Conventional and functionalised nanostructured lipid carriers for pulmonary-targeted delivery systems

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ABSTRACT

Pulmonary delivery systems are potential routes for numerous lung-related disease treatments. The pulmonary delivery system can be utilised for local deposition and systemic application due to its large surface area and high vascularisation within the alveolar epithelium. This can lead to high permeability and bioavailability of drugs. This review explores the utilisation of conventional nanostructured lipid carriers in pulmonary delivery system applications. Due to their high entrapment efficiency, stability, and biocompatibility, nanostructured lipid carriers can be used as drug carriers through the pulmonary delivery system. Using nanostructured lipid carriers can enhance drug deposition into deeper lungs, improve bioavailability and efficacy, provide sustained and controlled release profiles of drugs, enhance antimicrobial activity, enhance cellular uptake and penetration, and improve bioavailability. However, conventional nanostructured lipid carriers have a major drawback: low selectivity in target cells. The non-selective properties of these carriers can lead to potential side effects, high toxicity, and reduced effectiveness. Therefore, recent applications of functionalised nanostructured lipid carriers have been evaluated through *in vitro* and *in vivo* studies to prove their safety and effectiveness in pulmonary-targeted delivery. Nanostructured lipid carriers have been functionalised to improve their selectivity and effectiveness. This review discusses various functionalised nanostructured lipid carriers through surface modification and their mechanism, including hydrophilic polymers, polysaccharides, peptides and proteins, small molecules, surfactants, genes, antibodies, and pH-sensitive polymers. Furthermore, key case studies in clinical translation are examined to illustrate the practical applications and progress of these advanced nanocarriers. This review also discusses the potential challenges in development, including pulmonary-specific targeting, toxicity, and immunogenicity concerns, as well as production and scalability challenges. Moreover, developing functionalised nanocarriers presents new opportunities by highlighting effective strategies to address existing challenges and accelerate their progression from experimental research to clinical translation.

Keywords:
Nanostructured lipid carriers; Pulmonary-targeted delivery system; Lipid nanoparticles, Functionalisation; Surface modification; Active targeting

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1. Introduction

The pulmonary delivery system has been studied for treating various lung-related diseases, including asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, tuberculosis, and lung cancer.[1] Pulmonary delivery can be applied for local and systemic action due to the large surface area, sufficient blood supply, and high permeability of the alveolar epithelium. Pulmonary delivery systems can also avoid systemic metabolism and enzymatic degradation. To deliver drugs into the lung, high deposition and accumulation of drugs should be achieved

at deeper or targeted lung sites. However, several biological barriers can limit drug absorption into the lungs, such as mucociliary clearance, pulmonary surfactant, and alveolar macrophage clearance.[2] Overcoming these barriers requires advanced drug delivery strategies to prolong residence time, enhance drug solubility, and facilitate penetration through the lung's defence mechanisms.

Nowadays, various inhalation technologies such as nebulisers, pressurised metered-dose inhalers, and dry powder inhalers are widely used for pulmonary drug delivery.[3] However, these technologies still present several limitations, significantly reducing their therapeutic efficacy and safety. The main challenge is that a large portion of the delivered dose is consistently lost in the upper airways, preventing it from reaching the deeper lungs and reducing treatment effectiveness.[4] In addition, many drugs used for pulmonary delivery fall under the Biopharmaceutical Classification System Class II, characterised by low solubility. For effective treatment, these drugs must be soluble both in the formulation, especially for nebulisers and metered-dose inhalers, and in the lung site before absorption. Poor solubility not only limits drug absorption in the lungs but also leads to non-uniform delivery when administered through inhalation devices.[5]

