Insights into the Self assembled Lipid-Polymer hybrid Nanoparticles as Drug Delivery system

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Abstract
The Use of polymeric nanoparticles and lipid carrier systems, including liposomes, solid lipid nanoparticles and nanostructured lipid carriers has limitations such as drug leakage and high water content of dispersions. Thus, lipid polymer hybrid nanoparticles have been explored by the researchers to provide a better effect using biomimetic characteristics of lipids and architectural advantage of polymeric core and finally producing a system which overcomes the limitations of both polymeric nanoparticles and lipid carrier systems. The system composed of biodegradable polymeric core surrounded by layers of phospholipids, additional compounds and mixtures may also be added to the phospholipids in the amphiphilic coating as for example fatty acids, steroids (such as cholesterol), triglycerides, lipoproteins, glycolipids, vitamins, detergents, and surface active agents. They are generally prepared by mixing liposomes and Polymeric nanoparticles to form lipid–polymer complexes in which a lipid bilayer or lipid multilayers cover the surface of the polymeric core. The space between polymeric core and lipid layer is usually occupied by water or aqueous buffer. The obtained particle size of the final particles remained in the desirable range with narrow distribution. The lipid-polymer hybrid nanoparticle by design has the capacity to co-encapsulate both hydrophobic and lipophilic drugs. The metabolic pathway of lipids in the body has led to the site specific delivery of such system with the modification of the pharmacokinetics and biodistribution of active ingredients for increased efficacy. This hybrid structure provides an advantages of controllable particle size, surface functionality, high drug loading, entrapment of multiple therapeutic agents, tunable drug release profile, and good serum stability. This review focuses on current research trends on Lipid Polymer hybrid nanoparticles including methods of preparation and physicochemical characteristics.

Keywords: Lipid hybrid nanoparticles, Hybrid nanoparticle, liposome, polymeric nanoparticles.

Introduction

In recent years, the application of nanotechnology has been translated to medicine. Nanotechnology encompasses the design, synthesis, and characterization of materials and or devices, which are functionally organized in at least one dimension on the nanoscale [1]. The use of these nanoscale or nanostructured materials in medicine, termed as nanomedicine, has unique medical properties and effects owing to the small size (1 –1000 nm) and structure [2]. The ability to engineer and control materials in this size rage results in new medical efforts, innovative chemistry techniques, and novel manufacturing approaches [2]. Nanomedicine has the capacity to change the landscape of healthcare and drug delivery by enhancing the developability of biologically active drug candidates with poor pharmaceutical properties such as solubility and circulation half-life [3]. Nanomedinces and nanomaterials are engineered to have specific functions, which utilize the physical properties

and characteristics for diagnosis and treatment of disease [4]. These materials are able to be used as carriers to cross membranes, mediate molecular interactions, and detect molecular changes [4]. Nanomaterials have a high surface to volume ratio. This increased surface area can be coated or tagged with other molecules, which results in the formation of multifunctional nanomaterials [4]. Nanomaterials can be engineered to have different shapes, sizes, surface chemistry, particle density, and chemical compositions [4]. Because of their design, nanomedicines have applications in drug delivery, in vivo and in vitro diagnostics, biomaterials, active implants, in vivo imaging, biosensing, cell labeling, and tissue engineering [1-3, 5,6]. In vivo imaging employs the use of magnetic nanoparticles, quantum dots, fluorophores, and carbon nanotubes [2,3,5,6]. An example is Gastromark (ferumoxsil®), 3 which is a marketed product composed of superparamagnetic iron oxide nanoparticles used as a contrast agent for magnetic resonance imaging [2]. Fluorescent quantum dots are nanocrystals that have higher extinction coefficients than traditional fluorophores, which makes this technology useful for imaging [6]. Carbon nanotubes can act as biocompatible supportive substrates that can incorporate fluorophores and other molecules [7]. Using nanomaterials for in vivo imaging is a faster, less invasive, and a more accurate way to diagnose diseases and to monitor disease states and progression [3]. In the future, these types of imaging probes may be able to assist surgeons in locating tumors within the body and to identify adjacent structures [3].

