Wiley
Advances in Polymer Technology
Volume 2025, Article ID 4707146, 18 pages
https://doi.org/10.1155/adv/4707146

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Review Article

Polymer–Lipid Hybrid Nanosystems: An Emerging Advanced Therapeutic Tool

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Received 6 March 2025; Revised 16 October 2025; Accepted 18 October 2025

Academic Editor: Srijoni Sengupta

Polymer–lipid hybrid (PLH) nanoparticles have become an appealing therapeutic delivery system owing to their special properties. These nanoparticles are made with polymer and lipid components and have garnered significant interest across therapeutic applications. The combined properties of the polymers and lipids enable improved drug delivery by enhancing the stability, biocompatibility, and controlled drug release of the nanoparticle. The versatility of this form of drug carrier, including biomimetic and biocompatible features, allows the encapsulation of a wide range of therapeutic agents, including hydrophilic and hydrophobic compounds, proteins, and nucleic acids. These drug carriers can be modified and adapted to target the desired site of action, specific cells, and tissues, while minimizing the possibility of off-target and adverse effects. Thus, this review provides an in-depth analysis into PLH nanoparticles as a novel delivery system, their inherent characteristics, the functionalization strategies, and their wide applications, while providing their potential for future possibilities.

Keywords: application; drug delivery; functionalization approaches; nanoparticles; polymer–lipid

1. Introduction

Polymer–lipid hybrid (PLH) nanoparticles (Figure 1) are nanoscale drug delivery systems with both polymer and lipid components. These are core–shell nanoparticles with the physical stability and biocompatibility of polymeric and liposomal nanoparticles, composed of a polymeric core and lipid/lipid–polyethylene glycol (PEG) shells [1] (Figure 1). While polymeric nanoparticles have good structural integrity, reduced polydispersity, and prolonged duration of plasma circulation, their drawbacks include biocompatibility issues due to polymer degradation and reduced drug encapsulation efficiency (EE) for hydrophilic drugs [2]. On the other hand, lipid nanoparticles can encapsulate both hydrophilic and hydrophobic drugs; however, their reduced biological stability, high polydispersity, and limited surface functionalization scope impact their utilization in drug delivery [2, 3]. PLH nanoparticles are, therefore, amongst the most studied and alternative delivery strategies aimed towards transforming treatments and overcoming the limitations associated with polymeric and lipid nanoparticles [4].

The distinctive characteristics of PLH nanoparticles such as, enhanced stability and adaptability in protecting the therapeutic agents, have enabled targeted delivery and increased bioavailability; hence, PLH nanoparticles have been investigated for a wide range of applications, including bioimaging, gene delivery, and vaccine delivery [5–7]. They are regarded as a promising drug delivery tool for several drugs, including

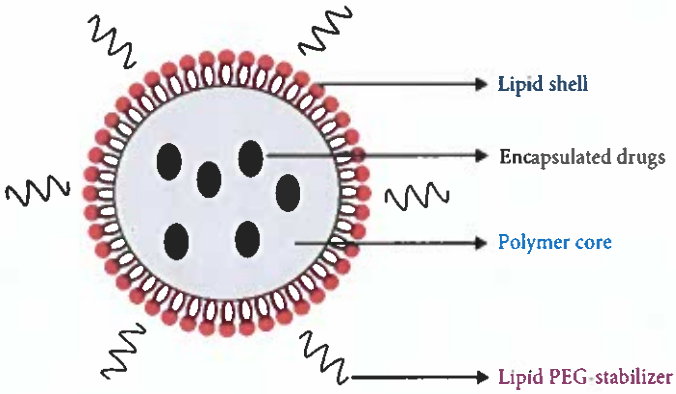

FIGURE 1: Diagrammatic presentation of drug-loaded PLH nanoparticles adapted from Sivadasan et al. [1].

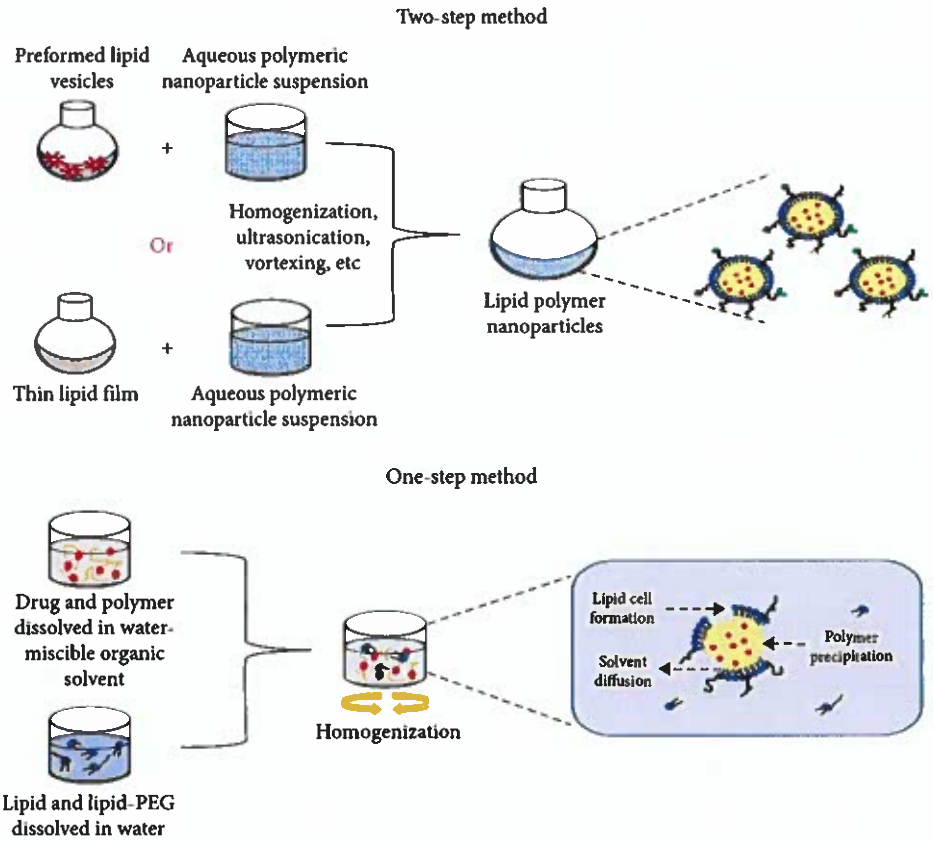

FIGURE 2: Schematic demonstration of formation of PLH nanoparticles using one-step and two-step nanoprecipitation methods [12].

anticancer drugs, due to their stable lipid and polymer composition, which also promotes enhanced biocompatibility and controlled drug release [1, 8]. The surface functionalization properties of these nanoparticles make them the perfect drug delivery device for targeted delivery, which is very appealing in the clinical field [9, 10]. This has enabled ease of drug release on demand, in response to environmental or physiological factors [10, 11]. PLH nanoparticles are very scalable and known to self-assemble via a one- or two-step nanoprecipitation process (Figure 2) in a reproducible and predictable manner and have been prepared using natural, synthetic, and semisynthetic polymers [1, 7]. The presented review has therefore paid keen attention to highlighting the properties, functionalization strategies, characterization approaches, and the different applications of PLH nanoparticles as emerging therapeutic delivery platforms.

2. Properties of PLH Nanoparticles and Their Role in Drug Delivery

PLH nanoparticles generally contain lipids and polymer components. They comprehensively combine the biological

Table 1: Summary of properties of PLH nanoparticles and their role in drug delivery.