To overcome the limitations of current pulmonary delivery systems, nanostructured lipid carriers (NLCs) can be utilised as carriers in pulmonary delivery systems.[6] NLCs consist of solid and liquid lipid cores with surfactants in the outer layers, which can mimic phospholipid bilayers.[7] NLCs have numerous advantages in drug delivery, such as high stability, extended release, controlled release, and high biocompatibility.[8] In addition, NLCs have been widely utilised to improve the solubility of various poorly soluble drugs to enhance their bioavailability.[9,10] Moreover, NLCs can escape clearance by mucociliary and alveolar macrophages due to their nanoscale properties. Therefore, NLCs can be internalised and taken up by various lung cells through endocytosis, which can lead to drug release and absorption into the systemic circulation.[11] However, NLCs can also be taken up by nonspecific cell types, leading to low selectivity in target cells. In addition, the decreased drug deposition and short half-life of drugs in targeted cells in the lungs can lead to ineffectiveness and multidrug resistance in various diseases. Therefore, pulmonary-targeted delivery into specific target cells should be designed to reduce systemic side effects and enhance therapeutic efficacy.[12]

Numerous studies have explored functionalisation through surface modification of NLCs to increase targeting effectiveness, improve selectivity, enhance therapeutic efficacy at specific lung sites, and decrease toxicity effects.[13] Surface modification can also be utilised to enhance drug penetration and control drug release.[14] Various functionalised agents, such as hydrophilic polymers, polysaccharides, peptides and proteins, small molecules, surfactants, genes, antibodies, and pH-sensitive polymers, can be integrated into NLCs through surface modification. The functionalised NLCs can attach and adhere to specific target cells, extending the contact time and increasing the drug concentration.[15]

To date, literature discussing the utilisation of conventional and functionalised NLCs for pulmonary delivery remains limited. While reviews have broadly covered NLCs for drug delivery, a systematic classification of functionalisation strategies specifically for pulmonary delivery and a critical comparison of their associated translational challenges are lacking. This comprehensive review provides a critical comparison between conventional and functionalised NLCs for pulmonary delivery, systematically classifies the functionalisation strategies, and integrates a practical, forward-looking framework for process development and clinical translation of both conventional and functionalised NLCs for pulmonary delivery systems. This review will discuss the application of traditional NLCs and the recent advancements in functionalised NLCs, including their *in vitro* and *in vivo* evaluations, to prove their effectiveness in pulmonary-targeted delivery systems. To provide a comprehensive review, this study involved searches on PubMed and ScienceDirect through keywords such as "nanostructure lipid carrier," "functionalised (or functionalized)," and "pulmonary-targeted."

2. Pulmonary-targeting delivery systems

The respiratory system comprises external respiration, the interchange of air between the alveoli and lung capillaries, and internal respiration, which entails the exchange of air between capillaries and tissues. The external respiratory system's anatomy is divided into two primary components: the upper respiratory system, including the nasal cavity and throat, and the lower respiratory system, which includes the larynx, trachea, bronchi, bronchioles, and alveoli.[16] The lungs include alveoli, capillaries, and other respiratory structures, including bronchi and bronchioles. A connective tissue stroma exists between the bronchi and alveoli, including lymphatic vessels, neurons, blood vessels, macrophages, fibroblasts, and diverse immune cells. The airway epithelium constitutes a continuous layer mostly comprised of many epithelial cell types, including ciliated cells, basal cells, goblet cells, club cells, and pulmonary neuroendocrine cells.[17] The lungs are encased in a pleural membrane and contain pleural fluid. The alveoli can be utilised as sites for gas exchange and are in direct contact with pulmonary capillaries. Within the alveoli, macrophages are tasked with eliminating infections by phagocytosis.[18] Alveoli are responsible for secreting surfactants from type II pneumocytes to reduce surface tension and prevent alveolar

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collapse. Moreover, alveoli also have type I pneumocytes that facilitate air exchange. In addition to playing a crucial role in gas exchange, lungs secrete the angiotensin-converting enzyme, which is involved in the renin-angiotensin-aldosterone system, regulating blood volume and pressure.

