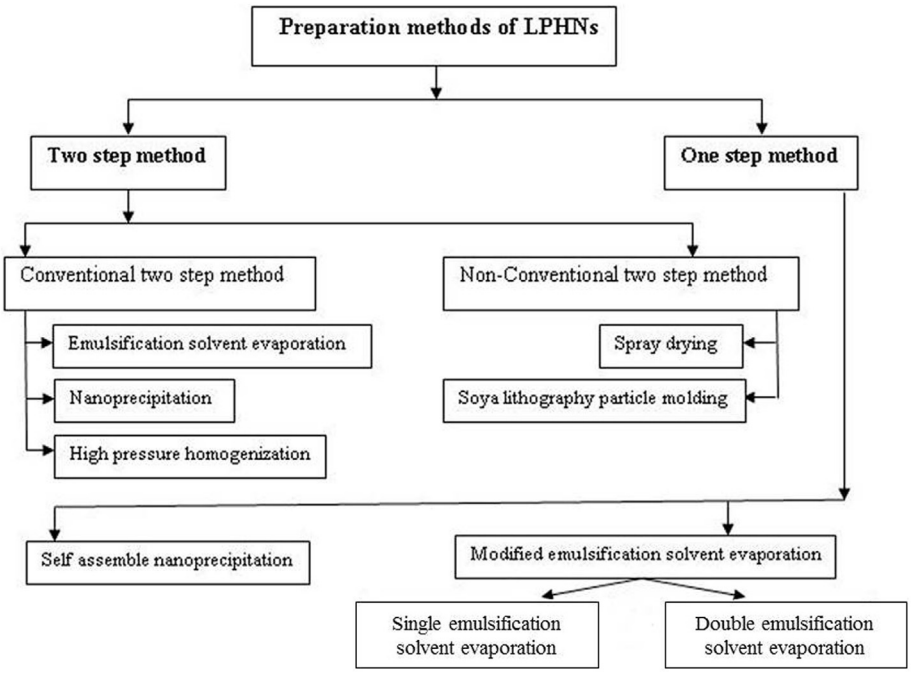

Fig. 4. Various method of preparation of LPHNs.

from the solution.

For obtaining the monodispersed form of LPHNs they were exposed to extrusion or homogenization steps (Troutier et al., 2005b; Wu et al., 2006; Messerschmidt et al., 2009). For example, Zhao et al., 2012 prepared LPHNs using PLGA polymer which forms the inner core and outer shell made up of cationic lipids of FA-OQLCS/ PEG-OQLCS/ Cholesterol. LPHNs were prepared by adding the PLGA nanoparticles with the vesicles of lipids at constant stirring or with the help of a bath sonicator at 30 °C resulting in the formation of LPHNs with 200 nm size and (+) 20–30 mV zeta potential. A similar procedure was also done by Sengupta et al. (2005) and Wang et al. 2010a.

3.1.2. Nonconventional two-step method

The non-conventional method is mainly used for large scale production of LPHNs. In this method, LPHNs were prepared with the help of various techniques like spray drying and soft lithography particle molding. In the spray drying technique polymers such as polyglutamic acid and polylysine were used to produce nanoparticles in the size ranging of 400–500 nm, after which they were dispersed in organic solvent (dichloromethane) containing various lipids. The lipoidal polymeric suspension was further spray dried to produce LPNHs. For example, Wang et al. worked on a relative study using spray drying (SD) and spray freeze-drying (SFD) to form LPHNs of levofloxacin in the dry powder form through an inhalation route (Wang et al., 2012). Another approach to synthesize LPHNs of the smaller size used a newly

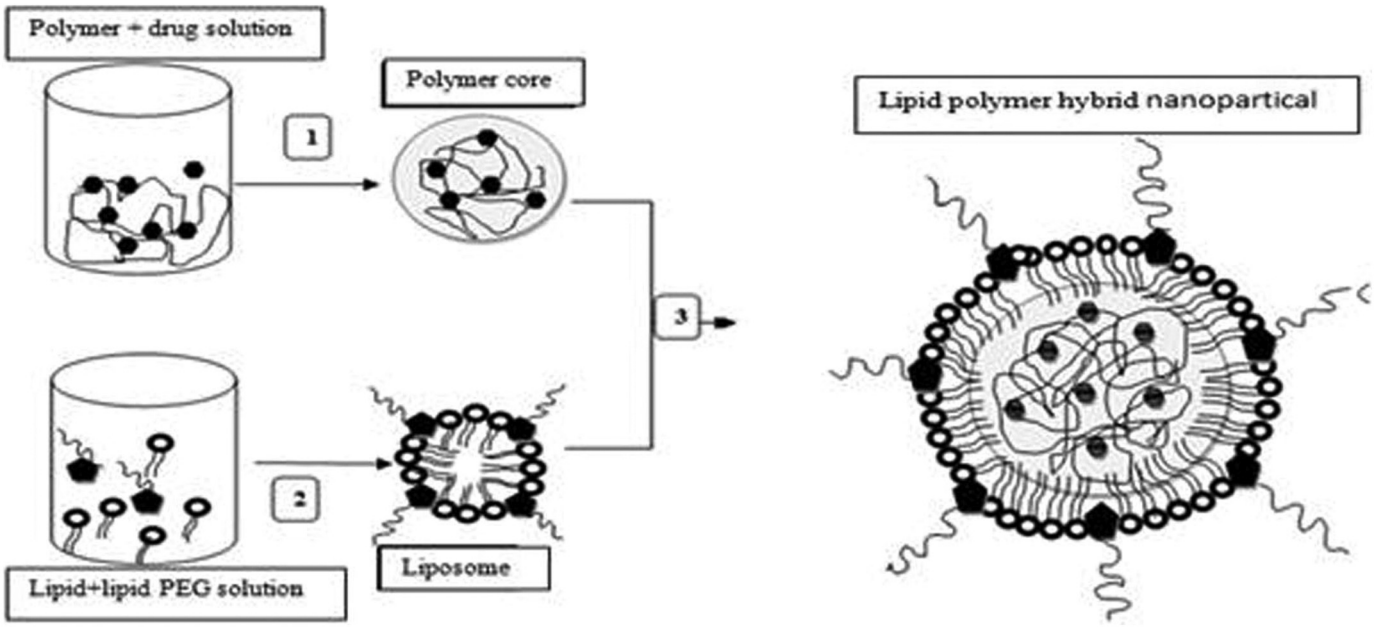

Fig. 5. Preparation of LPHNs by two-step method; (1) polymer core containing drug prepared separately, (2) lipoidal shells (liposomes) with lipid PEG prepared separately; (3) polymer core and liposomes mixed together by different techniques.

commercialized nano spray dryer (Hitzman et al., 2006; Li et al., 2010). A soft lithography molding technique also is known as particle replication in nonwetting templates (PRINT) was used to prepare LPHNs for genetic material delivery (Hasan et al., 2011) In this method, organic solvent polymer PLGA or genetic material such as siRNA were dissolved and then shed into a polyethylene terephthalate (PET) sheet, then heated in conformal contact with a PRINT mold, allowing the polymer to pour into the mold and leave to solidify upon being returned to ambient temperature, then polymeric nanoparticles were formed. After this, nanoparticle were removed from the mold and with the help of aqueous solution of lipids nanoparticles were separated from PVA coated PET sheets leading to the formation of LPHNs. After the freeze drying of LPHNs the particles had needle shapes with 200 nm length and (+) 5 mV zeta potential (Hasan et al., 2011).

3.2. One step method

One step method for the preparation of LPHNs shows various benefits such as cost-effectiveness, highly scalable, and the method of preparation was conventional. The drawback of two-step methods is separate preparation of polymeric nanoparticles and lipid vesicles which are very time consuming requiring higher energy expenses. To overcome these problems a one-step method was developed which involves a simple mixing of lipid solution and polymeric nanoparticles by nanoprecipitation or emulsification solvent evaporation method which is described in Fig. 6.

3.2.1. Self-assembled nanoprecipitation

The self-assembled nanoprecipitation method showed high production yield of sub 100 nm lipid-polymer particles. In this method LPHNs were prepared by firstly dissolving the polymers and drugs to be encapsulated in water or any organic solvent then lipid or lipid PEG was dissolved in water by heating at 65–70 °C to form a uniform dispersion. The solution was then continuously stirred or sonicated, the polymeric drug solution was added dropwise to precipitate the polymer and the lipid or lipid PEG molecule self-assembled around the core of the polymer with hydrophobic interactions. To the polymeric core a water-immiscible lipid tail was attached and to the external environment, a water-miscible head was attached forming LPHNs sterically stabilized by lipid or lipid PEG. The LPHNs were then centrifuged to remove extra lipid and polymer and to evaporate the solvent (Schafer et al., 2009; Su et al., 2011). The size and polydispersity index of the hybrid nanoparticles depends on polymeric material concentration, solvent volume ratio and mixing speed of the solution. The lipid-polymer ratio indirectly affects the encapsulation efficiency, drug loading and drug release kinetics (Zhang et al., 2008; Schafer et al., 2009). Hybrid nanoparticles of dextran sulphate PLGA of vincristine sulfate were prepared by the self-assembled nanoprecipitation method to increase the encapsulation efficiency and oral bioavailability of vincristine sulfate (Ling et al., 2010). Recently microfluidic nanoprecipitation and multi-inlet vortex microreactors were two advanced methods of nanoprecipitation which included high production and improve size homogeneity of nanoparticles.