S/N	Properties	Description	Reference
1	Biodegradability and biocompatibility	Highly biodegradable due to the polymer core in their structure acting as a cytoskeleton, which provides mechanical stability, regulates morphology, and offers a larger specific surface area with a small particle size. Their biocompatibility is due to the lipid shell surrounding the core, which behaves similarly to cell membranes. This lipid shell is versatile in interacting with other membranes or surfaces of various molecules.	[21–24]
2	Encapsulation of hydrophilic and hydrophobic drugs	Compared to liposomes and polymeric nanoparticles, they can encapsulate more hydrophobic drugs with a therapeutically high drug loading. At the same time, the amphiphilic nature of incorporated lipids can facilitate the adsorption of hydrophilic compounds on a bilayer surface, allowing multiple hydrophilic and hydrophobic therapeutic agents to be coloaded when needed.	[22–24]
3	Modifiable drug release profile	The drug release profile can be modified by optimizing the core, shell material, or both, to achieve a controlled, sustained, or prolonged release pattern.	[7, 25]
4	Better stability	Compared to liposomes, other lipid-based nanoparticles, and polymeric nanoparticles, PLH nanoparticles can be stored longer and maintain their serum stability.	[26]
5	Capability for passive and active targeting	In addition to their passive targeting ability, which depends on particle size, they can be functionalized for smart-active targeting, especially with targeting ligands such as aptamers, peptides, folic acid, transferrin, and antibody.	[27]

compatibility of lipid-based systems with the mechanical robustness and controlled-release features of polymeric systems [13]. The biodegradability, biocompatibility, modified release ability, better stability, capability for encapsulation of hydrophilic and hydrophobic compounds, and the ability for passive and active targeting make PLH nanoparticles optimal platforms for advanced drug delivery. Each of their cardinal properties significantly contributes to a functional advantage, including prolonging the circulation time, reducing toxicity, improving drug loading, enhancing tissue penetration, and ensuring precise control over drug release [14–16]. These benefits have made PLH nanoparticles very desirable in areas of cancer therapy, mRNA, and gene delivery as well as in poorly soluble drug delivery [17–20]. Table 1 summarizes the cardinal properties of PL nanoparticles and their specific role in drug delivery.

3. Functionalization Approaches for PLH Nanoparticles Modification

The process of functionalizing PLH nanoparticles is essential for modifying their characteristics and actions for particular drug delivery and targeting uses [28, 29]. Some well-known functionalization approaches that have been explored for these nanoparticles include the conjugation of different targeting ligands, PEGylation of the nanoparticle, attachment of stimuli elements, or even imaging agents to the nanoparticle surface (Figure 3) [30]. These strategies can enhance the uptake of the nanoparticles by the targeted cells while further maximizing the therapeutic efficacy and minimizing possible adverse and off-target effects [31]. The main functionalization approaches for the modification of PLH nanoparticles are summarized below.

3.1. Conjugation of Targeting Ligands. This is one of the functionalization techniques that has proven to enhance the properties of the conventional nanoparticles to specifically target and bind to cells through the conjugation of the targeting ligands to the surface of the nanoparticles [6, 31]. This involves attaching specific molecules like peptides, small molecules (e.g., folate), aptamers, or antibodies to the surface of the nanoparticles (Figure 4). These ligands are selected based on their binding affinity to specific receptors or biomarkers expressed on the target cells or tissues [33, 34]. Therefore, this method enables PLH nanoparticles to be specifically designed to target the desired biological sites, optimizing the delivery of therapeutic medicines to the targeted site [6, 35]. This strategy achieves a targeted delivery by improving the absorption or uptake of the nanoparticles by the intended cells, thus, improving the efficacy of the treatment and lowering possible off-target consequences [3, 7]. Moreover, the conjugation of targeted ligands approach tends to avoid the rapid clearance mechanism of the nanoparticles from the body, thus, bypassing the biological barriers and extending the circulation half-life [36]. This has been demonstrated to improve the overall drug delivery efficacy and helps minimize any possible adverse effects assigned with non-specific distribution [37, 38]. Conjugation of the targeting ligands is a potent approach for ameliorating the performance of PLH nanoparticles and further enhancing their properties as highly efficient drug carriers with precise targeting [33, 35].

3.2. Stimuli-Responsive Functionalization. The stimuli-responsive functionalization has been adopted to augment the PLH nanoparticles' performance [3]. These nanoparticles are modified by utilizing this technique, enabling them to react to specific physiological conditions due to the attachment of certain stimuli-responsive elements, thus, enhancing targeted, controlled, and stimulated drug release [39, 40]. The stimuli-responsive PLH nanoparticles can be designed to combine with temperature-responsive lipids or pH-

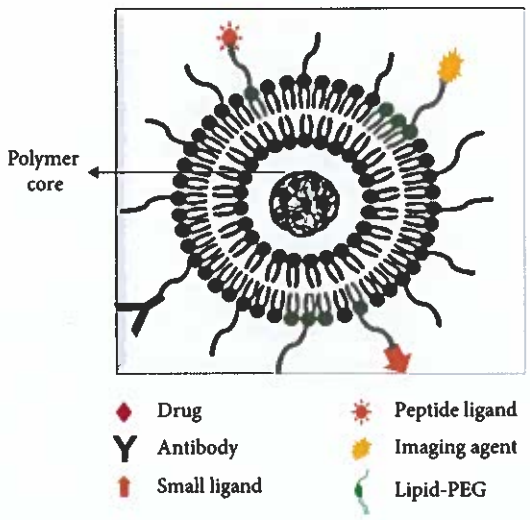

FIGURE 3: Diagrammatic demonstration of PLH nanoparticle functionalization, adapted from Gajbhiye et al. [7].

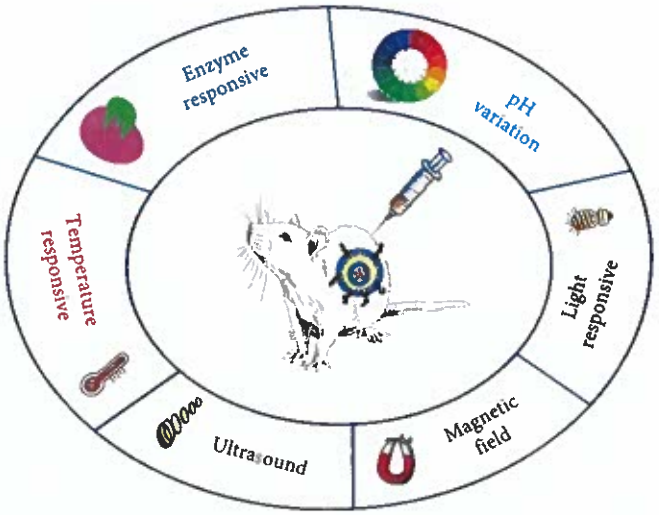

FIGURE 4: Representation of the various stimuli-responsive functionalization strategies, adapted from Tahir et al. [4] and Baghel and Bhattacharya [32].

sensitive polymers to particularly control the release of the drugs in response to changes in the physiological environment. These pH-sensitive polymers vary in their shape in acidic or alkaline environments and, therefore, have become one of the strategies for stimuli-responsive modifications [41]. These nanoparticles, when in contact with acidic conditions, may be in tumor microenvironments or endosomal compartments within cells, enable pH-triggered release of the drugs [10]. One other form of the stimuli-responsive functionalization is the incorporation of temperature-responsive lipids into the nanoparticles, which in turn can facilitate the release of drugs in response to modest hyperthermic conditions, frequently observed in infected and diseased tissues, or by the application of the regulated hyperthermia externally [42, 43]. Other external and internal stimuli have been investigated, including light, magnetic, enzyme, redox, and ultrasound, during the functionalization of these nanoparticles (Figure 4). A high level of accuracy and noninvasive drug release control can be achieved by adding the light-sensitive, enzyme, redox, or magnetically responsive components into the nanoparticle design [37, 44]. This may result in the design of novel PLH nanoparticles for external stimulation-assisted targeted delivery, with prospective uses in deep-seated tissue-controlled release or localized cancer therapy [45].