The lungs contain over 300 million alveoli, comprising type I and type II pneumocytes. These alveoli are connected to an extensive network of capillaries within the interstitial space, which leads to a substantial surface area of approximately 70 m^2. This large surface is a highly effective site for the exchange of gases between the blood and the alveoli. The respiratory membrane connects the alveoli and blood vessel walls and has a thickness ranging from 0.5 to 1.0 μm^2. The thin barrier can be a potential site for drug absorption through the pulmonary route. The respiratory membrane showed excellent permeability, and the lungs receive sufficient blood flow, which can lead to drugs bypassing the initial liver metabolism that often occurs with systemic drug administration.[19] Consequently, pulmonary drug delivery presents an alternative and attractive prospect for systemic action. Furthermore, localised drug delivery directly to the lungs holds promise for enhancing drug accumulation and efficacy in treating pulmonary diseases.[20]

Pulmonary delivery systems have been widely utilised to treat lung-related diseases, such as asthma, COPD, cystic fibrosis, tuberculosis, and acute lung injury.[21] In addition, pulmonary delivery can also be applied in the treatment of several lung cancers, including non-small cell lung cancer (NSCLC) and metastatic lung cancer.[22] Pulmonary delivery systems offer several benefits for delivering drugs into the lungs. The lungs have a large surface alveolar area of 70–100 m^2 with thin respiratory membranes ranging from 0.5–1.0 μm, which can be potential sites for drug absorption through pulmonary administration. The respiratory membrane exhibits good permeability with sufficient blood flow. These properties can prevent first-pass metabolism and enzymatic degradation, often occurring during systemic drug administration. In addition, the pulmonary route can also increase drug accumulation and retention time in deep lungs and, hence, can be utilised to increase drug efficacy in treating pulmonary diseases.[1,2]

The effectiveness of pulmonary delivery can be influenced by several factors, such as the aerodynamic particle size, inhalation device, and formulation. The aerodynamic diameter of a particle is determined by considering a sphere with a density of water that settles in an air stream at the same speed as the given particle. An optimum aerodynamic particle size ranging from 1 to 5 μm should be considered to deliver drugs into deeper lung sites and avoid clearance by the mucociliary system.[23,24] Furthermore, several types of inhalation devices can be utilised in pulmonary delivery systems, including medical nebulisers, metered-dose inhalers, soft-mist inhalers, aqueous droplet inhalers, and dry powder inhalers.[3,25] However, conventional inhaled devices are designed for rapid drug dissolution and absorption. On deposition, the drug particles dissolve in the lung fluids and are quickly absorbed into the highly vascularised pulmonary circulation, leading to a rapid peak in systemic plasma concentration followed by a relatively fast clearance. Therefore, these systems often result in a short duration of action, requiring frequent dosing to maintain therapeutic levels. This not only reduces patient compliance but also increases the risk of systemic side effects.

Therefore, numerous formulation strategies have been explored to enhance pulmonary drug delivery. Lipid-based nanoparticles have gained significant attention due to their biocompatibility, ability to encapsulate hydrophilic and lipophilic drugs, and potential to improve drug solubility, stability, and controlled release. Lipid-based nanoparticles have been utilised in various pulmonary drug delivery systems, including liposomes,[26,27] nanoemulsions,[28,29] lipid–polymer hybrid nanoparticles,[30,31] solid lipid nanoparticles,[32,33] and NLCs.[34] Among these, NLCs represent an advanced generation of lipid nanoparticles specifically designed to overcome the limitations of solid lipid nanoparticles, such as limited drug loading and drug expulsion during storage. By incorporating a mixture of solid and liquid lipids, NLCs form an imperfect lipid matrix that improves drug entrapment efficiency, particularly for poorly water-soluble drugs. Due to their hydrophobic core, NLCs can effectively encapsulate lipophilic drugs, creating a drug reservoir at the site of pulmonary deposition. Moreover, the lipid matrix can also make a diffusion barrier that significantly slows the release of the encapsulated drug, which can lead to a sustained-release profile and prolonged local residence time.[35,36]