3.2.2. Modified emulsification solvent evaporation [ESE]

The ESE method is divided into two types, single and double emulsification methods which were firstly reported by Gurny et al., 1981. In these methods, the lipid is dispersed in water through various processes like stirring, bath sonication and for the uniform dispersion of lipid in water, it is heated at a particular temperature to dissolve the organic solvent, polymer and drug. Then the organic solution was added drop wise in the aqueous dispersion and afterwards the mixture was probe sonicated for 5 min to convert polymeric particles into small particles and thus spherical shaped nanoparticles with a lipid layer coating were formed. With the help of rotary evaporator, the organic solvent was vaporized at room temperature then the solution was stirred overnight. The suspension was cold centrifuged to purify along with repeatedly washing with distilled water. To obtain the nanoparticle in solid form the suspension was lyophilized (Mandal et al., 2013).

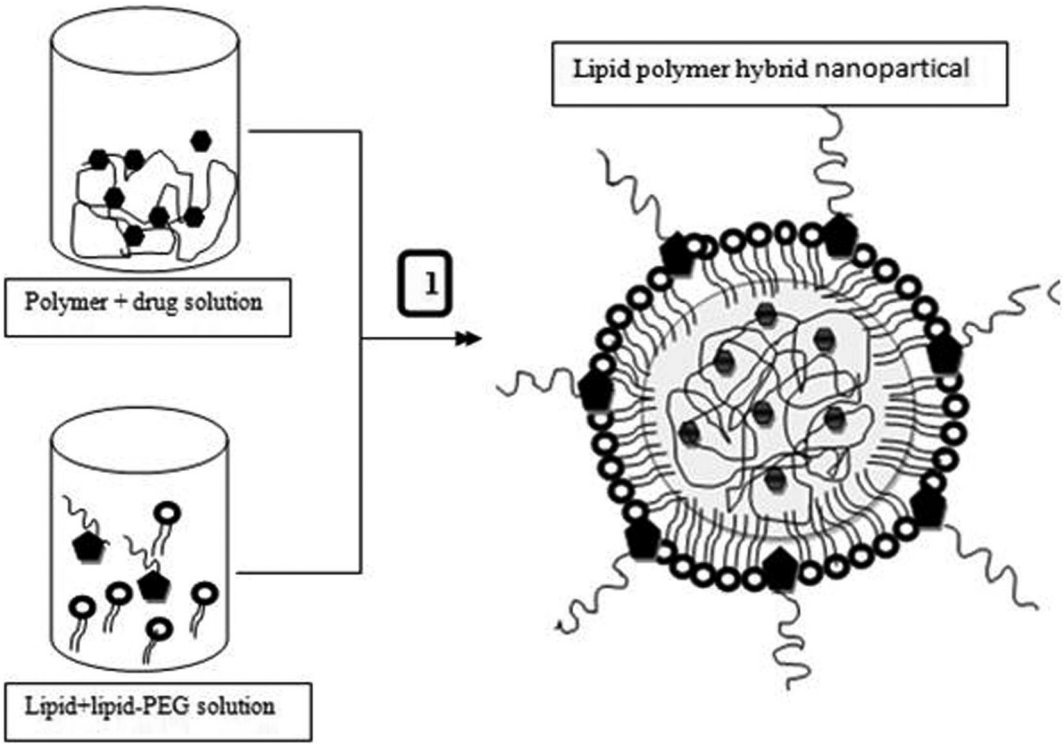

Fig. 6. A one-step method forming hybrid nanoparticles through a nanoprecipitation and self-assembly method mixing drug containing polymer solution with lipid and lipid PEG aqueous solution.

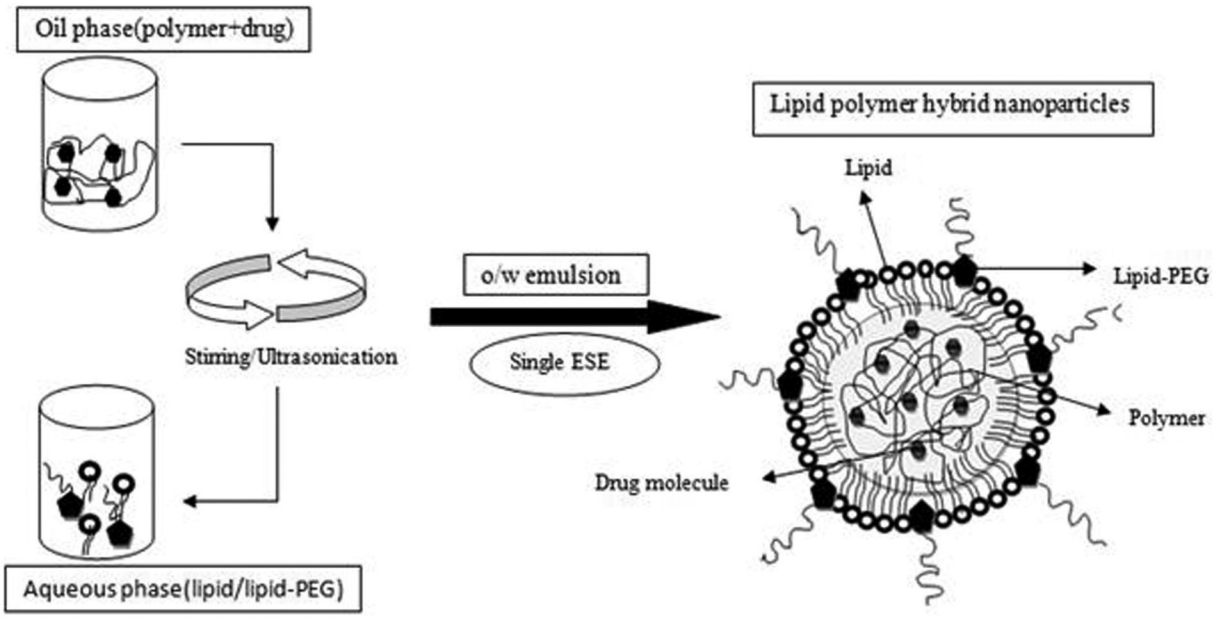

Fig. 7. Hybrid nanoparticle preparations by single emulsification solvent evaporation method.

3.2.2.1. Single emulsification solvent evaporation. A single ESE method (o/w) is shown in Fig. 7. In the single ESE process the therapeutic material to be encapsulated and the polymer is dissolved in a lipophilic solvent like an oil phase, then it is transferred, into a lipid-water dispersion to formulate an oil in water (o/w) emulsion under constant stirring, probe or ultra-sonication. The organic solvent is removed with the help of a rotary evaporator under reduced pressure; producing the polymer core and at the same time, the lipid or lipid PEG self assembles in the surrounding region of the polymer core (Cheow and Hadinoto, 2011; Bershteyn et al., 2008). The method formulates a large number of LPHNs as compared to the nanoprecipitation method due to the formation of a stable emulsion.

3.2.2.2. Double emulsification solvent evaporation. This method is employed when the drug is insoluble in an organic solvent. In this method the drug is first dissolved in water and then in the oil phase (containing lipid and polymer) it is emulsified to form a w/o. Afterwards this emulsion is added into the aqueous phase (containing lipid PEG) to formulate w/o/w emulsion. With the help of a rotary evaporator, the oil phase of the emulsion was evaporated leading to the formation of LPHNs. The method of preparation is shown in Fig. 8. In double ESE method, LPHNs contain a lipid layer inside which covers the hollow core of the aqueous media and the polymer layer is present between these two layers with the lipid-PEG layer present outside (Zhao et al., 2012).

4. Various routes for therapeutic delivery of LPHNS system

Advances in nanomedicine have mainly been attained in the use of nanoparticles for the delivery of therapeutic substances, but in delivery systems, nanoparticles in the range of 50–500 nm are suitable depending on the administration route. Many routes are suitable for deliveries of LPHNPs. With the help of nanoparticles, the pharmacokinetics of the drug is changed without changing the active drug. Significant effect on drug efficacy is completely dependent on the method by which a drug is delivered.

4.1. Oral therapeutic delivery system for LPHPs

The oral route is the most suitable route for the delivery of therapeutic substances because it is highly accepted by patients. As manufacturing of oral formulations does not require a sterile environment

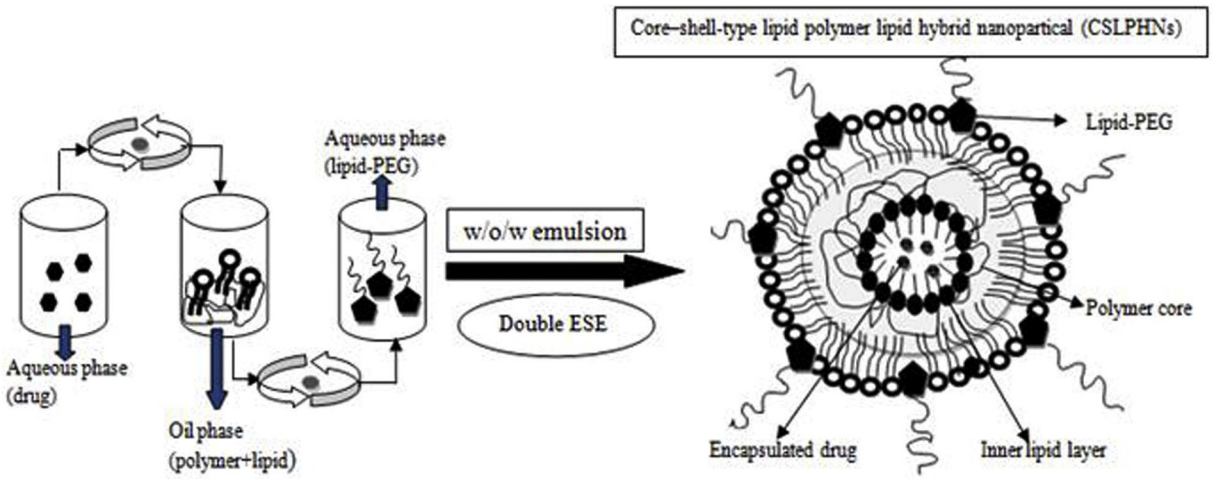

Fig. 8. Hybrid nanoparticle preparation by double emulsification solvent evaporation method.