3.3. PEGylation. PEGylation, which simply refers to surface functionalization of nanoparticles with PEG, represents the next frontier in improving the performance of PLH nanoparticles for efficient and targeted delivery of drugs and gene materials to the intended cells, tissues, and organs [1, 2, 46]. Regarding drug delivery, PEGylation, which entails the covalent attachment of PEG chains to the surface of nanoparticles, offers several advantages [47]. One of its advantages includes preventing the immune system identification and clearance of the nanoparticles, prolonging their systemic circulation and enhancing their general pharmacokinetics [46, 48]. PEGylation of the nanoparticles has the ability to drastically minimize nonspecific interactions with biological components, thereby limiting off-target effects and improving the accuracy of targeted drug delivery [46]. By designing PEGylated PLH nanoparticles, extended circulation, biodistribution, and concentration at the targeted biological areas can be achieved through the modification of the nanoparticles with PEG chains [49]. Similarly, the PEGylation process has been shown to enhance and increase the stability of the nanoparticles by decreasing aggregation and strengthening colloidal stability, which is essential for long-term drug delivery system deployment and storage [9, 50]. PEG chains added to the surface of the nanoparticles can also help conjugate stimuli-responsive moieties or targeting ligands, enabling the smooth integration of various functionalization techniques to produce synergistic effects in precision-targeted drug delivery [16]. The possibility of developing advanced drug delivery systems with increased efficacy and fewer off-target effects is becoming increasingly appealing as studies continue to investigate the possibilities of PEGylation functionalization in PLH nanoparticles [51]. The overall biocompatibility of the nanoparticles is increased by the PEGylation process [36].

In general, the goal of functionalization strategies for PLH nanoparticles is to maximize their potential for transforming therapeutic delivery and targeting in clinical settings by improving their performance, targeting capabilities, and biological system compatibility [6]. Table 2 summarizes the PLH nanoparticles functionalization approaches, the polymer and lipids used, therapeutic applications, and outcomes.

4. Characterization of PLH Nanoparticles

4.1. Physicochemical Characterization: Dimensions and Structural Morphology. Understanding the morphological properties of PLH nanoparticles from a microscopic perspective, such as size dimensions, zeta potential, polydispersity index, and shape, is essential to developing recommendations for creating innovative PLH nanoparticles [27]. Observing

Table 2: Summarized PLH nanoparticles functionalization approaches for various therapeutic applications.

Functionalization approaches	Examples	Polymer/lipid used	Therapeutic applications	Outcome	References
PEGylation	CD44, hyaluronic acid conjugated PLH nanoparticles	Poly(glycolic acid), hyaluronic acid (sodium salt), maleimide poly(ethylene glycol), polyvinyl alcohol, cholesteryl hemi succinate, 4-(dimethylamino) pyridine, n-hydroxy succinate, tocopheryl succinate	Breast cancer	Potential for targeted delivery of herbal extracts for cancer treatment and potential for translation in in vivo experiments	[52]
	PEGylated PLH nanoparticles	Polyvinyl alcohol, polycaprolactone, polyethylene glycol 6000	Breast cancer	A stable encapsulated system, enhanced drug solubility, and induction of apoptosis in MCF-7 cells	[53]
Conjugation	Surface modification with methoxyl, carboxyl, and amine groups	Poly(D,L-lactide-co-glycolide), poly(ethylene glycol), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N- [carboxy (polyethylene glycol)-2000]	Drug delivery	Strong protein binding patterns	[54]
	Vitamin D3 functionalized PLH nanoparticles	Poly(lactic-co-glycolic acid), cholesterol, hydrogenated soy phosphatidylcholine (HSPC), 1,2-disteroyl-sn-glycero-3-phosphaethanolamine-N- [succinyl (polyethylene glycol)-2000 (DSPE-PEG-2000) and 1,2-dis-tearoyl-sn-glycero-3-phosphoethanolamine-N-] cholecalciferol (polyethylene glycol)-2000] (DSPE-PEG-2000-VD)	Melanoma treatment	Efficiently targeted B16 melanoma cells	[15]
	P-gp inhibitors (D-α-tocopherol polyethylene glycol succinate, pluronic, and Solutol HS15) incorporated PLH nanoparticles	Glyceryl monooleate, chitosan, soluplus TPGS, P123, and HS15	Drug delivery	Enhanced mucoadhesion and stability of paclitaxel	[55]
	Fucose-conjugated PLH nanoparticles	Phospholipid S100, polycaprolactone, l-fucose, 7,12-dimethylbenz[a]anthracene, stearyl amine, 1,2-disteroyl-sn-glycero-3-phosphaethanolamine-N- [succinyl (polyethylene glycol)-2000 (DSPE-PEG-2000)-NH_2, stearoyl polyoxyl-32 glycerides	Breast cancer	Prophylactic and anticancer activity, synergistic effect	[56]
	Folate-modified PLH nanoparticles	Poly(ε-caprolactone), poly(ethylene glycol), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*- [methoxy (polyethylene glycol)-2000] (DSPE-PEG-2000), folate	Tumor-targeted therapy	Promising nanosized formulation for targeted delivery of paclitaxel	[57]
	Anticarcinoembryonic antigen (CEA) half-antibody conjugated PLH nanoparticles	PLGA, soybean lecithin, carboxyl-terminated 1,2-distearoyl-sn-glycero-3-phosphoethanol-amine-N-carboxy (polyethylene glycol)-2000), maleimide-terminated 1,2-disteroyl-sn-glycero-3-phosphaethanolamine-N-[succinyl (polyethylene glycol)-2000	Pancreatic cancer therapy	Enhanced cancer-killing effect	[58]
	Cholic acid functionalized star PLH nanoparticles	Soybean lecithin, 1,2-disteroyl-sn-glycero-3-phosphaethanolamine-N- [succinyl (polyethylene glycol)-2000, cholic acid	Cancer chemotherapy, multidrug resistance	Promising in synergistic drug delivery to overcome cancer drug resistance	[59]
	Folic acid/peptide (cRGDfK) decorated PLH nanoparticles	1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), poly(d, l-lactide-co-glycolide (PLGA), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine [methoxy (polyethylene glycol) (DSPE-PEG-2000), folic acid and soybean lecithin	Glioma/brain tumor targeting	High in vitro inhibitory effect, cell apoptosis, and cell uptake, extended survival time	[60]
	Arg–Gly–Asp (RGD) modified PLH nanoparticles	Monomethyl poly(ethylene glycol)–poly(lactic-co-glycolic acid), copolymers, lecithin, cholesterol	Active tumor targeting for cancer treatment	Enhanced antitumor activity against melanoma, excellent tumor-targeted formulation for cancer therapy	[61]

Table 2: Continued.