3. Nanostructured lipid carriers for pulmonary-targeting delivery systems

3.1. Nanostructured lipid carriers

Nanostructured lipid carriers constitute a second generation of solid lipid nanoparticles with nanosized and high surface areas. NLCs consist of solid lipids and liquid lipids on the inner side and surfactants with cosurfactants on the outer side.[37] Adding liquid lipids can lead to an imperfection in the core structure of NLCs. This structure has several advantages to overcome the drawbacks of solid lipid nanoparticles, such as high loading efficiency, negligible drug expulsion, and high stability.[36] NLCs have a smaller particle size on the nanometres scale and a high surface area-to-volume ratio, which can lead to high solubility and stability of drugs. Due to their similar hydrophobicity, poorly water-soluble drugs can dissolve well in a lipid phase, consisting of solid and liquid lipids. The hydrophobic drugs are entrapped in a lipid matrix and surrounded by a surfactant. Using surfactants in NLC formulation can decrease the interfacial tension between hydrophobic drugs, lipids, and water, improving the solubility of drugs. In addition, cosurfactants can increase the stability of NLCs and help surfactants reduce interfacial tension. Hydrophobic drug-loaded NLCs can be loaded into the core of the matrix system to improve stability.[35] Moreover, NLCs can also control the release of drugs by diffusion mechanism across lipid barriers, after which NLCs can be internalised through endocytosis and transcellular and paracellular pathways.[38,39]

3.2. Application of nanostructured lipid carriers in pulmonary delivery systems

Nanostructured lipid carriers have been utilised as a lung-targeted delivery system using both passive and active

mechanisms with unmodified NLCs and functionalised NLCs, respectively. The mechanism of NLCs can involve active and passive targeted delivery systems. In passively targeted delivery systems, conventional or unmodified NLCs, as nanocarriers with small particle sizes ranging from 10–100 nm, can enhance permeability and retention at lung-targeted sites, which is called the enhanced permeability and retention (EPR) effect.[40] In addition, NLCs can also prevent mucociliary and macrophage clearance, which can lead to improved cellular uptake and internalisation. Pulmonary administration through inhaler technology can directly target NLCs in lung cells. In a cancer cell environment, there is leaky vascularisation and rapidly increasing angiogenesis, so NLCs can increase permeability to blood vessels, increase extravasation, and escalate retention in tumour cells.[41] The passive targeting mechanism of unmodified NLCs is shown in **Figure 1**.

Furthermore, NLCs can be easily integrated with ligands through surface modification to actively target cells, as shown in **Figure 2**. Functionalised NLCs with specific ligands can increase the affinity of NLCs for the receptor of target cells; hence, the selectivity, permeability, and retention of NLCs can be improved.[42] These surface modification properties can enhance the cellular uptake and internalisation of NLCs with low immunogenicity effects. Therefore, the bioavailability and stability of drugs encapsulated in NLCs can be improved. Specific targeting of the disease site can reduce toxicity to normal cells, which can prevent systemic side effects and improve patient compliance.[43] NLCs have been widely utilised to deliver and target drugs into the lungs and specific sites to treat various lung-related diseases. The current application of NLCs through a pulmonary delivery system is tabulated in **Table 1**.