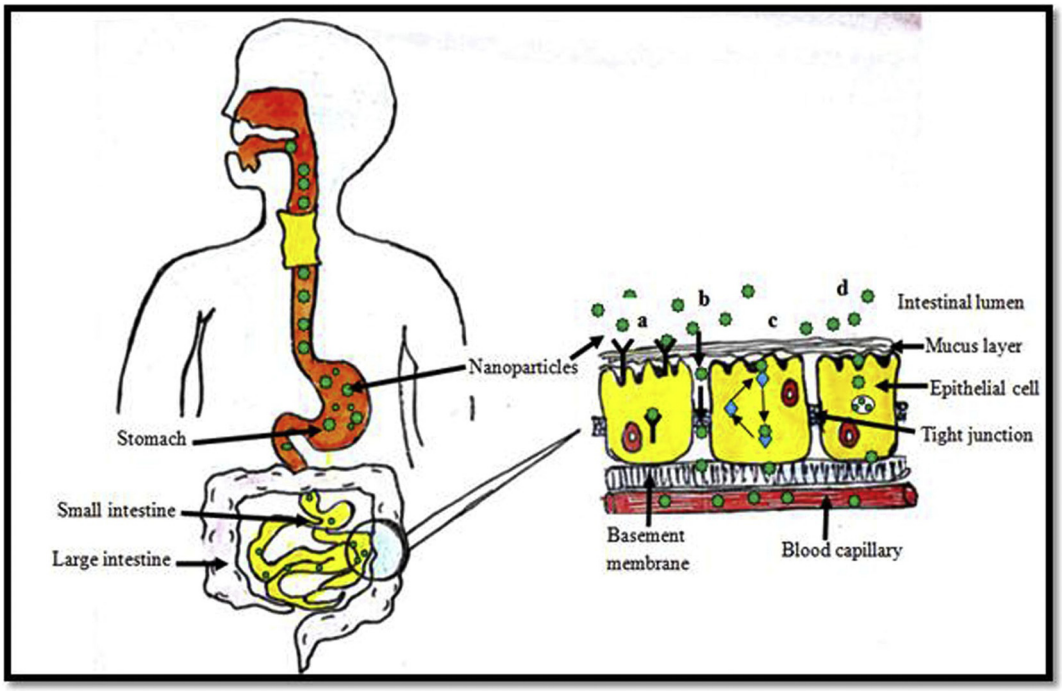

Fig. 9. Oral delivery entry points of nanoparticles into the GIT by various mechanisms: (a) receptor-mediated transport; (b) paracellular transport; (c) carrier-mediated transport; (d) transcellular transport.

they are cheaper than other formulations. Encapsulation of drugs in nanoparticles reduces disadvantages of the drug such as degradation from the gastric environment is prevented, delivery of the drug is only at the targeted site thus achieving sustained release of the drug (Yun et al., 2012). With the help of four mechanisms nanoparticles can easily cross the biological membrane *i.e.* paracellular transport, transcellular transport, receptor-mediated and carrier-mediated transport as shown in Fig. 9. In the GI tract, drug absorption depends on various factors like hydrophobicity, molecular weight, pH stability of the drug and biological membrane, hydrophobicity (Ensign et al., 2012).

4.2. Transdermal or topical therapeutic delivery system for LPHNPs

In this type of drug delivery system, the therapeutic substance is applied on the skin to achieve the therapeutic level of drug in the blood. This type of drug delivery provides various advantages like first pass metabolism of the drug is avoided, easy to administer the drug, maintain the concentration of drug in a steady state manner in blood *etc.* This leads to enhanced patient compliance, particularly when long term treatment is required, as in chronic pain treatment like cancer therapy and smoking cessation therapy. In the case of toxicity during treatment, the transdermal or topical therapy can easily be discontinued by the patient (Valenzuelaa and James, 2012). The study report was performed by González et al. produce particulate hybrid carriers containing a glyceryl monostearate (Lumulse® GMS-K), a waxy triglyceride (Cutina® HR), silanized TiO_2 and caffeine were investigated with the aim of producing sunscreens with UV-radiation protection properties for topical application (Troutier et al., 2005a).

Nanoparticles in the topical administration provide various advantages like provide sustained release of the drug, protects the drug from the chemical degradation. In topical drug delivery system, the drug enters the skin through the intracellular mechanism, transcellular mechanism, transappendageal pathway as shown in Fig. 10. The entry of nanoparticle in the skin depends on various factors like size, polymers and lipid properties used in the preparation, surface charge, encapsulation of the drug, lamellarity of the particles. (Chen-yua et al., 2012).

4.3. Pulmonary therapeutic delivery system for LPNPs

In the recent years, pulmonary delivery of the active drug substance through nanoparticles has recently gained important interest in the drug delivery system because of the high bioavailability, avoids lung phagocytic and mucociliary clearance mechanisms of lung resulting in elongated drug residence time. Moreover, hybrid nanoparticles in general exhibit higher hydrophobicity than polymer nanoparticles resulting in enhanced cell intake and lower cytotoxicity. In the case of drugs which have poor bioavailability, the pulmonary route is the preferred route (Wang et al., 2012). Wang et al. worked on comparative studies of employing spray drying (SD) and spray freeze-drying (SFD) to produce an inhalable dry powder form of drug loaded lipid-polymer hybrid nanoparticles of levofloxacin for the treatment of lung infection. The mechanism of drug delivery through the pulmonary route is shown in Fig. 11.

After orally inhaled, there are three established mechanisms for retrieving the particles in the lung: gravitational sedimentation, Brownian diffusion, and inertial impaction. For the deposition of agglomerates and microparticle impaction and sedimentation play an important role. The particles with the diameter less than 5 nm get a deposit in the flat form in oropharynx as compared to the particle which has diameter ranged from 1 nm to 5 nm *i.e.* nonporous particle can attain the pulmonary deposition deeply but they get engulfed by an alveolar macrophage. Because of the low density of the porous large particle, they get a deposit in the lung deeply and due to their larger diameter, they escape the phagocytosis phenomena (Liang et al., 2014; Olsson et al., 2011).

4.4. Ocular therapeutic delivery system for LPNPs

As compared to the conventional delivery system nanoparticle show more benefits to treat the various eye-related disease as they do not cause any type of irritation to the parts of the eye like cornea, iris, pupil *etc.* Delivery of drugs to the eye in the form of nanoparticle reduces the dose, shows a good drug release profile providing prolonged release of time, concentration of the drug at target site was increased; residence

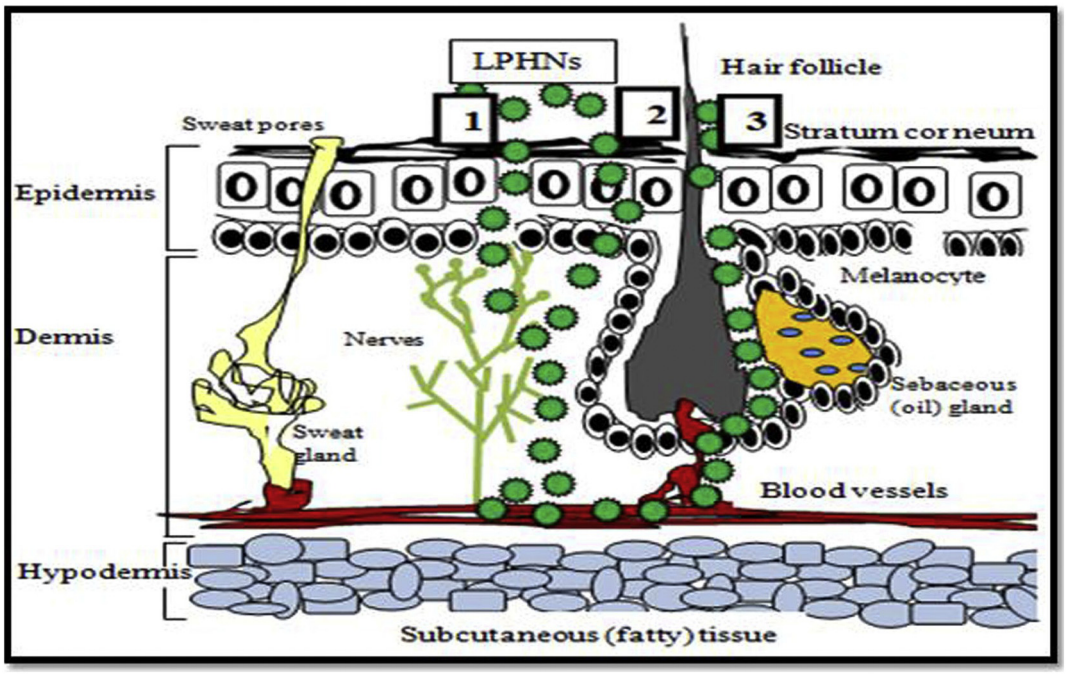

Fig. 10. Transdermal or topical delivery pathway for entry of nanoparticles into the skin. Nanoparticles entering the stratum corneum by (1) transcellular route, (2) intracellular route (across the intact horny layer), (3) transappendageal pathway (the hair follicles with the associated sebaceaous glands) reaching the epidermis, dermis and blood vessels.

time of drug on the surface of cornea is increased and reduces the systemic toxicity of drug (Das and Suresh, 2010). For the delivery of nanoparticle through intraocular tissue two factors need to be considered *i.e.* cornea or blood-ocular barrier as it is difficult to cross this barrier and reach the drug at the site of action. By topical (drops, ointment *etc.*) application, the drug gets diffuses around the tear film, cornea, iris, cilliary body, and vitreous humor before it reaches to the target tissue in the posterior eye. The systemic delivery of drug shows a poor response in the retina. Intravitreal or Subconjunctival implant through transscleral diffusion increases the concentration of drug in the retina (Zimmer and Kreuter, 1995). The deliveries of the drug in the eyes through various routes were shown in Fig. 12.