In vitro diagnostics is another application for nanomedicine, which uses nanoparticles, nanowires, nanotubes, nanoarrays, and cantilevers [2,3]. Lateral flow assays are marketed products that utilize colloidal gold to test ovulation, HIV infection, and pregnancy [2]. In this case, an antibody for a specific analyte is conjugated to the nanoparticle surface. Gold nanoparticles are widely used because they have good stability, which avoids the chance of false positive readings [2]. With the use of these materials, disease detection can be quick, high throughput, and more accurate by using biomarkers with higher sensitivity [3]. In the future, novel analytes could be measured such as Alzheimer's plaques [2]. Using nanomaterials for in vitro diagnostics is advantageous because they can improve sensitivity, reduce cost, and consume less of the sample [2,3]. Biomaterials have mechanical properties than can be used as medical implants, dental restoratives, and bone substitutes [3]. One example of a biomaterial is the nanoparticle composite found in the dental restorative Filtek Supreme®, which is a marketed product produced by 3M [2]. Vitoss® is a marketed nano-hydroxyapatite based product that is used in the repair of bone defects [2]. Another example of biomaterials in the market is Anticoat®, which is a silver nanoparticle based wound dressing [2]. Nanomedicines have been especially successful as drug delivery vehicles. This may be due to the fact that diseases originate at the molecular level, which is on the nanoscale and can be caused by

gene mutations, misfolded proteins, viral and bacterial infections, cell misfunction, and cell miscommunications [4]. In order to address these modes of disease, nanocarrier delivery systems were developed. Nanotechnology formulation platforms include liposomes, nanoparticles, polymeric micelles, dendrimers, nanocantilevers, carbon nanotubes, aptamers, quantum dots, and polymer conjugates [8]. Liposomes consist of a phospholipid bilayer and an aqueous core for drug encapsulation of water-soluble molecules. Marketed liposomal products include Doxil and Myocet (liposomal doxorubicin), Ambisome (liposomal Amphotericin B), DaunoXome (liposomal daunorubicin) and Depocyt (liposomal cytarabine) [2]. There are also several examples of marketed products that are polymer conjugates. Polyethylene glycol (PEG) is conjugated to a molecule in order to increase circulation time [9]. Pegasys (PEG-α-interferon-2a) and PEG-Intron (PEG-α-interferon-2b) are both therapies for hepatitis C in which PEG is conjugated to a protein [2]. These marketed products are considered first generation nanosystems because the drug is contained within a system used for passive targeting [3]. Nanomedicine can be a solution for cancer therapy where the current treatments have some problems that include non-specific systemic distribution of the drug, inadequate drug concentration reaching the target site, normal tissue toxicity, and drug resistance [8,10]. Nanomedicines can be used to overcome these obstacles that conventional medicines cannot address. Because of their size and surface properties, nanomedicines can accumulate in tumor sites due to the enhanced permeability and retention (EPR) effect [4]. Nanomedicines have the capacity to encapsulate multipledrugs in order to yield combinatorial delivery, increase circulation time, and exhibit controlled drug release kinetics [11]. This allows for improved dose scheduling, which leads to patient compliance. The impact of nanotechnology for drug delivery is that the characteristics of the vehicles such as size, charge, surface hydrophobicity, ligand type, and density of ligands on the surface can enhance pharmacokinetic properties such as circulation half-life and biodistribution while also improving pharmaceutical properties such as drug solubility [12]. Because of this, nanotechnology is beneficial for the pharmaceutical industry since it can provide life-style extensions for drugs after patents have expired, new classes of drug therapeutics can be developed, and the biologically active molecules that have poor pharmaceutical properties can be re-investigated [3,12] Pharmaceutical substances may be administered either in the absence or in the presence of a carrier, such carriers based delivery is used for the controlled release of biologically active molecules, and the targeting of biologically active molecules to specific tissues. The nanostructure carrier has been extensively explored in the past decade to develop a moiety to facilitate the delivery of therapeutic and imaging agents at or near one or more target regions in an organism so that to expose the target tissue or cells to the desired active ingredients for a predetermined time and concentration. This site