Functionalization approaches	Examples	Polymer/lipid used	Therapeutic applications	Outcome	References
	Aptamer-functionalized curcumin and cabazitaxel PLH nanoparticles	L-α-phosphatidylcholine, A10-3.2 aptamer, poly(L-lactide-co-glycolide)-poly(ethylene glycol)	Prostate cancer therapy	Good cell inhibition ability, high tumor accumulation, remarkable tumor inhibition efficiency, promising co-drug delivery, and potential synergistic combination therapy for prostate cancer	[62]
	Chitosan hydrogel beads functionalized with thymol-loaded PLH nanoparticles	Precirol ATO 5, dextran, thymol, sodium periodate, chitosan, bovine serum albumin	Antimicrobial treatment	Promising controlled-release features as an antibacterial approach for improving food safety	[63]
	Gambogic acid engineered urolithin A-laden PLH nanoparticles	Glyceryl tristearate (tristearin), D-α-tocopherol polyethylene glycol 1000 succinate, N-boc ethylenediamine	Tubular injury hemotherapy-induced acute kidney injury	Superior intracellular accumulation, excellent anti-inflammatory properties	[64]
	Antioxidant enzymes sequestered PLH nanoparticles	Carboxy-terminated poly (lactide-co-glycolide, 1,2-dioleoyl 3-trimethylammonium-propane, 2-distearoyl-sn-glycero-3-phosphoethanolamine- [methoxy (poly(ethylene glycol)-2000], 1,2-dis-tearoyl-sn-glycero-3-phosphoethanolamine-N-[folate(poly(ethyleneglycol))-2000]	Treatment of inflammatory bowel disease	Excellent mucus-penetrating ability, inflammation targeting via inhibition of pro-inflammatory cytokines	[65]
	Chitosan functionalized PLH nanoparticles	Poly(lactic-co-glycolic acid), chitosan, soybean lecithin, 1,2-distearoyl-sn-glycero-3-phosphoethanol-amine-*N*-methoxy (polyethylene glycol)-2000	Nonalcoholic fatty liver disease treatment	Reduced blood lipid levels, improved liver function, reduced lipid accumulation, and reduced macrovesicular steatosis	[66]
Stimuli responsive	pH-sensitive PLH nanoparticles	Eudragit E100, eudragit RS100, 3β-[N-(N′, N′-dimethylaminoethane)-carbamoyl] cholesterol hydrochloride (DC-cholesterol), phosphoethanolamine-N-methoxy polyethylene glycol, lipofectamine 3000	Plasmid DNA transfection	High pDNA encapsulation efficiencies, increased expression of targeted RFP, and favorable biocompatibility	[67]
	Magnetic field–activated PLH nanoparticles	Poly(lactic-co-glycolic acid), soybean lecithin, phosphoethanolamine-N-carboxy (polyethylene glycol), iron oxide	Cancer chemotherapy	Significant reduction in MT2 breast cancer cells, controlled drug release	[68]
	Polymetformin and penetratin-based PLH nanoparticles	Chitosan, 2-distearoyl-sn-glycero-3-phosphoethanol-amine-N-methoxy (polyethylene glycol)-2000	Nonalcoholic fatty liver disease treatment	Alleviated hepatic steatosis, restored insulin sensitivity, improved metabolic syndrome	[69]

such morphological influences has increased prospects for developing PLH nanoparticles for distinct applications. The size, shape, and surface zeta potential of nanoparticles are crucial physical factors affecting their in vivo performance, serum, and storage stability. These factors are generally influenced by process parameters such as mixing rate, the ratio of solvent used in preparation, the type and polarity of solvent and drug encapsulated, and the general method applied in preparing the nanosystem [49, 70]. Elkateb et al. [71] studied the effects of different synthesis parameters and ways to optimize the formulation of PLH nanoparticles dual-loaded with darunavir and ritonavir for HIV treatment. They established an optimum stabilizer to polymer core ratio at 20% *w/w* and 70% *w/w* of the total stabilizer (Brij 78) mass was required to stabilize phosphate-buffered saline. Moreso, using PEG, cryoprotectant, and freeze-drying yielded PLH nanoparticles with a size of 150 nm after redispersing the nanoparticles in water [71].

Generally, spherically shaped nanoparticles spontaneously stimulate the connections between cell membrane receptors, induce novel attachment-dependent phenotypes in cell membranes, and increase drug accumulation at target sites of interest. These results have provided valuable opportunities to customize new PLH nanoparticle applications. Moreover, the surface charge also greatly affects the nanoparticle interactions with cell membranes, circulation duration, potential for targeted drug delivery, and extent of intracellular penetration. Compared to neutrally and negatively charged nanoparticles, positively charged nanoparticles can increase intermolecular cross-links, allowing medications to escape from the lysosome and demonstrate perinuclear localization. Also, positively charged nanoparticles favor the formation of ionic interactions with negatively charged cell membranes to increase the absorption of drug-loaded nanoparticles. The ability of cell membranes to reversibly expand and contract in response to protonation and deprotonation processes at varying pH values causes a change in cell shape and facilitates the uptake of medicines. Studies by Pho et al. [72], Guo et al. [73], and Zhao et al. [74] suggested that negatively and neutrally charged nanoparticles can delay the circulation time relative to positively charged nanoparticles by blocking plasma protein adsorption.

Therefore, to obtain detailed information on the physicochemical characteristics of PLH nanoparticles, a combination of techniques involving electrophoretic light scattering, dynamic light scattering, nanoparticle tracking analysis (NTA), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) has been well-reported. Dynamic light scattering and NTA have been used to determine the particle size, PDI, and zeta potential. However, because the light scattering techniques do not obtain the core–shell structural configuration, structural homogeneity, and related dynamic integrity, the development of microscopic imaging technology has aided in collecting structural proof for the nanometric dimensions. High-resolution SEM has been used to see the hybrid nanoparticles' topological shape. TEM and atomic force microscopy have also been beneficial in successfully characterizing individual hybrid nanoparticles. The internal core–shell lipid polymer distribution, along with the thickness of the lipid shell over the polymer core, could be observed with the help of TEM. In addition, Fourier transform infrared (FTIR) and UV–Vis spectroscopy (UV–Vis) are versatile surface-sensitive techniques for studying nanomaterials [75]. They provide quantitative information on functional group compositions and structural changes upon intermolecular interactions. Furthermore, attenuated total reflection (ATR)-FTIR spectroscopy and X-ray diffraction (XRD) have also been used to investigate the surface chemistry and structure of PLH nanoparticles on an atomic scale [76].

4.2. Drug EE, Drug Loading Capacity, and Drug Content Analysis. Many factors must be considered when designing PLH nanoparticles to encapsulate different molecular weight therapeutics. The therapeutic agents can be entrapped within the nanocarrier, or they may be conjugated onto the surface of nanostructures. The encapsulation of therapeutics into hybrid vesicles can improve their pharmaceutical properties by decreasing drug leakage and maximizing circulation time. The drugs can be loaded into nanoparticles of polymer-rich or lipid-rich domains, which work as sorting platforms. In addition, the choice of preparation methods plays an important role in optimizing the loading properties of PLH nanoparticles by providing optimal intermolecular interactions and binding strength. Various weak and strong drug interactions and anionic polymeric core or cationic lipids drive the fusion process. However, strong and irreversible interactions make it difficult to release drugs, while weak bonds allow quick dissociation of drugs and may affect formulation performance in vivo.

There are two established methods for drug content determination. The direct method involves the separation of the nanoparticles from the free drug using techniques such as centrifugation, ultracentrifugation, or ultrafiltration, after which the amount of drug contained in the nanoparticles is broken down using a suitable organic solvent and the drug content is quantified using suitable analytical methods. Yalcin et al. [77] used this method to determine the EE of gemcitabine hydrochloride-loaded PLH nanoparticles. They mixed 5 mg of the lyophilized PLH nanoparticles with 5 mL of dichloromethane, then added 10 mL of phosphate buffer solution (pH 7.4) and sonicated in an ultrasonic bath for 60 min. Finally, the remaining aqueous dispersion was filtered, and high-performance liquid chromatography (HPLC) was used to analyze the samples at a wavelength of 268 nm. An average EE of 45.2 % was reported [77].

In comparison, the indirect method estimates the free drug concentration in the solution after removing the nanoparticles. It is important to note that the choice of method depends on the characteristics of the loaded drug. Muso-Cachumba et al. [78] investigated the accuracy of both methods in calculating the EE of polymerosomes. They reported a reasonable agreement between the two methods and further recommended that a combination of both methods would produce a more reliable result [78]. Schober et al. [79] recently described a method involving the determination of

EE for RNA-loaded PLH nanoparticles via modified Quant-it RiboGreen Assay (Thermo Fisher Scientific).

Depending on the method used, EE is calculated using the formula below.

$$\text{For direct method}: \%\,\text{EE} = \frac{\text{Actual amount of drug in PLH nanoparticles}}{\text{Total initial amount of drug added}} \times 100.$$

$$\text{For the indirect method}: \%\,\text{EE} = \frac{\text{Total amount of drug added} - \text{free or unloaded drug}}{\text{Total amount of drug added}} \times 100.$$

Drug loading capacity is the amount of drug loaded or encapsulated per unit weight of the nanoparticle. This parameter depends on the structure of the loaded drug and excipients, as well as the physical and chemical interactions between the drug molecules and the carrier materials [80]. Limited compatibility between the drug and excipients in the formulation results in very low drug loading; therefore, it is important to optimize these factors during the preparation of the PLH nanoparticles [81]. The direct method protocol is most preferred for determining drug loading capacity. Shafique et al. [82] used this method to determine the % DLC of doxorubicin-loaded PLH nanoparticles and reported a drug loading of 0.391%.