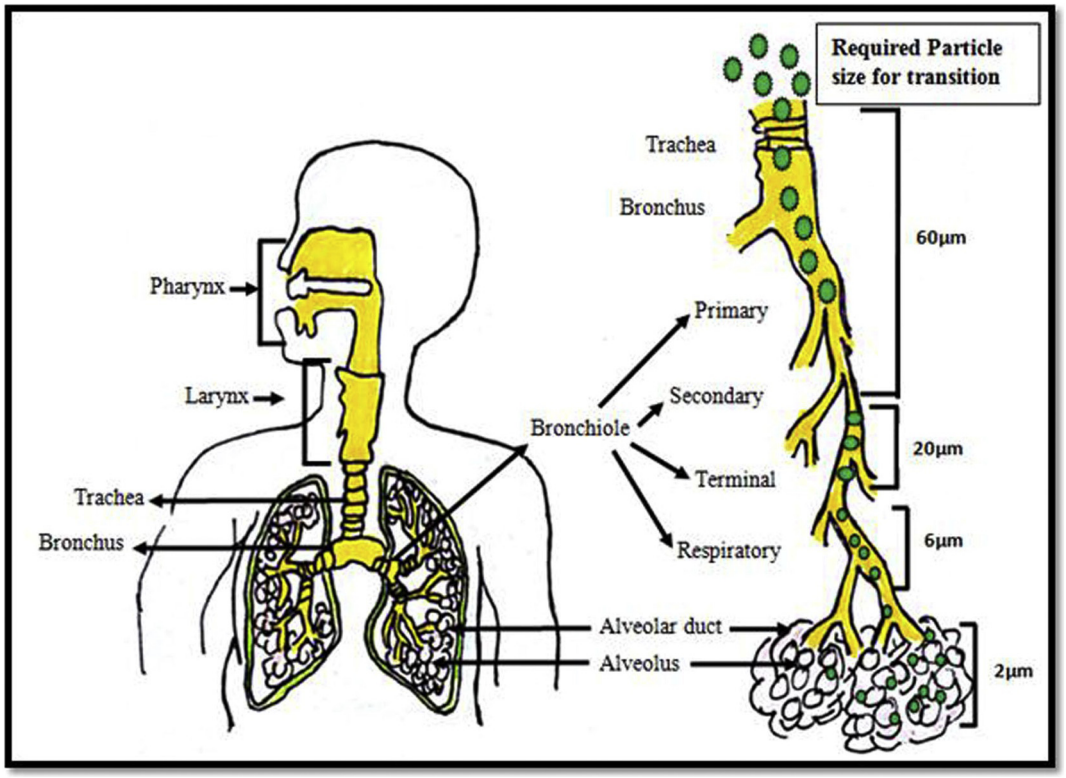

Fig. 11. Pulmonary delivery pathways for entry of nanoparticles according to particle size deposit into the lungs.

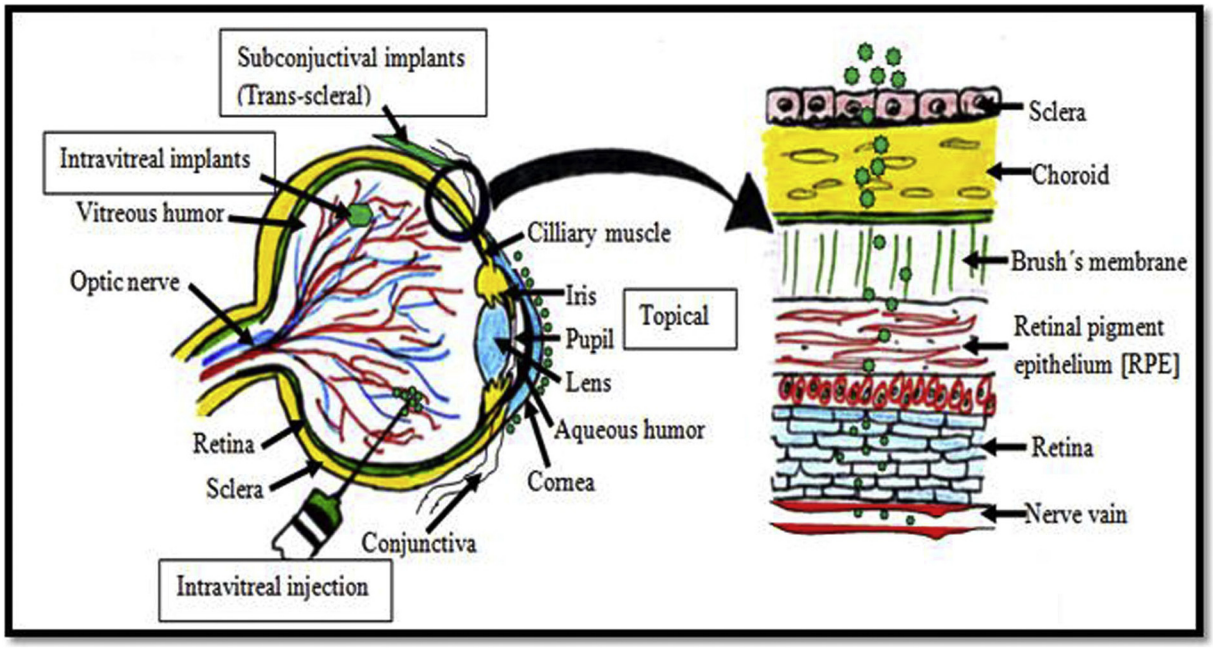

Fig. 12. Ocular delivery pathways for entry of nanoparticles into the eye by a topical, systemic and intravitreal or subconjuctival implant.

4.5. Lipid polymer hybrid nanoparticles surface functionalization

The outer lipid PEG layer is necessary to keep the stability of the LPHNs both *in vitro* by decreasing aggregation and *in vivo* by allowing the particles to recognize by the reticuloendothelial system (RES) and other immune cells. Additionally, the lipid-PEG layer also makes available space for functional groups for further modification of the hybrid nanoparticles with targeting ligands like monoclonal antibodies, antibody fragments, aptamers, peptides, and small molecules such as folic acid. For cell or tissue-specific drug delivery targeting ligands are conjugated to the nanoparticles through covalent bonds with the functional groups at the end of the lipid-PEG chains (Zhang and Zhang, 2010). Various studies performed to enhance the active drug substances by conjugating the ligands on the surface attachment of nanoparticles were shown in Table 1 (Hu et al., 2010; Liu et al., 2010b; Chang et al., 2011). In nanoparticles, these targeting ligands can be conjugated with the lipid-polymer hybrid nanoparticles to enhance delivery efficiency at targeting site.

5. Lipid polymer hybrid nanoparticles characterization

5.1. Physicochemical characteristics

5.1.1. Particle size distribution, Zeta potential, and Surface morphology

It has been well documented that nanoparticles with a size range of between 10 and 150 nm were useful and desirable for the delivery of the drug in blood circulation. To determine the surface morphology of the particles scanning electron microscopy (SEM), transmission electron microscopy (TEM) and Atomic force microscopy study was performed (Salvador-Morales et al., 2009; Garcia-Gonzaleza et al., 2009; Troutier et al., 2005a; Peyrot et al., 1994). Zhang et al. study the surface morphological of the LPHNs by TEM using negative staining (Zhang et al., 2008).The particle size and their distribution were determined by using the principle of dynamic light scattering (DLS) (Pinto et al., 2006; Wong et al., 2006). Valencia et al., 2010 effectively prepared highly monodisperse LPHNs with 40 nm particle size and (−) 20 mV zeta potential by using the nanoprecipitation method in a microchannel (Salvador-Morales et al., 2009). Surface zeta potential is determined by Malvern zetasizer which determine electrokinetic potential among the surface of the particle and the solution present in bulk quantity (Garcia-Gonzaleza et al., 2009). The charge present on the surface of nanoparticle determines the stability of the formulation equally *in vitro* and *in vivo*. With the help of laser doppler velocimetry and photon correlation principle zeta potential of prepared nanoparticle was determined. The zeta potential of the surface of the nanoparticle can vary by varying the end functional group of PEG molecule *i.e.* by COOH, NH_2, and CH_3. Higher the value of zeta potential towards the positive or negative side more stable is the formulation.