3.2.1. High particle deposition in the lungs

The important parameter in pulmonary-targeted delivery is high deposition in the deeper lungs. Mostly, the desired parameter is determined by aerodynamic particle size, ranging from 1–5 μm, to obtain high deposition in the lateral stage, with low particle deposition in the mouth-throat region.[72] The particle size more than 5 μm can be deposited into the mouth-throat or upper respiratory tract, which can be eliminated through the mucociliary clearance pathway, whereas particles less than 1 μm can be exhaled back through the expiratory system.[73] The NLCs can be utilised to mitigate losses to mouth-throat deposition and achieve improved lung deposition.[74]

Beclomethasone dipropionate-loaded NLCs, which have a smaller particle size, lower polydispersity index, higher entrapment efficiency, and enhanced stability during 6-week stability studies, can be utilised as potential carriers for asthma and COPD. In addition, superior aerosolisation performance was achieved through an air jet nebuliser with Next-Generation Impactor at 60 and 15 L/min airflow rates. Notably, the air jet nebuliser achieved the lowest beclomethasone deposition in the initial stages, and the highest concentration of beclomethasone was deposited in the lateral stages of the Next Generation Impactor. Therefore, the combination of beclomethasone dipropionate-loaded NLCs and an air jet nebuliser can be an effective delivery system to the lower regions of the lungs.[46] In another study, ciprofloxacin-loaded NLCs had an optimum mass median aerodynamic diameter (MMAD) of 3.9–5.1 μm, which indicated that the formulation could be deposited into deeper lungs with a high fine particle deposition of 49.2%.[50] A lower MMAD (<5 μm) could lead to a greater fraction deposited into deeper lungs. Montelukast-loaded NLCs dry powder inhalers with MMADs of 2.83–3.24 μm could achieve more than 90% fraction deposition into deeper lungs at an airflow rate of 60 L/min.[58] Similarly, rosuvastatin-loaded NLCs have an MMAD value of 2.56–2.98 μm with a high fine particle fraction of 87.65–91.25%.[65] In addition, the administration of itraconazole-loaded NLCs for pulmonary aspergillosis remained stable during nebulisation through air jets and ultrasonic nebulisers, with a high entrapment efficiency of more than 97% and no significant difference in entrapment efficiency before and after inhalation.[56]

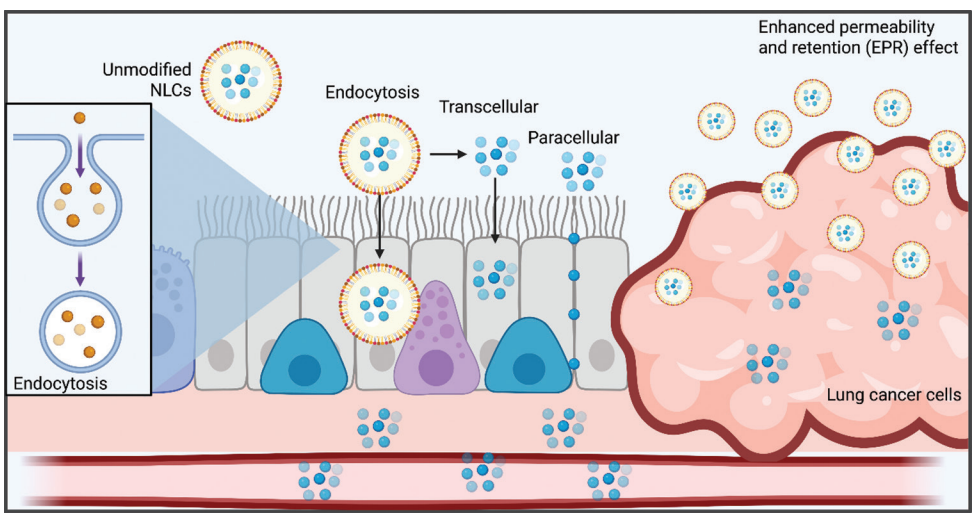

Figure 1. The cellular uptake mechanism of conventional nanostructured lipid carriers through endocytosis, transcellular, paracellular, and EPR effect. Created in BioRender. Gunawan, M. (2025) https://BioRender.com/59m8zg9.
Abbreviations: EPR: Enhanced permeability and retention; NLCs: Nanostructured lipid carriers.