specific delivery led to the modification of the pharmacokinetics and biodistribution of active ingredients for increased efficacy. To date, the most common cancer treatments are chemotherapy, radiation, and surgery. The challenging aspects involved with cancer therapy include nonspecific systemic distribution, low drug levels reaching the tumor site, cytotoxicity, poor stability, and multidrug resistant tumor cells [8,10,13]. Multidrug resistance (MDR) is a major hurdle in cancer therapy because it decreases the efficacy of drugs through multiple mechanisms [14]. This phenomenon involves an active efflux of a large range of cytotoxic drugs out of the cytoplasm by membrane-bound transporters [15]. One example is the P-glycoprotein (P-gp), which is an active membrane-bound efflux pump [16]. Over-expression of P-gp and other membrane transporters can lead to MDR [17]. Other cellular mechanisms that contribute to MDR are drug molecule reactions with intracellular nucleophiles like glutathione, repair of drug-induced damage to the DNA, altered proteins that affect apoptotic pathways, and an altered drug target [16,17]. Noncellular events that can lead to MDR include high interstitial pressures at the tumor site, which decreases drug permeability, lower pH, hypoxia (drugs generating free radicals), and the extracellular matrix effect [18]. Therapeutic materials can also be removed from the systemic circulation by the mononuclear phagocyte system that comprises of kupffer cells in the liver and macrophages in the spleen and bone marrow [19, 20]. Nanoparticle drug delivery vehicles can be designed to address these challenges associated with current cancer treatment. Nanosystems are distinct from other cancer therapeutics because the nanocarrier itself can also have therapeutic effects along with the actual drug [21,22]. Nanoparticles can be designed to carry large payloads, have attached targeting ligands, encapsulate multiple drugs for combinatorial therapy, and have the ability to bypass drug resistance mechanisms [23,24]. Material selection is an important consideration for nanomedicines. Biodegradable, biocompatible, and physiological lipids are chosen for formulation development in an attempt to reduce immunogenicity and minimize toxicity [25]. Colloidal drug carriers such as micelles, nanoemulsions, nanosuspensions, polymeric nanoparticles, and liposomes are formulation platforms that are used to address drug solubility and stability issues [26]. One example of a nanoparticle delivery vehicle is a dendrimer, which is a biodegradable branch-like structure that consists of a core (two or more reactive groups) with repeated units covalently bound to the core and peripheralfunctional groups [27]. Drugs can be encapsulated or conjugated to the dendrimer and delivered to tumor sites through the EPR effect or by using targeting ligands like peptides and antibodies [20]. Another example is quantum dots, which are nanocrystals that have improved fluorescent properties over traditional fluorophores and can be used as drug carriers or as tags for other drug carriers [21]. Liposomes have a lipid bilayer in which the surface characteristics can be modified by lipid type and lipid charge. Cationic liposomes are established in the literature to have antimicrobial action due to the adsorption

of the positively charged lipid bilayer onto the bacterial cell membrane, which changes the membrane surface charge from negative to positive and induces apoptotic cell death [19]. With liposomes and polymeric nanoparticles, multiple drugs can be coencapsulated in a single system for combination delivery. Nanoparticles can be considered as an intermediates between bulk materials and molecules, these are generally synthesized using two approaches, the "top-down" approach and the "bottom-up" approach. The "top-down" approach is a method of breaking bulk material into small pieces, but it is difficult to make less than 50 nm of nanoparticles. For this reason, the "bottom-up" approach, a method of assembling atoms or molecules into nanoparticles, has recently received attention, such nanoparticles are unstable as they aggregate with the passage of time and thus lose their nanoparticle properties. Thus, for the synthesis of nanoparticles, to be stable in solution and even after drying, requires a method capable of preventing these nanoparticles from aggregating with each other. The lipid-polymer hybrid nanoparticles with characteristics of forming multilayer structure through self assemblage technique have came forward to ovecome the limitations associated with the nanoparticles formation [28].

Lipid-polymer hybrid Nanocarrriers

The system comprises of a core, comprises of a natural hydrophilic polymer or a hydrolysate of a natural biodegradable polymer made up of cross-linked polysaccharide or a cross-linked oligosaccharide, the core is partially coated or completely coated with a layer which is either a lipid layer or an amphiphillic compound such as phosphplipid covalently bound to the inner core. Therapeutic agents, can be entrapped, adsorbed, or covalently attached to such hybrid system. The amphiphilic coating may also comprise a derivative of a phospholipid as PEG-phospholipids, and phospholipids grafted to other molecules or polymers. Additional compounds and mixtures may be added to the phospholipids in the amphiphilic coating as for example fatty acids, steroids (such as cholesterol), triglycerides, lipoproteins, glycolipids, vitamins, detergents, and surface active agents. PEG-phospholipids or grafted phospholipid has an additional property of stealth action which is basically responsible for the inhibition of enzymatic action over the lipid molecules. The metabolic pathway of lipids depicts that earlier breakdown of lipids into its metabolites takes place when enzymes acts on C_{10} position of the hydrocarbon chain of the lipids. The grafted phospholipids are mainly designed to aim the binding of C_{10} position so that enzymes would not act upon it. The PEG coating of lipid is also responsible for EPR effect [28].