5.2. Drug loading and entrapment/encapsulation efficiency

They were determined by various methods like high-performance liquid chromatography (HPLC), dialysis, and membrane filtration (Chan et al., 2009; Liu et al., 2010b; Li et al., 2008). The encapsulation efficiency (EE) is defined as the total amount of the drug that is encapsulated inside the nanoparticle. To determine it briefly, a known amount of the nanoparticle formulation was placed in Eppendorf tube and centrifuged the formulation. The procedure was repeated multiple times to obtain a clear supernatant. Further, the concentration of unentrapped drug was determined Using Ultra Violet/visible-

Table 1
Examples of conjugated ligands on lipid PEG layer on nanoparticles and their application.

Conjugated ligands	Encapsulated material	Application	Reference
Half-antibody	Paclitaxel	Pancreatic cancer	Hu et al., 2010
A10 RNA aptamer	Docetaxel, indium111 and yttrium 90	Prostate cancer	Wang et al., 2010a
Folate molecule	Docetaxel	Breast cancer	
Peptide	Paclitaxel	Injured vasculature	Chan et al., 2011

spectrophotometry or through HPLC. The %EE is determined by the given formula

$$(\%)EE = \frac{Amount\ of\ encapsulated\ matrial\ in\ nanoparticle\ pellet\ (\mu g)}{Amount\ of\ encapsulated\ matrial\ in\ nanopartical\ dispersion\ (\mu g)} \times 100$$

The LPHNs signify a new drug delivery vehicle for hydrophobic drugs with high encapsulation efficiency and controlled release rates. The lipid or lipid PEG layer retards the diffusion of small drug molecules from the polymeric core and thus improves the encapsulation efficiency. Mandal et al. found that many factors that affects drug loading and EE which depends on hydrophilic nature of the drug, drug miscibility and affinity in the lipid and polymeric phase, concentration of lipid or lipid PEG used, charge interaction between the drug and lipid, pH of the aqueous phase, and preparation method (Mandal et al., 2013)

5.3. Drug release studies

The release of drug in the solvent medium was determined by calculating the remaining drug content in the nanoparticles by various method like dialysis followed by high performance liquid chromatography (HPLC) and UV–Visible spectrophotometry method (Hu et al., 2010; Liu et al., 2010b; Wong et al., 2006; Zheng et al., 2010; Pinto et al., 2006). Most commonly dialysis method was used for the drug release study. In this previously separated nanoparticle resuspended and dispersed in release medium and transferred into the prewashed and preconditioned (by equilibrating with release medium for 30–40 min) cellulose ester dialysis membrane bags and sealed. Sealed bag was placed in release medium at 37 °C with continuous stirring at 120 rpm. At fixed time interval particular amount of release medium is withdrawn and is replaced by the same volume of the fresh medium to maintain the sink condition and the quantification of drug is measured in each release by UV-VIS spectrophotometer or high-performance liquid chromatography (HPLC) (Wang et al., 2010a; Narvekar et al., 2012).

Several factors influence drug release profile of the LPHNs which includes the interaction between the drug and polymer, the solubility of the drug, rate of degradation of polymer and size of particles. The release of the drug from the LPHNs which is physically encapsulated occurs through drug diffusion and erosion of the polymer. For chemically encapsulated drugs, the release depended on the linker hydrolysis (the link between polymer chain and drug). The uniform distribution of the drug molecules in nanoparticles improves the release kinetics of the drug with expressively reduced burst release effects (Gary et al., 2007).

5.4. Stability studies of nanoparticles

Stability study for both nanoparticle suspension and the lyophilized sample was performed to study the loss of drug on storage of nanoparticle at different-different temperature. Accelerated stability study of the optimized formulation was performed as per International Council for Harmonization guidelines (ICH guidelines “Q1A (R2) 2003, Stability Testing of New Drug Substances and Products”). Lyophilized nanoparticle and nanoparticles suspension samples were stored at 20 °C and at 4 °C for 3 months in the glass vials. After storage duration of 7, 15, 30, 60 and 90 days, lyophilized nanoparticles were redispersed in distilled water and nanoparticles suspension was directly sonicated for 10–15 s and then particle size, PDI, zeta potential and % encapsulation efficiency was calculated. (Wu et al., 2006).

5.5. Cellular intake and cytotoxicity

Cellular intake and cytotoxicity are the characteristics *in vitro* assay to measure the efficacy of drug encapsulated nanoparticles against target tissue earlier and correlate to *in vivo* estimations. Cellular intake of hybrid nanoparticles is inspected by tagging the hybrid nanoparticles with suitable fluorescent probes like fluorescence isothiocyanate (FITC) and with the help of confocal laser scanning microscope (CLSM) tissue imaging is done.

The cellular intake of nanoparticles *in vitro* depends on two mechanism endocytosis or nonspecific inundate. To assist cellular intake of the LPHNs, ligands were typically united for targeting LPHNs surface to trigger the receptor facilitated endocytosis. In this, a variety of targeting ligands have been used to help binding specificity and cellular intake of the LPHNs (Sengupta et al., 2005; Wang et al., 2010b).

Cellular cytotoxicity examination was generally performed by the incubation process for a specific time. After the 72 h of supplementary culture, cell ability is evaluated with suitable assays such as ATP assay and MTT assay. When compared to free drugs, the nanoparticles expressively decrease the side effects associated with the drug. Liu et al. worked on paclitaxel hybrid nanoparticles and MCF-7 breast cancer cells were employed to evaluate the cellular intake and cytotoxicity. After incubation with MCF-7 cells at 0.250 mg/ml nanoparticles concentration, the paclitaxel-loaded PLGA nanoparticles of DLPC shell showed more successful cellular intake *versus* those of PVA shell. The analysis of IC50 (the drug concentration at which 50% of the cells are killed) demonstrated that paclitaxel nanoparticles could be 5.88-, 5.72-, 7.27-fold effective than the commercial formulation Taxol® after 24, 48, 72 h treatment, respectively (Liu et al., 2010b).

5.6. In vivo evaluation

Major problems of drug delivery in the blood circulation nanoparticles are their limited circulation lifetime. On reaching the nanoparticle in the systemic circulation plasma proteins get rapidly adsorbs on the particle surface and they get rapidly cleared from the circulation through mononuclear phagocyte system (MPS) in the liver and spleen by opsonization process.

The *in-vivo* behavior of the nanoparticle was depended on the particle size, charge present on the surface of the nanoparticle, modification of lipid PEG and a targeting ligand. It is common that conventional and PEGylated nanoparticles with 10–150 nm size and slightly negatively surface charged are capable to present in the blood circulation for a specific time and specifically penetrate in tumor tissues by the passive diffusion and active targeting effects. In the research area targeted delivery is an important aspect. The study performed by Wong et al. (2007) to evaluate *in vivo* efficacy, unwanted toxicity and site-specific distribution of a doxorubicin loaded LPHNs formulation in a murine solid tumor model after intratumoral injection resulted in 70% and 100% tumor growth delay for doxorubicin doses of 0.1 and 0.2 mg in mice, respectively and showed no signs of toxicity (Garcia-Gonzaleza et al., 2009).

6. Lipid polymer hybrid nanoparticles applications

6.1. Therapeutics drug delivery

LPHNs were formulated to efficiently incorporate and deliver a wide range of therapeutic materials with the loading of different types of drugs alone or in a combination. Water-soluble drugs get encapsulated in the polymeric core through the nanoprecipitation method and water-insoluble drugs were encapsulated into the lipid or lipid PEG shell. For additional controlled release kinetics of the therapeutic substance, it was covalently attached to the polymer chains. Among their adaptable applications, some key areas with important medical implications were discussed in Table 2. The LPHNs application in drug delivery was classified into three subclasses that are single drug deliveries, combinatorial drug deliveries, and actively targeted drug delivery by LPHNs functionalization with targeting ligands (Pinto et al., 2006). The sub-100 nm lipid-polymer hybrid nanoparticles with a single drug

Table 2
Some examples and applications of formulated hybrid nanoparticles for delivery of important therapeutic agent.

S. No.	Type of drug delivery	Encapsulated material	Material used (polymer/lipid)	Method of preparation	Disease targeted	References
1.	Single delivery	Paclitaxel	PLA, TPGS, Lecithin, targeting peptide	Nanoprecipitation	Vasculature	Chan et al. (2011)
2.	Single delivery	Doxorubicin	PLGA, PEG-PE	Emulsification solvent evaporation	Cervical cancer	Shi et al. (2011)
3.	Single delivery	Paclitexal	PLGA, DLPC	Emulsification solvent evaporation	Breast cancer	Liu et al., 2010b
4.	Combinational delivery	Doxorubicin combretastatin	PLGA, PC, Cholesterol, DSPE-PEG	Two-step conventional method	Lewis lung carcinoma	Sengupta et al. (2005)
5.	Combinatorial delivery	Camptothecin, Fe3O4 nanoparticles	PLGA, lecithin, DSPE-PEG	Nanoprecipitation	Breast cancer	Kong et al. (2013)
6.	Combinatorial delivery	Doxorubicin, pEGFP DNA	PLGA, FA-OQLCS, Cholesterol, PEG-OQLCS	Two-step conventional method	Breast cancer	Wong et al. (2006)
7.	Combinatorial delivery	Gemmcitabine HCL, Paclitaxel	PLGA, lecithin, DSPE-PEG	Nano precipitation	Pancreatic cancer	Aryal et al. (2010)
8.	Targeted delivery	Docetaxel	PLGA, DLPC, DSPE-PEG	Emulsification-solvent evaporation	Breast cancer	Liu et al., 2010a
9.	Targeted delivery	Norfloxacin	PIA, Soyalecithin	Emulsification-solvent evaporation	Antimicrobial activity	Dave et al. (2017)
10.	Targeted delivery	Aromatase inhibitor	PLGA, EPC, DOPE-Tf, TPGS	Nanoprecipitation	Breast cancer	Zheng et al. (2010)
11.	Targeted delivery	Paclitaxel	PLA, Lecithin, DSPE-PEG	Nano precipitation	Protein & peptides delivery	Chan et al. (2009)
12.	Gene delivery	DNA (pLuc)	PLGA, DOTAP, DC-Chol.	Emulsification-solvent evaporation	Vaccination	Li et al. (2010)
13.	Gene delivery	SiRNA (anti-GFP, anti-Luc, GAPDH	PLGA, lecithin, PC, DSPE-PEG	Emulsification-solvent evaporation	Cervical & liver cancer	Shi et al. (2011)
14.	Gene delivery	mRNA	PBAE, DOPC, DOTAP, DSPE-PE	Nano precipitation	Dendritic	Su et al. (2011)
15.	Gene delivery	SiRNA (anti-luc, KIF11 *etc.*)	PLA, DC-Chol, DSPE-PE	Emulsification solvent evaporation	Cervical, Prostate, Liver cancer	Al-Dosari and Gao (2009)
16.	Gene delivery	DNA (pEGFP-N2)	PEI, PC triolein, DSPE-PEG	One step emulsification solvent evaporation	Breast cancer	Heyes et al. (2007)