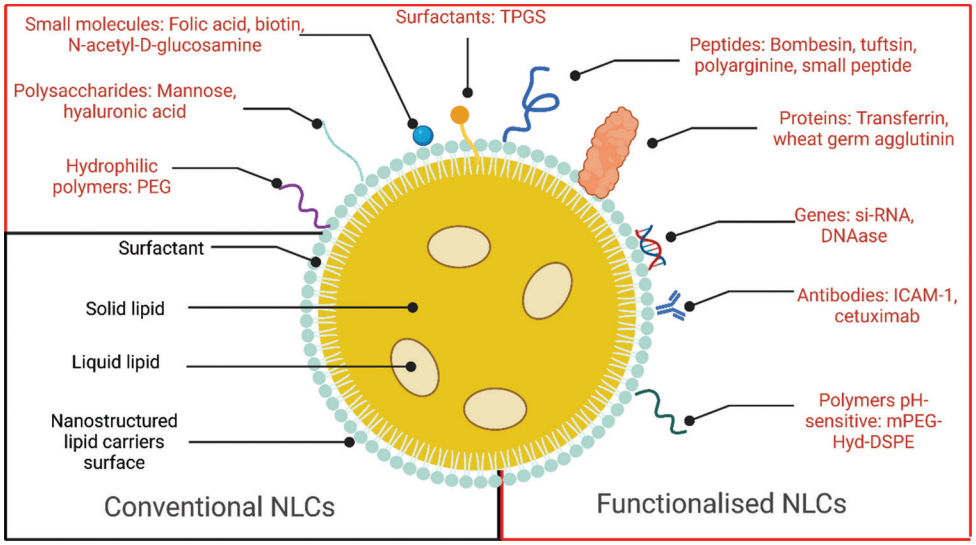

Figure 2. Schematic diagram of conventional and functionalised nanostructured lipid carriers with various modalities, including hydrophilic polymers, polysaccharides, small molecules, surfactants, peptides, proteins, genes, antibodies, and pH-sensitive polymers. Created in BioRender. Gunawan, M. (2025) https://BioRender.com/q34x42y.
Abbreviations: DNase: Deoxyribonuclease; ICAM-1: Intercellular adhesion molecule-1; mPEG-Hyd-DSPE: Methoxy (polyethylene glycol) 2000-hydrazone- 1,2-stearoyl-sn-glycerol-3-phosphoethanolamine; NLCs: Nanostructured lipid carriers; PEG: Polyethylene glycol; siRNA: Small interfering RNA; TPGS: D-α-tocopheryl polyethylene glycol succinate.

3.2.2. Sustained and controlled release by in vitro drug release kinetics

Nanostructured lipid carriers can be effectively utilised in pulmonary drug delivery systems to control and sustain the release of drugs. By encapsulating drugs within their lipid matrix, NLCs enhance and control the drug release profile over an extended period.[75] This lipid matrix slows drug diffusion and protects the drug from rapid clearance and degradation, allowing for prolonged therapeutic effects and reduced dosing frequency. The controlled release mechanism aligns with the Noyes-Whitney equation, where the drug is encapsulated into the NLCs matrix system. This encapsulation can increase the diffusion layer and reduce direct exposure to the dissolution medium, resulting in a slower and more controlled release in the lungs. Therefore, NLCs are highly effective for pulmonary drug delivery due to their ability to encapsulate drugs within a lipid matrix that controls and sustains drug release over time. This sustained release helps maintain therapeutic drug concentrations in the lungs, reduces dosing frequency, minimises systemic side effects, and improves patient compliance.