DLC can then be calculated from the equation below.

$$\%\,\text{DLC} = \frac{\text{Total amount of drug in PLH nanoparticles}}{\text{Total amount of drug added} + \text{total amount of excipients used}} \times 100.$$

Quantifying the amount of drug in the supernatant or amount present in PLH nanoparticles can be achieved using different methods involving HPLC and UV–Vis spectroscopy instruments. In addition to these, other techniques such as mass spectroscopy (MS), gel electrophoresis, and fluorescence measurements have also been used to quantify loaded drugs.

4.3. Stability Assessment. The stability and integrity of PLH nanoparticles in serum and saline solutions are other important factors that must be carefully considered in PLH nanoparticles' physicochemical characterization. These are done to predict the physical and chemical stability in vivo utilizing in situ approaches. Factors such as concentration of nanoparticles, surface charge and polarity, surface hydrophobicity, and lipid concentration at the interface have been reported to influence the stability and integrity of PLH nanoparticles. The lipid coating on the entire nanoparticles' interface and steric repulsion imparted by the PEG layer over the nanoparticle surface also impact the stability of the nanoparticles. PEG concentration, molecular weight, and length of chain influence nanoparticle stability [83]. The physical stability of PLH nanoparticles is also assessed to determine the effects of temperature, pH, light, moisture, and reactive oxygen species on the developed formulation to determine the shelf life or best storage condition. Usually, the finished formulation is exposed to the desired test conditions for a determined time, which may last up to 3 months, for long-term stability measurement and 1 month for short-term stability measurement. As such, researchers have consistently established stability and integrity parameters by assessing the average size, PDI, zeta potential, and entrapment efficiency of the PLH nanoparticle formulations. These parameters are monitored and assessed overtime. Shafique et al. [82] assessed the physical stability of prepared DOX-PLH nanoparticles formulation at refrigerated and room temperatures. They determined the size over 90 days and reported an increase in size at the initial 30 days, followed by stabilization overtime at both temperature conditions. This may be attributed to the amorphous nature of the loaded drug and, consequently, the dissolution of smaller particles while being deposited onto the surface of the larger ones, a common phenomenon with amorphous particles [82]. More so, the higher the concentration of nanoparticles, the higher the tendency for aggregation; hence, a higher PDI will be imminent. This also depends on the stabilization mechanism, density, and surface charge of the nanoparticles [84].

Nanoparticles in the systemic circulation rapidly form a protein corona over their surface by selective adsorption of serum proteins based on hydrophobic interactions, Van der Waals forces, and electrostatic interactions, forming aggregates with greater particle size and polydispersity [85]. This phenomenon established the need for serum or plasma stability assessment of the nanoparticles. Serum and plasma stability could be evaluated in vitro using 10%–100% fetal bovine serum and extracted blood plasma, respectively [86, 87]. An accelerated stability study of optimized formulations can also be performed per the International Council for Harmonization guidelines for Stability Testing of New Drug Substances and Products [88].

4.4. Surface Functionalization/Association Efficiency (AE) Assessment. An AE assay calculates the percentage of targeted ligands successfully incorporated onto the nanoparticle surface. In this assay, after functionalization, nanoparticle dispersions are centrifuged at room temperature at 9300 × g for 10 min using suitable ultrafilters to separate nanoparticles from the nonincorporated fraction of the targeting ligand, which remains in the subnatant. Afterwards, the fluorescence or amount of this subnatant can be determined using a

suitable analytical technique. The microplate reader and UV–Vis spectroscopy have been extensively used [89]. The surface functionalization efficiency is then calculated using the equation below.

$$\text{AE}(\%) = \frac{\text{The total amount of targeting ligand added} - \text{free ligand in the supernatant}}{\text{Total amount of targeting ligand added}} \times 100.$$

HPLC is another technique used to quantify uncoupled ligands in supernatants after centrifugation of predialysis solution. The main disadvantage of the HPLC method is the indirect quantification of bound fractions based on the direct estimation of unbound ones. This may result in over- or underestimation of the amount of targeting, especially due to improper purification processes during the HPLC technique.

Despite the mentioned techniques, other complementary methods, including FTIR, circular dichroism (CD), MS, SDS-PAGE, and SPR, can detail the structural changes of proteins and peptides adsorbed onto the surface of the PLH nanoparticles. Among them, MS and SDS-PAGE have been widely used to investigate protein corona composition and electrophoretic mobility of each component in the surface-decorated nanoparticles, respectively [90]. Notwithstanding the benefits of SDS-PAGE to separate coated PLH nanoparticles domains according to the electrophoretic mobility, the comigration of macromolecules with similar size distribution in the complex mixture may still occur.

Moreover, a combination of these techniques can be used depending on the desired result, nature, and constituents of the nanovesicle. In combination with LC, MS is a widely used method to qualitatively and quantitatively study the interactions between large proteins such as albumin and various nanoparticles [91]. The CD techniques with the FTIR method can be used to probe secondary and tertiary structural changes of proteins or peptides after association with nanovesicles [92].

4.5. Drug-Release Characterization. Drug-release characterization usually involves an in vitro assay carried out to study the release behavior of the drug from the formulation, commonly called the in vitro dissolution studies [76]. For this study, the drug-loaded PLH nanoparticles are incubated in a dialysis sac in the presence of dissolution media in constant sink conditions at 37 ± 0.5°C under agitation, and samples are collected at predetermined intervals. The samples are analyzed using HPLC, UV–Vis, and other analytical tools to quantify the loaded drug amount and determine the cumulative drug release overtime. The drug released with time is then subjected to various data fitting kinetics models like Peppas–Korsemeyer, Higuchi, Hixon–Crowell, zero order, and first order to gain further insights into the drug release mechanisms [93]. The drug release assay has also been modified by scientists to mimic different physiological conditions and to release loaded drugs to target sites distinctly. Shen et al. [94] described a release assay containing 1% Tween 80 as a surfactant, a reduced form of nicotinamide adenine dinucleotide phosphate to establish a hypoxic condition, and H_2O_2 to establish a peroxidative condition.

Several factors influence the drug release profile of the PLH nanoparticles, including the interaction between the drug and polymer, the drug's solubility, the polymer's rate of degradation, and the particles' size. The release of the drug from the PLH nanoparticles, which is physically encapsulated, occurs through drug diffusion and depends on the polymer's erosion rate. For chemically encapsulated drugs, the release depends on the linker hydrolysis (the link between the polymer chain and the drug). The uniform distribution of the drug molecules in nanoparticles improves the release kinetics of the drug with expressively reduced burst release effect [76].

4.6. The Cell-Based Assays. Cell uptake, transport mechanisms, apoptosis, cell cycle arrest, and cytotoxicity assays are commonly carried out to study the effect of the nanoparticles on in vitro cultured cell lines. These cell-based assays are used to assess the efficacy, safety, and mechanism of activity of the drug-loaded PLH nanoparticles in vitro, and the results are correlated to in vivo estimations. Cytotoxicity assays assess the cytocompatibility of the PLH nanoparticles. The cell viability and cytotoxicity experiments are generally performed by incubating the test samples (the nanoparticles and free drug) with the desired cell lines for a specified period, usually 24–72 h. After which, the cell viability is established using a suitable ATP, XTT/MTT–based assay. MTT assays assess colorimetric reactions whereby viable cells reduce yellow tetrazolium salt to purple formazan crystals, indicating their metabolic activity. This assay mostly uses normal healthy cells [93].