Abbreviations: PLGA, poly lactic co glycolic acid; PLA, poly lactic acid, DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; DOPE, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; DSPE-PEG, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine–polyethylene glycol; PBAE, poly b-amino ester; FA-OQLCS, folic acid conjugated octadecyl-quaternized lysine modified chitosan; GFP, green fluorescent protein; PEG-OQLCS, polyethylene glycol conjugated octadecyl-quarternized lysine modified chitosan; Luc., luciferase gene; PC, phosphatidylcholine; DC-Chol, 3b-[N-(N0,N0-dimethylaminoethane)-carbamyl] cholesterol; siRNA, small interfering RNA; Tf, transferrin; TPGS, D-a-tocopherol PEG 1000 succinate; mRNA, messenger RNA; DLPC, 1,2-dilauroyl-sn-glycero-3-phosphocholine; PEG–PE, polyethylene glycol–phosphoethanolamine; PEI, poly(ethyleneimine); pEGFP-N2, plasmid DNA encoding green fluorescent protein.

specifically deposited in damaged vasculature *in vivo* animal model and release the drug in a continuous manner for 2 weeks. It is useful for delivery of various single chemotherapeutic agent incorporated into the polymeric core or lipid substances or surface of lipid PEG layer, to the specific targeting for the treatment of different type of cancers (Schafer et al., 2009; Valencia et al., 2010; Wang et al., 2010a). The outcomes of a widely held of the single drug delivery studies were restricted to *in vitro* cytotoxicity of LPHNs, biocompatibility of LPHNs with free drug, cellular intake, and release kinetics of drug (Hadinoto et al., 2013; Kularatne and Low, 2010). Additionally, the LPHNs have shown huge perspective for combinatorial drug delivery. Various drug merging strategies for combinatorial drug delivery was used to treat disease in a more effective way resembling for LPHNs prepared by the one-step method using different precursors of the lipid and polymer through covalent conjugation. For LPHNs prepared by the two-step process, one drug substance is encapsulated inside the polymeric while the other is in caged into the lipid vesicles (Kularatne and Low, 2010). The study performed by Wang et al., 2010a a targeted LPHNs system to simultaneously deliver chemotherapy and radiotherapy agents for the treatment of prostate cancer. The resulting double drug-loaded nanoparticles showed separate release profiles for both drugs and improved killing of tumor cells. The synergistic effect was attained by the sequential release of the two anticancer agents (Kong et al., 2013). For the LPHNs delivery, the active targeted drug delivery is attained by functionalizing the LPHNs with various active targeting moieties like small folate molecules, transferrin (Tf), (Cheow and Hadinoto, 2012) half antibody,(Hu et al., 2010) aptamers, (Zhang et al., 2008) single chain variable fragment, (Messerschmidt et al., 2009) and peptides (Valencia et al., 2010; Bivas-Benita et al., 2004). Active targeted delivery reduces the toxic effects on the healthy cells as caused by chemotherapy and increases the exposure of the drug to the tumor cell (El-Aneed, 2004). Folic acid is over expressed in a large number of cancerous cells hence it is considered as a targeted moiety (Glover et al., 2005). Through pre-insertion technique targeting substance is attached to the lipid-PEG material.

6.2. Nucleic acid (gene) delivery

Delivery of gene like DNA, RNA showed a challenge and huge probabilities to relief from various genetic diseases, different chronic diseases, genetic disorders, and many types of cancers (Lee and Kim, 2005). In gene delivery, use of the biodegradable nanoparticles and of cationic liposomes have been extensively investigated (Li and Rana, 2012) between various non-viral based methods, polymer and lipid-based non-viral carriers which shows variety of benefits like less immunogenicity, less harmfulness, absence of viral recombination, less manufacturing cost, and the option of repetitive administration (Oh and Park, 2009; Papanicolaou et al., 2004). But these carriers also suffers from various limitation like they were not stable in serum, show cytotoxicity, particle size is larger. Recently, LPHNs has emerged as a substitute for the delivery of a vector system as they are stable, long-lived and biodegradable (Feng et al., 2007; Heyes et al., 2007).

Due to the high stability and high biocompatibility of this carrier system LPHNs have been used recently for the delivery of genes.

The transport of genes through some non-viral vehicles (like cationic lipid DOTAP (*i.e.*, lipoplex) and cationic polymers like PEI (*i.e.*, polyplex)) is more favorable as compared to the delivery through viral route (Feng et al., 2007). For lipoplexes, one such method is that the lipoplex is covered through the polymer to prevent the binding of any non-specific protein to the lipoplex (Al-Dosari and Gao, 2009). All the above methods have shown efficient *in vivo* effect by decreasing the non-specific binding of the protein to the DNA complexes, hence increasing the movement in systemic administration with decreasing the intake in non-targeted tissues.

siRNA plays an important role in gene delivery by conquering the expression of particular genes with RNA interference process. The

Delivery of siRNA to cancerous cell begins the interference mechanism of RNA to prevent the expression of proteins, involved in the tumor beginning and tumor progression (Ambegia et al., 2005). Various formulations like lipoplexes, polyplexes have been established for the delivery of siRNA same as delivery of DNA (Chin-Hang et al., 2011).

6.3. Diagnostic and imaging agent delivery

In addition delivering therapeutic agents, the LPHNs can also be used in the area of bioimaging agents for medicinal diagnostic as delivery vehicles like Iron oxide, Quantum dots (QDs) Fluorescent dyes and inorganic nanocrystals commonly used in magnetic resonance imaging (MRI) and computed tomography(CT) by encaged them inside the polymer core (Troutier et al., 2005a). Valencia et al. used QDs to substitute the hydrophobic polymer to formulate lipid-QD hybrid nanoparticles by a quick mixing method within a microfluidic system (Salvador-Morales et al., 2009). The encaged QDs remained their fluorescence properties and exhibited high stability in aqueous solutions. Mieszawska et al. studied LPHNs delivery, large amount payloads of gold nanocrystals (Au NC) and QD through nanoprecipitation, where the Au NC and QD were inundated to the polymer (*i.e.*, PLGA) through esterification reactions, before the mixing of lipid and polymer. The *in vitro* outcomes in mouse macrophage cells displayed useful bioimaging applications of the Au NC loaded and QD loaded LPHNs, therefore demonstrating their capable abilities for CT and visual imaging, correspondingly (Troutier et al., 2005a).

7. Conclusion

LPHNs have been considered as a smart and robust drug delivery system as they were easy to prepare and shows good stability as compared to other types of drug delivery systems. LPHNs are the drug nanocarriers which were synthesized from various natural, semi-synthetic and synthetic polymers. LPHNs possess small size due to which they can be used into a variety of applications such as anticancer therapy, lung infection treatment, delivery of vaccines, delivery of genes, gene delivery and bioimaging. Moreover, LPHNs protect the drug molecule such as proteins, DNA or peptides from the degradation by shielding the drug molecule with the help of lipid PEG layer. The upcoming research trend should show the possibility of converting the LPNHs to the dry powder form, deprived of affecting the significant changes on the LPNHs' physical characteristics so as to maintain their stability. A number of LPHNs formulations have been accepted under clinical trials and many of them are beneath the pre-clinical trials.

Conflict of interest

The authors declare no conflict of interest.

References

Albanese, A., Tang, P.S., Chan, W.C.W., 2012. The effect of nanoparticle size, shape, and surface chemistry on biological systems. Annu. Rev. Biomed. Eng. 14, 1–16.

Al-Dosari, M.S., Gao, X., 2009. Nonviral gene delivery: principle, limitations, and recent progress. AAPS J. 11, 671–681.

Ambegia, E., Ansell, S., Cullis, P., Heyes, J., Palmer, L., MacLachlan, I., 2005. Stabilized plasmid–lipid particles containing PEG-diacylglycerols exhibit extended circulation lifetimes and tumor selective gene expression. Biochim. Biophys. Acta 1669, 155–163.

Aryal, S., Hu, C.M.J., Zhang, L., 2010. Combinatorial drug conjugation enables nanoparticle dual-drug delivery. Small 6, 1442–1448.