The drug release kinetics for beclomethasone-loaded NLCs followed the Higuchi model, which strongly indicated that drug release was influenced by diffusion-controlled release from the NLCs matrix.[47] In addition, the release of montelukast from the NLCs matrix was fitted with the Higuchi model with a diffusion-controlled sustained release profile.[58] Similarly, an *in vitro* release study of celecoxib-loaded NLCs revealed that the release of celecoxib was controlled and sustained for up to 72 h compared with that of the celecoxib solution. This could be caused by the thickness of the diffusion lipid matrix in the core of the NLCs.[48] Furthermore, 34.22% of the ciprofloxacin-loaded NLCs were released in the first 2 h, followed by a sustained release pattern for 10 h. The initial release could be caused by the presence of the drug on the surface of the NLCs, and the diffusion mechanism could control the release of the drug from the inner side of the NLCs matrix.[50] A similar burst release pattern was also experienced by gefitinib-loaded NLCs, with 20% rapid release in 2 h, followed by sustained release up to 50% in 24 h. This pattern might be caused by the disruption of solid lipids by the addition of liquid lipids to the NLCs matrix system, which could lead to changes in the crystallinity arrangement; hence, sustained release from the lipid core was expected.[55] Paclitaxel- and doxorubicin-loaded NLCs exhibited prolonged drug release for 48 h through several mechanisms, including surface erosion, disintegration, diffusion-controlled, and desorption. A higher lipid concentration could increase the sustained release profile due to the extended lipid matrix barriers that occur in the diffusion mechanism.[61] Surprisingly, the release of paclitaxel and doxorubicin-loaded NLCs from dry powder inhalers could be extended for 20 days, with 65% of the drug released.[62]

3.2.3. Enhanced antimicrobial activity

Nanostructured lipid carriers have been utilised for the pulmonary delivery system of numerous drugs, with both local and systemic action. NLCs can be utilised as a carrier in the treatment of localised lung diseases, including bacterial pneumonia, tuberculosis, and fungal infections, by enhancing antimicrobial efficacy.[76,77] NLCs can improve the solubility and stability of drugs and facilitate controlled release, specifically at the site of infection within the lungs. Their nanoscale size enables deep lung penetration and efficient uptake by alveolar macrophages, targeting intracellular pathogens more effectively. In addition, NLCs can navigate the mucus barrier and prolong drug retention, ensuring sustained antimicrobial action at the infection site while reducing systemic toxicity.

Ciprofloxacin-loaded NLCs could enhance antimicrobial activity against Gram-positive and Gram-negative bacteria,

Table 1. Conventional nanostructured lipid carriers for pulmonary-targeted delivery systems