Cellular uptake studies can quantitatively and qualitatively investigate the ability of the drug-loaded PLH nanoparticles to be internalized within the target cells and induce biological effects [76]. To qualitatively assess the uptake of loaded PLH nanoparticles, the nanoparticles are tagged with suitable fluorescent dyes/probes such as fluorescence isothiocyanate (FITC), coumarin-6 (C-6), and 2′-7′-dichlorodihydrofluorescein diacetate (DCFDA), which demonstrates cell internalization at specific absorbance and excitation wavelengths under fluorescent conditions. Imaging can be done using a confocal laser scanning microscope (CLSM) or a fluorescent microscope. Rajana et al. [76] described a quantitative assay for cell uptake investigation in which the cells were incubated with the test samples and collected at different time intervals, washed, and broken down. Then, the quantity of the loaded drug, Palbociclib, in the cells was determined using a suitable RP-HPLC procedure at 356 nm [76]. Pang et al. [95] used flow cytometry to quantitatively assess the cellular uptake of PLH nanoparticles for delivery of erlotinib plus bevacizumab to non-small cell lung cancer. The cellular

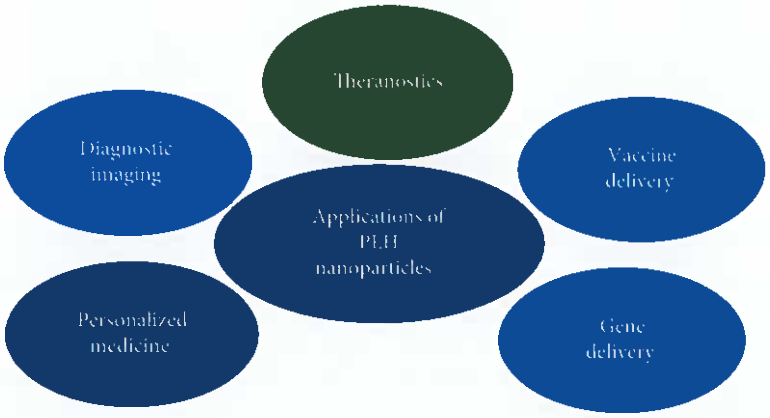

FIGURE 5: Schematic illustration of different applications of PLH nanoparticles.

uptake of nanoparticles in vitro depends on two mechanisms: endocytosis or nonspecific inundation. Therefore, to assist in cellular uptake of the PLH nanoparticles, targeting ligands have been typically conjugated to the surface of PLH nanoparticles to trigger receptor-facilitated endocytosis. In this regard, a variety of targeting ligands, such as proteins, peptides, and aptamers, have been used to facilitate cellular uptake and binding specificity [15, 26, 76].

Cell apoptosis and cell cycle arrest, on the other hand, could be estimated using flow cytometry studies. Here, the capability of the PLH nanoparticles and free drug to induce apoptosis and the mechanism by which they induce cell death is investigated. Rajana et al. [76] investigated cell apoptosis using a standard externalization assay of phosphatidyl serine in the cancer cell membrane with FITC-labeled Annexin V/PI dual staining assay. MCF-7 and MDA-MB-231 cells were incubated with the test samples for 48 h, washed and stained, and then, subjected to FACS Calibur flow cytometry to determine the live and dead cells [76].

4.7. Release Mechanisms of PLH Nanoparticles. The drug release mechanisms of nanoparticle-based delivery systems differ depending on their structure, composition, and physicochemical characteristics [50]. As a result, common drug release mechanisms involving diffusion, degradation, swelling, stimulus triggers, and fusion have been reported. Moreover, for PLH nanoparticles, the most reported mechanisms are diffusion, fusion, and stimuli-controlled release mechanisms. Diffusion-controlled drug release is driven by the difference in concentration gradient across membranes [96]. Here, the drug is dissolved in the central part and diffuses through the membrane to exert activity. This is the most common mechanism of drug release for most PLH nanoparticles, where the drug molecules are usually encapsulated within the core polymer matrix [97].

On the other hand, the degradation-controlled drug release mechanism involves the release of the drug through enzymatic breakdown [98, 99]. Certain enzymes break down the amide or ester bonds in the nanosystem or induce hydrolysis. This is typically a feature for carriers containing biodegradable polymers and lipids. For the stimuli-controlled release mechanism, drug release from the PLH nanoparticles is controlled by temperature, pH, light, ultrasound, and magnetic or electric fields. Such carriers have been extensively explored for targeted delivery [100]. In addition to the described mechanisms, different mathematical models have been developed and used to predict and further explain drug release mechanisms. To this end, mathematical models such as the Higuchi, Korsmeyer–Peppas, first-order and zero-order release kinetics, Weibull release model, the two-film model, biomembrane, and the toroidal models have been studied [93].

5. Different Applications of PLH Nanoparticles

PLH nanoparticles have been applied across diverse fields for different applications and have shown enormous advantages across these fields. By combining two nanocarriers (organic to inorganic or inorganic to organic nanoparticles), these nanoparticles have demonstrated interesting properties, synergistic effects due to the combinational features of the polymer and lipids, and their ability to foster targeted drug delivery. As a result, they have been explored most significantly as drug carriers for delivering vast therapeutic agents and treating different diseases [12]. Given the adaptable and surface modification features of PLH nanoparticles, they have been investigated in various applications, including targeted drug delivery, gene delivery, vaccine delivery, biomedical and multifunctional imaging, and personalized medicine (Figure 5).

5.1. Targeted Drug Delivery. The hybrid architecture of PLH nanoparticles allows for both passive and active targeting drug delivery approaches. Passive targeting exploits the enhanced permeation and retention (EPR) effect, particularly in tumor tissues. In contrast, active targeting is achieved by functionalizing the nanoparticle surface with certain stimuli-responsive moieties or biomolecules such as folic acid, antibodies, aptamers, or peptides that bind to overexpressed receptors on target cells [5]. The application of PLH nanoparticles in targeted cancer therapy is one of the most extensively investigated areas. Their ability to deliver chemotherapeutic agents such as doxorubicin, paclitaxel, gemcitabine,

or combinations thereof, specifically to tumor sites, reduces off-target toxicity and improves the therapeutic index of anticancer drugs [6, 101]. Moreover, dual-ligand strategies, where two different targeting moieties are simultaneously attached, have been developed to improve selectivity further and overcome the heterogeneity of receptor expression in tumors [6]. Studies have demonstrated that nanoparticles such as folate-modified, antibody-conjugated, and peptide-targeted PLH nanoparticles significantly enhance internalization by cancer cells through receptor-mediated endocytosis, leading to improved tumor regression and survival outcomes in animal models [57, 102]. Beyond cancer therapy, PLH nanoparticles have been investigated for antimicrobial applications, particularly in addressing the challenge of antibiotic resistance [103]. The design of PLH nanoparticles allows for the targeted delivery of antimicrobial agents to infection sites, thereby reducing the effective dose required and minimizing systemic toxicity [101, 104]. Studies have demonstrated that encapsulating antibiotics within PLH nanoparticles leads to sustained drug release profiles and improved penetration into biofilms, critical for eradicating persistent infections associated with resistant bacterial strains [6].

In treating neurological disorders, PLH nanoparticles offer significant advantages for delivering therapeutic agents across the blood–brain barrier (BBB), a major challenge in neuropharmacology. The small size and surface modifications of these nanoparticles enable them to penetrate the BBB efficiently, thereby facilitating the delivery of neuroprotective drugs, peptides, or genes to target sites in the brain [1]. This capability is particularly beneficial in the management of neurodegenerative diseases such as Alzheimer's and Parkinson's, where conventional drug delivery approaches have historically shown limited efficacy. Similarly, in cardiovascular applications, PLH nanoparticles have been engineered to deliver drugs specifically to diseased vasculature or myocardial tissues, which can improve treatment outcomes in conditions such as atherosclerosis, myocardial infarction, and other heart diseases. By offering controlled release and targeted delivery, these hybrid nanoparticles help to mitigate systemic side effects and enhance therapeutic efficacy in these sensitive organs [5]. The potential of PLH nanoparticles in oral drug delivery has also been demonstrated. This involves the incorporation of enteric coatings and pH-sensitive lipid layers that protect the loaded drug from the harsh gastric environment and promote absorption in the intestine [105]. In essence, modification of PLH nanoparticles using passive, active, or endogenous mechanisms to circumnavigate the biological barriers to target the intended cells, tissues, or organs would enable using these nanoparticles as a targeted drug delivery platform for treating diverse diseases.