Bershteyn, A., Chaparro, J., Yau, R., Kim, M., Reinherz, E., Ferreira-Moita, L., 2008. Polymer-supported lipid shells, onions, and flowers. Soft Matter 4, 1787–1791.

Bivas-Benita, M., Romeijn, S., Junginger, H.E., Borchard, G., 2004. PLGA–PEI nanoparticles for gene delivery to pulmonary epithelium. Eur. J. Pharm. Biopharm. 58, 1–6.

Chan, J., Zhang, L., Yuet, K., Liao, G., Rhee, J., Langer, R., 2009. PLGA-lecithin-PEG core-shell nanoparticles for controlled drug delivery. Biomaterials 30, 1627–1634.

Chan, J.M., Rhee, J.W., Drum, C.L., Bronson, R.T., Golomb, G., Langer, R., Farokhzad, O.C., 2011. In vivo prevention of arterial restenosis with paclitaxel encapsulated targeted lipid-polymeric nanoparticles. Proc. Natl. Acad. Sci. 108, 19347–19352.

Chang, W.K., Tai, Y.J., Chiang, C.H., Hu, C.S., Hong, P.D., Yeh, M.K., 2011. The comparison of protein entrapped liposomes and lipoparticles: preparation, characterization, and efficacy of cellular uptake. Int. J. Nanomedicine 6, 2403–2417.

Chen-yua, G., Chun-fena, Y., Qi-luc, L., Qia, T., Yan-weia, X., Wei-nad, L., Guang-xia, Z., 2012. Development of a quercetin-loaded nanostructured lipid carrier formulation for topical delivery. Int. J. Pharm. 430, 292–298.

Cheow, W.S., Hadinoto, K., 2011. Factors affecting drug encapsulation and stability of lipid-polymer hybrid nanoparticles. Colloids Surf. B: Biointerfaces 85, 214–220.

Cheow, W.S., Hadinoto, K., 2012. Lipid-polymer hybrid nanoparticles with rhamnolipid-triggered release capabilities as anti-biofilm drug delivery vehicles. Particuology 10, 327–333.

Chin-Hang, C., Yu-Chao, W., Hsin-Ying, H., Li-Chen, W., Chung-Shi, Y., 2011. Ultrafine PEG-coated poly(lactic-co-glycolic acid) nanoparticles formulated by hydrophobic surfactant-assisted one-pot synthesis for biomedical applications. Nanotechnology 22, 185–601.

Das, S., Suresh, P.K., 2010. Drug delivery to eye: special reference to nanoparticles. Int. J. Drug. Deliv. 2, 12–21.

Dave, V., Yadav, R.B., Kushwaha, K., 2017. Lipid-polymer hybrid nanoparticles: development & statistical optimization of norfloxacin for topical drug delivery system. Bioact. Mater. 2, 269–280.

El-Aneed, A., 2004. An overview of current delivery systems in cancer gene therapy. J. Control. Release 94, 1–14.

Enlow, E.M., Luft, J.C., Napier, M.E., DeSimone, J.M., 2011. Potent engineered PLGA nanoparticles by virtue of exceptionally high chemotherapeutic loadings. Nano Lett. 11, 808–813.

Ensign, L.M., Cone, R., Hanes, J., 2012. Oral drug delivery with polymeric nanoparticles: the gastrointestinal mucus barriers. Adv. Drug Deliv. Rev. 64, 557–570.

Feng, S.S., Zhao, L.Y., Zhang, Z.P., Bhakta, G., Win, K.Y., Dong, Y.C., Chien, S., 2007. Chemotherapeutic engineering: vitamin E TPGS-emulsified nanoparticles of biodegradable polymers realized sustainable paclitaxel chemotherapy for 168h in vivo. Chem. Eng. Sci. 62, 6641–6648.

Gao, H.Y., Schwarz, J., Weisspapir, M., 2007. Hybrid lipid-polymer nanoparticulate delivery composition. US Patent App. 20, 1–7.

Garcia-Gonzaleza, C.A., Sousab, A.R., Argemic, A., Periago, A.L., Saurina, J., Duarte, C.M.M., Domingo, C., 2009. Production of hybrid lipid-based particles loaded with inorganic nanoparticles and active compounds for prolonged topical release. Int. J. Pharm. 382, 296–304.

Gary, D.J., Puri, N., Won, Y.Y., 2007. Polymer-based siRNA delivery: perspectives on the fundamental and phenomenological distinctions from polymer-based DNA delivery. J. Control. Release 121, 64–73.

Glover, D.J., Lipps, H.J., Jans, D.A., 2005. Towards safe, non-viral therapeutic gene expression in humans. Nat. Rev. Genet. 6, 299–310.

Gregoriadis, G., 1995. Engineering liposomes for drug delivery: progress and problems. Trends Biotechnol. 13, 527–537.

Gurny, R., Peppas, N., Harrington, D., Banker, G., 1981. Development of biodegradable and injectable latices for controlled release of potent drugs. Drug Dev. Ind. Pharm. 7, 1–25.

Hadinoto, K., Sundaresan, A., Cheow, W.S., 2013. Lipid-polymer hybrid nanoparticles as a new generation therapeutic delivery platform: a review. Eur. J. Pharm. Biopharm. 85, 427–443.

Hans, M.L., Lowman, A.M., 2002. Biodegradable nanoparticles for drug delivery and targeting. Curr. Opin. Solid State Mater. Sci. 6, 319–327.

Hasan, W., Chu, K., Gullapalli, A., Dunn, S.S., Enlow, E.M., Luft, J.C., Tian, S., Napier, M.E., Pohlhaus, P.D., Rolland, J.P., DeSimone, J.M., 2011. Delivery of multiple siRNAs using lipid-coated PLGA nanoparticles for treatment of prostate cancer. Nano Lett. 12, 287–292.

Heyes, J., Palmer, L., Chan, K., Giesbrecht, C., Jeffs, L., MacLachlan, I., 2007. Lipid encapsulation enables the effective systemic delivery of polyplex plasmid DNA. Mol. Ther. 15, 713–720.

Hitzman, C.J., Elmquist, W.F., Wattenberg, L.W., Wiedmann, T.S., 2006. Development of a respirable, sustained release microcarrier for 5-fluorouracil I: *in vitro* assessment of liposomes, microspheres, and lipid-coated nanoparticles. J. Pharm. Sci. 95, 1114–1126.

Hu, C.M.J., Kaushal, S., Cao, H.S.T., Aryal, S., Sartor, M., Esener, S., Bouvet, M., Zhang, L., 2010. Half-antibody functionalized lipid-polymer hybrid nanoparticles for targeted drug delivery to carcinoembryonic antigen presenting pancreatic cancer cells. Mol. Pharm. 7, 914–920.

Hu, C.M.J., Zhang, L., Aryal, S., Cheung, C., Fang, R.H., 2011. Erythrocyte membrane-camouflaged polymeric nanoparticles as a biomimetic delivery platform. Proc. Natl. Acad. Sci. 108, 10980–10985.

Kim, B.Y.S., Rutka, J.T., Chan, W.C.W., 2010. Current concepts: nanomedicine. N. Engl. J. Med. 363, 2434–2443.

Kong, S.D., Sartor, M., Hu, C.M., Zhang, W., Liang, W., 2013. Magnetic field activated lipid-polymer hybrid nanoparticles for stimuli-responsive drug release. Acta Biomater. 9, 5447–5452.

Kularatne, S., Low, P., 2010. Targeting of nanoparticles: folate receptor. In: Grobmyer, S.R., Moudgil, B.M. (Eds.), Cancer Nanotechnology. 624. pp. 249–265.

Lee, M., Kim, S.W., 2005. Polyethylene glycol-conjugated copolymers for plasmid DNA delivery. Pharm. Res. 22, 1–10.

Lee, S.M., Chen, H., Dettmer, C.M., O'Halloran, T.V., Nguyen, S.B.T., 2007. Polymer-caged liposomes: a pH-responsive delivery system with high stability. J. Am. Chem. Soc. 129, 15096–15097.

Li, Z., Rana, T.M., 2012. Molecular mechanisms of RNA-triggered gene silencing machineries. Acc. Chem. Res. 45, 1122–1131.