Advantages of Lipid-polymer hybrid nanoparticles [29]

• The solid polymeric core acts as a cytoskeleton that provides mechanical stability, controlled morphology,biodegradability, narrow size distribution, and high available specific surface area.

• The lipid shell enveloping the core is biocompatible and exhibits behavior similar to that of cell membranes.

• Improved encapsulation of hydrophobic drugs with therapeutically effective drug entrapment efficiency and drug loading has been reported for a number of drugs compared to liposomes or Polymeric nanoparticles.

Methods of preparation [24]

Methods used to prepare Lipid-polymer hybrid nanoparticles broadly fall into two categories: the two-step method and the single-step method.

Two-step method

The polymeric core and lipid shell are prepared separately using two independent processes after that these two components are combined by direct hydration, sonication, or extrusion to obtain the desired lipid shell–polymer core structure . The dry lipid film can be hydrated with the polymeric nanoparticles dispersion ultimately allows the introduction of these into polymeric nanoparticles into preformed lipid vesicles. This mixture is heated at a temperature above phase transition temperature (Tc) of the lipid to facilitate the reorganization of the lipid onto the particle surface. The nonadsorbed lipids, micelles, and free polymeric nanoparticles are separated by centrifugation to obtain a final lipid-polymer hybrid dispersion.
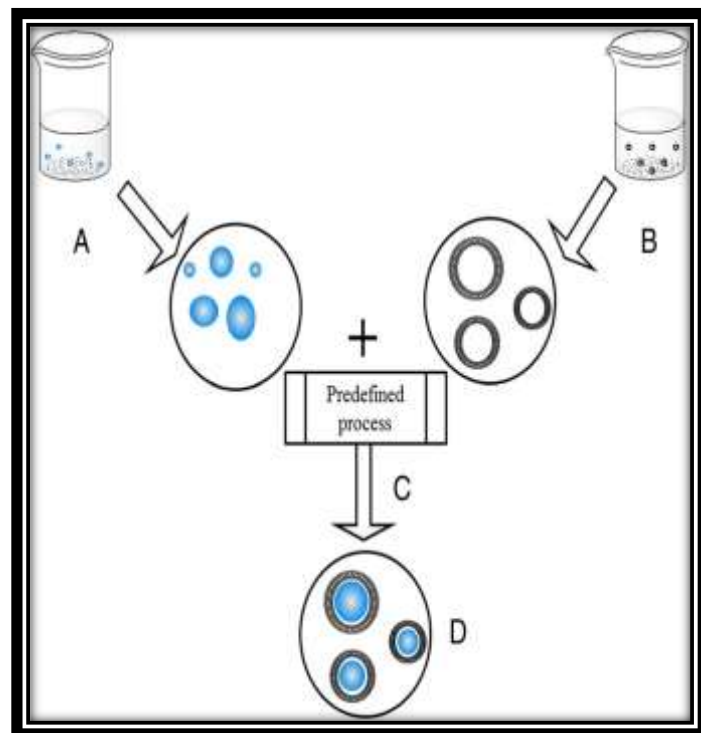
Single step method

The relatively simple approach that combines the dual steps of the two-step method into a single step has been evaluated.
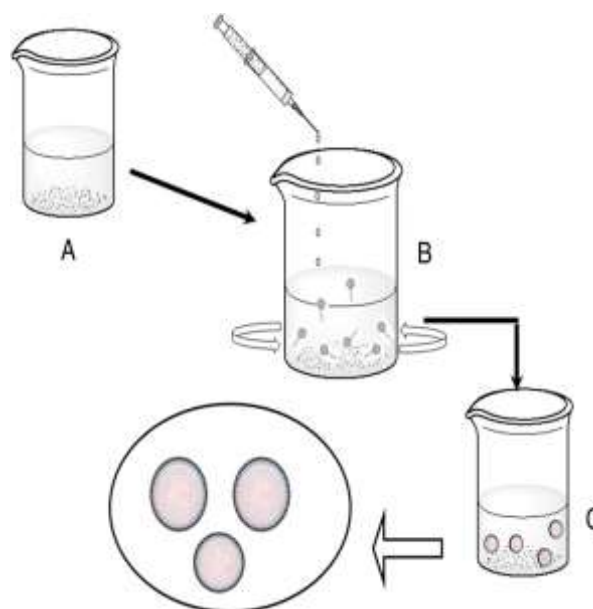
Modified solvent extraction/evaporation method