5.2. Stability Enhancement. PLH nanoparticles are capable of shielding encapsulated therapeutics from degradation and premature leakage, even under harsh physiological conditions, thereby extending storage stability and simplifying long-term transport of pharmaceutical formulations [1, 8]. Stimuli-responsive functionalization offers an additional advantage by enhancing nanoparticle stability and shelf-life [2]. Optimizing these design features enables the creation of systems that remain inert during storage yet release their drug payload efficiently when exposed to specific biological triggers [106]. Achieving an optimal balance between stability and responsiveness is essential for translating such smart PLH nanoparticles into clinical applications [2]. Surface modifications such as PEGylation further contribute to prolonged circulation, improved stability, and reduced immune clearance, minimizing off-target effects and enhancing therapeutic precision [107]. Collectively, these strategies highlight the significant promise of PLH nanoparticles in advancing drug delivery, particularly through stimuli-responsive engineering and PEGylation, to achieve enhanced stability, extended half-life, and improved therapeutic outcomes [7, 35].

5.3. Diagnostic Imaging and Theranostics. PLH nanoparticles have also been explored for diagnostic imaging [1]. The integration of imaging moieties, such as fluorescent dyes or contrast-enhancing agents, into the structure of nanoparticles enables them to act as imaging probes in advanced diagnostic techniques. This dual functionality enhances visualization of pathological tissues and facilitates the detection and monitoring of various medical conditions [1, 12]. Therefore, through careful engineering and surface functionalization, PLH nanoparticles hold significant potential for improving the early detection and accurate assessment of various diseases, notably cancer and inflammatory conditions [12]. Given this, fluorescent-labeled antibodies, ligands, and peptides have been reported as surface markers in tumor detection [108]. Different imaging agents can be incorporated into PLH nanoparticles, such as gadolinium for magnetic resonance imaging (MRI), quantum dots for fluorescence imaging, and gold nanoparticles for computed tomography (CT), to provide complementary diagnostic perspectives. Embedding these agents within the nanocarriers enables real-time visualization of nanoparticle biodistribution and supports the monitoring of therapeutic outcomes in cancer and other pathological conditions [101, 109]. Furthermore, PLH nanoparticles can be coloaded with therapeutic and diagnostic payloads, rendering them effective platforms for multifunctional theranostic applications that integrate disease detection with treatment [12]. These systems are often designed to respond to physiological triggers, including pH variations or redox imbalances, thereby facilitating controlled drug release while simultaneously enhancing imaging contrast—features that are highly valuable for prognostic assessment [110].

5.4. Gene Delivery. Another prominent application of PLH nanoparticles is in gene delivery, where they act as nonviral carriers for the transport of nucleic acids, including siRNA, DNA plasmids, and mRNA, to specific cells [5]. PLH nanoparticles protect these sensitive genetic materials from enzymatic degradation and other sources of instability in the systemic circulation, while improving cellular uptake and intracellular trafficking to achieve effective gene silencing or expression [5]. They are considered good candidates for gene delivery applications due to their adaptability, high

stability, and biocompatibility properties [2, 111]. Their core–shell architecture, particularly when combined with cationic polymers such as polyethylenimine, facilitates the formation of stable ionic complexes with nucleic acids, thereby further enhancing delivery efficiency through the proton sponge effect that encourages endosomal escape [112]. The surface or structure of these PLH nanoparticles may be altered to improve target cells' effective uptake of therapeutic genes [1, 113]. For instance, PLH nanoparticles can be modified with targeting ligands to achieve cell-specific gene delivery, which is especially important in treating genetic disorders and cancer, where therapeutic gene modulation is critical [6]. By modifying the surface and fine-tuning the size and charge of the nanoparticles, an efficient contact with cell membranes, possible internalization of the nanoparticles and subsequent transfer of the therapeutic genes to the targeted intracellular locations could be achieved [114, 115]. Applying PLH nanoparticles in gene delivery can also reduce possible side effects and prolonged delivery of the encapsulated genetic drug in a controlled approach [37, 116]. Studies have reported significant improvements in both in vitro and in vivo gene transfection efficiency using PLH nanoparticles, thereby highlighting their potential to serve as reliable and safe alternatives to viral vectors in clinical gene therapy for both inherited and acquired diseases [6, 117].

5.5. Vaccine Delivery. The use of PLH nanoparticles in vaccine delivery has attracted considerable interest, given their special properties. By encapsulating protein antigens, peptides, or even mRNA within their polymeric cores and coating them with lipids that can include immunostimulatory components, PLH nanoparticles facilitate a more potent and sustained immune response [118]. These nanoparticles are ideal for the entrapment of adjuvants and antigens, protecting vaccine constituents and promoting specific immune reactions [1, 119]. PLH nanoparticles can be designed to target immune cells and improve vaccine efficiency by manipulating vaccine components' release kinetics through exploring the nanoparticles' adaptable features [12, 120]. They enable enhanced antigen stability, improved delivery to antigen-presenting cells (APCs), and prolonged immune activation [6]. They can protect antigens and adjuvants from degradation and stabilize them throughout storage and delivery [121]. Several experimental vaccine formulations based on PLH nanoparticles have demonstrated enhanced immunogenicity in preclinical trials, setting the stage for future clinical applications in combating infectious diseases as well as in cancer immunotherapy [1]. Moreover, their ability to co-deliver adjuvants alongside antigens further improves the quality and durability of the immune response, demonstrating significant potential in next-generation vaccine platforms [12].

5.6. Tissue Engineering. The extensive properties of PLH nanoparticles make them a useful instrument for various biomedical fields beyond drug delivery [115, 122]. Tissue engineering and regenerative medicine are other areas where PLH nanoparticles have been explored. The regenerative medicine field involves replacing organs or tissues that have been lost or destroyed because of diseases, trauma, or congenital disabilities [123]. It offers the possibility to address healing issues resulting from a variety of disorders or ailments that were once thought to be incurable. In this way, the challenges of the transplant therapy, including the donor tissue's limited availability and the possibly damaging host immune responses, are highly minimized [123]. To this effect, regenerative medicine adopts different strategies, including cell therapy, tissue engineering, and the delivery of therapeutic agents such as drugs, proteins, and even genes to the targeted tissue that aid in the process of healing and repair [123]. The adaptability of the PLH nanoparticles makes it possible to transport the bioactive molecules, such as growth factors and cytokines, to the exact location of the tissue regeneration or injury [50, 123, 124]. These nanoparticles enable the controlled and localized release of these therapeutic substances, aiding tissue repair and regeneration.

5.7. Other Applications of PLH Nanoparticles. Application of PLH nanoparticles has also been explored for certain purposes beyond above stated. In the realm of immunotherapy, PLH nanoparticles have been applied to modulate immune responses in autoimmune diseases and inflammatory conditions by targeted delivery of immunomodulatory agents. This opens up new therapeutic avenues for disorders that require precise immune system regulation [125, 126]. In addition, the utility of PLH nanoparticles in photothermal and photodynamic therapy has been demonstrated, wherein these nanoparticles are combined with photosensitizers or metallic nanoparticles to achieve site-specific thermal ablation of diseased tissues upon light irradiation [125]. PLH nanoparticles have also been applied for codelivery and synergistic therapeutic strategies, such as the simultaneous encapsulation of chemotherapeutic drugs and gene-silencing agents like siRNA, thereby enabling combined modalities of treatment to overcome multidrug resistance and improve treatment outcomes [6]. Dual-drug-loaded formulations have been used to achieve synergistic anticancer effects, for example, by codelivering doxorubicin with a chemosensitizer or radiotherapeutic agent to coordinate chemotherapy and radiotherapy modalities [5]. These strategies enhance the pharmacokinetic profile of the drugs and facilitate a more controlled spatial and temporal release potential for synergistic activity [102].