Li, Y., Wong, H.L., Shuhendler, A.J., Rauth, A.M., Wu, X.Y., 2008. Molecular interactions,

internal structure and drug release kinetics of rationally developed polymer-lipid hybrid nanoparticles. J. Control. Release 128, 60–70.
Li, X.A., Anton, N., Arpagaus, C., Belleteix, F., Vandanune, T.F., 2010. Nanoparticles by spray drying using innovative new technology: the Buchi Nano spray dryer B-90. J. Control. Release 147, 304–310.
Liang, Z., Ni, R., Zhou, J., Mao, S., 2014. Recent advances in controlled pulmonary drug delivery. Drug Discov. Today 20, 380–389.
Ling, G., Zhang, P., Zhang, W., Sun, J., Meng, X., Qin, Y., Deng, Y., He, Z., 2010. Development of novel self-assembled DS-PLGA hybrid nanoparticles for improving oral bioavailability of vincristine sulfate by P-gp inhibition. J. Control. Release 148, 241–248.
Liu, Y., Li, K., Pan, J., Liu, B., Feng, S.S., 2010a. Folic acid conjugated nanoparticles of mixed lipid monolayer shell and biodegradable polymer core for targeted delivery of docetaxel. Biomaterials 31, 330–338.
Liu, Y., Pan, J., Feng, S.S., 2010b. Nanoparticles of lipid monolayer shell and biodegradable polymer core for controlled release of paclitaxel: effects of surfactants on particles size, characteristics and *in vitro* performance. Int. J. Pharm. 395, 243–250.
Mandal, B., Bhattacharjee, H., Mittal, N., Sah, H., Balabathula, P., Thoma, L.A., Wood, G.C., 2013. Core-shell-type lipid-polymer hybrid nanoparticles as a drug delivery platform. Nanomedicine 9, 474–491.
Messerschmidt, S.K.E., Musyanovych, A., Altvater, M., Scheurich, P., Pfizenmaier, K., Landfester, K., 2009. Targeted lipid-coated nanoparticles: delivery of tumor necrosis factor-functionalized particles to tumor cells. J. Control. Release 137, 69–77.
Mieszawska, A.J., Gianella, A., Cormode, D.P., Zhao, Y., Meijerink, A., Langer, R., Farokhzad, O.C., Fayad, Z.A., Mulder, W.J., 2012. Engineering of lipid-coated PLGA nanoparticles with a tunable payload of diagnostically active nanocrystals for medical imaging. Chem. Commun. 48, 5835–5837.
Moon, J.J., Suh, H., Polhemus, M.E., Ockenhouse, C.F., Yadava, A., Irvine, D.J., 2012. Antigen-displaying lipid-enveloped PLGA nanoparticles as delivery agents for a plasmodium vivax malaria vaccine. PLoS One 7, 1–9.
Müller, R.H., Mader, K., Gohla, S., 2000. Solid lipid nanoparticles (SLN) for controlled drug delivery– a review of the state of the art. Eur. J. Pharm. Biopharm. 50, 161–177.
Mullera, R.H., Radtkeb, M., Wissingb, S.A., 2002. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv. Drug Deliv. Rev. 1, S131–S155.
Narvekar, M., Xue, H.Y., Wong, H.L., 2012. A novel hybrid delivery system: polymer-oil nanostructured carrier for controlled delivery of highly lipophilic drug all-trans-retinoic acid (ATRA). Pharm. Nano. Tech. Int. J. Pharmaceutics. 436, 721–731.
Oh, Y.K., Park, T.G., 2009. siRNA delivery systems for cancer treatment. Adv. Drug Deliv. Rev. 61, 850–862.
Olsson, B., Bondesson, E., Borgstorm, L., Edsbacker, S., Eirefelt, S., Ekelund, K., Gustavsoon, L., Hegelund-Myrback, T., 2011. Pulmonary drug metabolism, clearance, and absorption. In: Smith, H., Hickey, A. (Eds.), Controlled Pulmonary Drug Delivery, pp. 21–50.
Papanicolaou, I., Briggs, S., Alpar, H.O., 2004. Increased resistance of DNA lipoplexes to protein binding in vitro by surface-modification with a multivalent hydrophilic polymer. J. Drug Target. 12, 541–547.
Peyrot, M., Sautereau, A., Rabanel, J., Nguyen, F., Tocanne, J., Samain, D., 1994. Supramolecular biovectors (SMBV): a new family of nanoparticulate drug delivery systems. Synthesis and structural characterization. Int. J. Pharm. 102, 25–33.
Pinto, R.C., Neufeld, R.J., Ribeiro, A.J., Veiga, F., 2006. Nanoencapsulation: methods for preparation of drug-loaded polymeric nanoparticles. Nanomedicine 2, 8–21.
Salvador-Morales, C., Zhang, L., Langer, R., Farokhzad, O.C., 2009. Immunocompatibility properties of lipid-polymer hybrid nanoparticles with heterogeneous surface functional groups. Biomaterials 30, 2231–2240.
Schafer, J., Sitterberg, J., Ehrhardt, C., Kumar, M., Bakowsky, U., 2009. A new drug vehicle-lipid coated biodegradable nanoparticles. Adv. Sci. Technol. 57, 148–153.
Sengupta, S., Eavarone, D., Capila, I., Zhao, G., Watson, N., Kiziltepe, T., Sasisekharan, R., 2005. Temporal targeting of tumour cells and neovasculature with a nanoscale delivery system. Nature 436, 568–572.
Sharma, A., Sharma, U.S., 1997. Liposomes in drug delivery: progress and limitations. Int. J. Pharm. 154, 123–140.
Shi, J., Xiao, Z., Votruba, A.R., Vilos, C., Farokhzad, O.C., 2011. Differentially charged hollow core/shell lipid-polymer-lipid hybrid nanoparticles for small interfering RNA delivery. Angew. Chem. Int. Ed. Eng. 123, 7165–7169.
Soppimath, K.S., Tan, D.C.W., Yang, Y.Y., 2005. pH-triggered thermally responsive polymer core–shell nanoparticles for drug delivery. Adv. Mater. 17, 318–323.
Su, X., Fricke, J., Kavanagh, D.G., Irvine, D.J., 2011. In vitro and in vivo mRNA delivery using lipid-enveloped pH-responsive polymer nanoparticles. Mol. Pharm. 8, 774–787.
Thevenot, J., Troutier, A.L., David, L., Delair, T., Ladavière, C., 2007. Steric stabilization of lipid/polymer particle assemblies by poly (ethylene glycol)-lipids. Biomacromolecules 8, 3651–3660.
Thevenot, J., Troutier, A.L., Putaux, J.L., Delair, T., Ladaviere, C., 2008. Effect of the polymer nature on the structural organization of lipid/polymer particle assemblies. J. Phys. Chem. B 112, 13812–13822.
Troutier, A., Delair, T., Pichot, C., Ladaviere, C., 2005a. Physicochemical and interfacial investigation of lipid/polymer particle assemblies. Langmuir 21, 1305–1313.
Troutier, A.L., Veron, L., Delair, T., Pichot, C., Ladaviere, C., 2005b. New insights into self organization of a model lipid mixture and quantification of its adsorption on spherical polymer particles. Langmuir 21, 9901–9910.
Valencia, P.M., Basto, P.A., Zhang, L., Rhee, M., Langer, R., Farokhzad, O.C., Karnik, R., 2010. Single-step assembly of homogenous lipid–polymeric and lipid- quantum dot nanoparticles enabled by microfluidic rapid mixing. ACS Nano 4, 1671–1679.
Valenzuelaa, P., James, A., 2012. Nanoparticle delivery for transdermal HRT. Nanomedicine 8, S83–S89.
Wang, A., Yuet, K., Zhang, L., Gu, F., Huynh-Le, M., Radovic-Moreno, A., Kantoff, P.W., Bander, N.H., Langer, R., Farokhzad, O.C., 2010a. ChemoRad nanoparticles: a novel multifunctional nanoparticle platform for targeted delivery of concurrent chemoradiation. Nanomedicine 5, 361–368.
Wang, H., Zhao, P., Su, W., Wang, S., Liao, Z., Niu, R., Chang, J., 2010b. PLGA/polymeric liposome for targeted drug and gene co-delivery. Biomaterials 31, 8741–8748.
Wang, Y., Kho, K., Cheow, W.S., Hadinoto, K., 2012. A comparison between spray drying and spray freeze drying for dry powder inhaler formulation of drug-loaded lipid-polymer hybrid nanoparticles. Int. J. Pharm. 424, 98–106.
Wong, H., Rauth, A., Bendayan, R., Manias, J., Ramaswamy, M., Liu, Z., 2006. A new polymer-lipid hybrid nanoparticle system increases cytotoxicity of doxorubicin against multidrug-resistant human breast cancer cells. Pharm. Res. 23, 1574–1585.
Wong, H., Rauth, A., Bendayan, R., Wu, X., 2007. In vivo evaluation of a new polymer–lipid hybrid nanoparticle (PLN) formulation of doxorubicin in a murine solid tumor model. Eur. J. Pharm. Biopharm. 65, 300–308.
Wu, C.F., Szymanski, C., McNeill, J., 2006. Preparation and encapsulation of highly fluorescent conjugated polymer nanoparticles. Langmuir 22, 2956–2960.
Yun, Y., Cho, Y.W., Park, K., 2012. Nanoparticles for oral delivery: targeted nanoparticles with peptidic ligands for oral protein delivery. Adv. Drug Deliv. Rev. 65, 822–832.
Zhang, L., Zhang, L., 2010. Lipid polymer hybrid nanoparticles: synthesis, characterization, and applications. Nano Life 1, 163–173.
Zhang, L., Chan, J.M., Gu, F.X., 2008. Self-assembled lipid-polymer hybrid nanoparticles: a robust drug delivery platform. ACS Nano 2, 1696–1702.
Zhao, P., Wang, H., Yu, M., Liao, Z., Wang, X., Zhang, F., Ji, W., Wu, B., Han, J., Zhang, H., Wang, H., Chang, J., Niu, R., 2012. Paclitaxel-loaded folic acid targeted nanoparticles of mixed lipid-shell and polymer-core: in vitro and in vivo evaluation. Eur. J. Pharm. Biopharm. 81, 248–256.
Zheng, Y., Yu, B., Weecharangsan, W., Piao, L., Darby, M., Mao, Y., Koynova, R., Yang, X., Li, H., Xu, S., Lee, L.J., Sugimoto, Y., Brueggemeier, R.W., Lee, R.J., 2010. Transferrin-conjugated lipid-coated PLGA nanoparticles for targeted delivery of aromatase inhibitor 7 [alpha]-APTADD to breast cancer cells. Int. J. Pharm. 390, 234–241.
Zimmer, A., Kreuter, J., 1995. Microspheres and nanoparticles used in ocular delivery systems. Adv. Drug Deliv. Rev. 16, 61–73.