The polymer and drug are dissolved in a water-immiscible organic solvent. A predetermined amount of lipid is then dispersed in water by bath

sonication, mechanical stirring, or sometimes heat. The organic solution is mixed into the aqueous phase, and the resulting dispersion is sonicated using a probe sonicator and ice bath. The organic dispersed phase is broken into tiny nanodroplets, which are solidified into nanospheres coated with a lipid layer. The organic solvent is usually removed by evaporation in a rotary evaporator under reduced pressure or stirred overnight. The particle suspension is purified by centrifugation followed by controlled washing. The washed particles are freeze-dried to obtain a dry powder.

Modified nanoprecipitation method

In this method, polymer(s) and hydrophobic drug(s) are dissolved in a water-miscible organic solvent. The organic solution is then added, drop by drop, to the aqueous dispersion containing lipid and/or lipid–PEG conjugate. The mixture is vortexed and subsequently homogenized or ultrasonicated to reduce the particle size to nanometer range. To ensure proper dispersion of lipid and lipid–PEG conjugate, it is necessary to heat the aqueous dispersion (generally~65 °C) before adding the organic solution. Particles formed were purified by ultracentrifugation, centrifugal ultrafiltration, or dialysis.

Characterization [25]

After successful synthesis of various lipid coated hybrid nanoparticles, the particles were characterized using different state-of-art analytical technique including dynamic light scattering (DLS), scanning electron microscope (SEM), and transmission electron microscopy (TEM).

- **Particle Size and Polydispersity Index (PDI)** Particle size measurements were performed by using dynamic light scattering (DLS) technique (Malvern Zetasizer, ZEN 3600). Three subruns were carried out per measurement, and the average values were taken.

- **Zeta Potential** Zeta potential measurements were taken using the Malvern Zetasizer (ZEN 3600) in which the electrophoretic mobility on the surface of the nanostructures was measured. The measurements were carried out at room temperature with the backscatter angle of 173°. Three subruns were carried out per measurement and the average values were taken.

- **Scanning Electr on Micr oscopic (SEM) Analysis** Scanning electron microscopy is the technique used to look at morphology and surface structure of the materials. Samples for SEM were prepared by dropping 5 µL of a nanoparticle solution onto a polished silicon wafer. After drying the droplet at room temperature overnight, the sample was coated with chromium and then imaged under Phillips XL 30 ESEM.

- **Transmission Electron Microscopic (TEM) Analysis** Transmission electron microscopy is the technique used to look at the internal structure of the materials. In order to understand the internal structure of hybrid nanoparticles, a drop of the nanoparticle solution at a concentration of 4 µg/mL was deposited onto a glow-

discharged carbon-coated grid. Five minutes after the sample was deposited the grid was rinsed with ten drops of distilled water. A drop of 1% uranyl acetate stain was added to the grid. The grid was subsequently dried and visualized using a FEI 200KV Sphera microscope.

Conclusion

This particle design uses an integrative approach by combining two classes of nanocarriers, namely polymeric nanoparticles and liposomes. These particles have several beneficial features for treating various diseases, particularly cancers. Often treatment of a single type of cancer requires administering multiple drugs, and, in this aspect, such system is promising because they have the potential to deliver multiple drugs simultaneously from a single platform. Specifically, incorporating two drugs into the core and lipid layer can offer a viable approach to treating life-threatening diseases.

The design and development of lipid-polymer hybrid particles as drug delivery platforms have been concentrated in the architecture and in vitro efficacy. More focused research is warranted, especially in key areas of development including stability, scale-up, optimization of targeting ligand density, in vivo fate, toxicity, and pharmacokinetic profiles.

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