5.8. Emerging Frontiers in Personalized Medicine. The modifiable and flexible features of PLH nanoparticles make them intriguing candidates for designing and developing personalized therapeutic interventions owing to the increased interest in personalized medicine [7]. Personalized medicine refers to a tailored and individualized approach to treatment management that aims to provide the appropriate drugs at the optimum dose for each patient [127]. This is necessitated by understanding the undesired drug adverse effects in different individuals and the variation in the pharmacological efficacy from person to person depending on the drug class [127]. Personalized medicine and nanotechnology have been reported to intersect across several fields. The first field of

application was diagnostics, where nanotechnology can be extremely helpful in pharmacogenetic testing, investigating the state of certain drug targets, and doing both in vitro and in vivo testing. Another aspect is in therapeutics, where nanomedicine is utilized in the treatment to achieve a targeted delivery for a specific disease in a particular patient [35, 127]. These nanoparticles can be designed to encapsulate specific drugs or genetic material, and their distribution optimized for individualized treatment plans. A significant margin can exceed the non-formulated drug's maximum tolerated dose because of its targeting capacity; hence, the dose can be adjusted following the unique circumstances of each patient [127]. PLH nanoparticles can be designed to boost customized drug response influenced by the heterogeneity in cytochrome P enzymes (CYPs) and drug transporters in various populations [128]. With the capacity to tackle patient-specific demands with improved efficacy and fewer side effects, advances in precision-targeted delivery and controlled release kinetics further contribute to the achievement of personalized medicine [129].

6. Challenges With the Application of PLH Nanoparticles

The application of the PLH nanoparticles has shown enormous therapeutic potential; however, they still face challenges that must be resolved, eliminated, or minimized before their broad clinical adoption can be realized [26, 125]. One primary concern is the issue of large-scale reproducibility and the maintenance of uniform physicochemical properties, such as particle size, surface charge, and drug loading capacity, which are critical for ensuring consistent therapeutic outcomes [5, 130]. Most PLH nanoparticles are currently produced in small batches in a lab, and it is not easy to produce PLH nanoparticles with the same properties in large batches in an industrial setting. Clinical translation and commercial feasibility depend on keeping a uniform size distribution, reproducibility of surface changes, and large-scale maintenance of functional structures [105, 131]. The development of scalable methodologies with highly dependable in vivo qualities would enable faster clinical translation [102]. The long-term stability and storage circumstances of these nanoparticles present another difficulty. For clinical application and shelf-life, it is essential to preserve the stability of encapsulated cargo and the integrity of nanoparticles for prolonged periods of time, particularly in different environmental conditions, through formulation refinement and advanced quality-by-design (QbD) approaches [132, 133].

Furthermore, while incorporating targeting ligands has improved the specificity of PLH nanoparticles toward certain cells or tissues, optimizing the density and orientation of these ligands to achieve effective targeting without compromising nanoparticle stability remains a critical area of ongoing research [5]. Another challenge involves the immunogenicity of the surface modifications, such as PEGylation, which, although beneficial for prolonging circulation time, may trigger the production of anti-PEG antibodies in some patients and reduce the stealth capability of the nanoparticles over time [101]. Regulatory challenges also persist, particularly related to the complexity of these multicomponent systems and the need for rigorous characterization methods that can comprehensively assess their safety, pharmacokinetics, and biodistribution in vivo [5, 45]. Effective and targeted delivery of these nanoparticles is also hampered by the intricate biological microenvironments in disease sites. One of the key goals for improving the effectiveness of targeted distribution is to get beyond biological barriers, including the extracellular matrix and physiological barriers in particular tissues [134]. A further consideration is the in vivo fate of PLH nanoparticles after drug delivery. Understanding their degradation pathways, clearance mechanisms, and potential accumulation in off-target tissues is essential for ensuring long-term safety and therapeutic efficacy [6]. Through overcoming these challenges, PLH nanoparticles can reach their maximum potential in biomedical applications, facilitating the progress of targeted therapeutics, precision medicine, and diagnostics [45, 135].

7. Conclusion and Future Outlook

The discovery of PLH nanoparticles has shifted attention toward multifunctional therapeutic vehicles capable of precisely targeting malignant cells, tissues, and organs while combining the complementary advantages of polymeric nanoparticles and liposomes. While preclinical studies highlight their promise, clinical acceptance requires bridging critical translational and regulatory gaps. Key challenges, including scalable manufacturing, batch-to-batch reproducibility, long-term storage stability, mitigation of potential immunogenicity, and regulatory bottlenecks, remain major barriers to their clinical application. Advances in synthesis technologies, particularly microfluidics and QbD approaches, are expected to mitigate these issues by enabling reproducible, cost-effective, and industry-compatible production [6]. Successful translation of PLH nanoparticles will also require closer alignment with regulatory frameworks. The U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) emphasize standardized characterization protocols, validated bioanalytical assays, and comprehensive preclinical toxicity evaluations before nanotherapeutic approval. Establishing globally harmonized guidelines for evaluating the pharmacokinetics, biodistribution, and immunogenicity of hybrid nanoparticles is essential to ensure their safety and efficacy in humans.

Looking ahead, PLH nanoparticles could be synergistically integrated with disruptive biomedical technologies such as gene editing, immunotherapy, 3D bioprinting, and personalized medicine platforms. This convergence may yield hybrid systems that not only deliver therapeutic payloads but also provide real-time diagnostic feedback, advancing the field of theranostics. Biomimetic strategies, such as cell membrane coatings and immune-evasive surface modifications, further hold promise for enhancing in vivo compatibility, extending circulation time, and reducing risks of rapid clearance or off-target effects [1].

Future research must also emphasize systematic evaluation of toxicity and biocompatibility, as understanding nanoparticle interactions with biological tissues and the immune system is critical for reducing adverse effects and improving therapeutic outcomes. In parallel, predictive computational modeling and artificial intelligence could accelerate nanoparticle design optimization, streamline regulatory assessment, and reduce translational bottlenecks.

To fully realize the promise of PLH nanoparticles, multidisciplinary collaboration will be indispensable, drawing on expertise from nanotechnology, clinical medicine, regulatory science, and bioethics. Clinical studies must extend beyond proof-of-concept and rigorously evaluate safety, tolerability, and therapeutic benefit in diverse patient populations. By integrating diagnostic, imaging, and therapeutic functionalities into a single platform, PLH nanoparticles can evolve into multipurpose tools for precision medicine, offering tailored therapies across a wide range of diseases. In essence, the future of PLH nanoparticles lies not only in scientific discovery but also in overcoming industrial, translational, and regulatory hurdles. Therefore, with continued innovation and harmonized regulatory guidance, PLH nanoparticles have the potential to redefine precision medicine and establish themselves as a cornerstone of next-generation therapeutics.

Data Availability Statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Nnamdi Ikemefuna Okafor: conceptualization, writing – original draft, writing – review and editing, supervision. **Nkeiruka N. Igbokwe**: writing – original draft, writing – review and editing. **Ngozi Francisca Nnolum-Orji**: writing – original draft, writing – review and editing. **Yahya E. Choonara**: writing – review and editing, supervision, funding acquisition, project administration.

Funding

The authors acknowledge the financial support of the National Research Foundation (NRF) of South Africa under the SARChI Chair program (Grant PPNT230823145247), awarded to Yahya E. Choonara.

Acknowledgments

The authors acknowledge the financial support of the National Research Foundation (NRF) of South Africa under the SARChI Chair program (Grant PPNT230823145247), awarded to Yahya E. Choonara.

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