Drug	Composition	Disease	Dosage forms	*In vitro* results	*In vivo* results	References
9-Bromo-noscapine	Stearic acid, egg phosphatidylcholine, sodium glycocholate, sodium carbonate, citric acid, and lactose	NSCLC cells	Dry powder inhalers	Enhanced cytotoxicity with half-maximal inhibitory concentration (IC_{50}) of 5.1 μM and cellular uptake (85.49%) in A549 cells	Improved drug deposition in lung cancer	44
Aloperine	Stearic acid, medium chain triglyceride, polyoxyethylene castor oil, sodium deoxycholate, and soya phosphatidylcholine	Pulmonary arterial hypertension	Solutions for medical nebulisers	Demonstrated good sustained-release characteristics *in vitro*, with a cumulative release of 90.98% at 48 h	Significantly increased lung accumulation of aloperine, with lung concentrations 1.79-, 3.78-, and 2.30-fold higher than the solution group at 0.25, 1.5, and 4 h, respectively, while prolonging circulation time and improving bioavailability	45
Beclomethasone dipropionate	Glycerol tri laurate, propylene glycol caprylate/caprate, Tween 80, sodium taurocholate hydrate, and soya phosphatidylcholine	Asthma	Solutions for medical nebulisers	High particle deposition (>80%) in lateral stages, high respirable fraction, and optimum aerodynamic diameter	-	46
Beclomethasone dipropionate	Stearic acid, Miglyol® 812, Tween 80, Poloxamer® 188	Asthma	Solutions for medical nebulisers	High entrapment efficiency (97–99%), sustained release properties, high fine particle fraction (66–82%), and optimum median mass aerodynamic diameter (2.98–3.81 μm)	-	47
Celecoxib	Compritol®, Miglyol®, and sodium taurocholate	NSCLC	Solutions for medical nebulisers	IC_{50} of 27.36 μg/mL against A549 cells	Fourfold higher bioavailability compared to solution	48
Celecoxib and docetaxel	Compritol®, Miglyol®, and sodium taurocholate	NSCLC	Solutions for medical nebulisers	Moderate synergistic properties (Combination index=0.63)	Tumour volume decreased by 67% compared to the control	49
Ciprofloxacin	Stearic acid, oleic acid, and Tween 80	Non-cystic fibrosis bronchiectasis	Dry powder inhalers	Improved bacterial growth inhibition compared to the solution	-	50
Colistin sulphate	Precirol® ATO 5, Miglyol® 812, Tween 80, and Poloxamer® 188	Cystic fibrosis	Dry powder inhalers	High antimicrobial activity (3% survival), enhanced biofilm eradication	-	51
Copper (II)-isoniazid complexes	Polyoxyethylene 40 stearate, caprylic/capric triglyceride, polyoxyl 40 hydrogenated castor oil, poloxamer 407, and cetyltrimethylammonium bromide	Tuberculosis	Oral	Twenty-sevenfold improved antimicrobial activity, no toxicity on Vero cells	Increased mouse survival by 67% at 1,000 mg/kg	52
Curcumin	Triglycerides, phosphatidylcholine, and Solutol®	Acute lung injury	Intravenous	Improved caveolar-dependent cellular uptake and penetration	-	53
Docetaxel and curcumin	Precirol ATO5®, Dynasan 114®, Labrafac lipophile WL1349®, Phospholipon 90G®, PEG 4000 monostearate, stearylamine, Solutol HS15®, and sucrose	NSCLC	Lyophilized powder	Controlled release properties with greater cytotoxicity against NCI-H460 cells than free docetaxel or free docetaxel and curcumin combination, indicating strong synergistic effects	-	54

(Cont'd...)

Table 1. *(Continued)*

Drug	Composition	Disease	Dosage forms	*In vitro* results	*In vivo* results	References
Gefitinib	Stearic acid, oleic acid, and Pluronic®-F68	Metastatic lung cancer	Oral	Enhanced cancer cell uptake, concentration-dependent cytotoxicity. IC_{50} against A549 cells was 15.05 μg/mL at 24 h, compared to the solution (11.16 μg/mL)	-	55
Itraconazole	Precirol® ATO 5, oleic acid, Eumulgin® SLM 20, and glycerol	Lung fungal infection	Solutions for medical nebulisers	High entrapment efficiency remained stable during nebulisation	-	56
Itraconazole	Precirol® ATO 5, oleic acid, Polysorbate 20, and glycerol	Pulmonary aspergillosis	Solutions for medical nebulisers	No toxic effect (97.4% viability) against A549 cells	High deposition in deeper lungs (gamma scintigraphy on falcons)	57
Montelukast	Precirol® ATO 5, Capryol®-90, and DL-Pyrrolidonecarboxylic acid salt of L-cocyl arginine ethyl ester	Chronic asthma	Dry powder inhalers	No toxicity found (96.89% A549 cell viability). Higher IC_{50} against A549 cells of 107.61 and 70.85 μg/mL at 24 h for NLCs and solution, respectively	Lung targeting and deposition improved 11.76-fold over the solution	58
Oleuropein	Precirol® ATO 5, olive oils, Tween 80, and Poloxamer® 188	Antioxidant for lung epithelial cells	Dry powder inhalers	Significantly higher antioxidant efficiency than pure drug for 61.22% and 55.33%, respectively. No toxicity against A549 cell lines	-	59
Paclitaxel	Stearic acid, glyceryl monostearate, oleic acid, and Tween 20	Lung cancer	Dry powder inhalers	High cellular uptake in Caco-2 cell lines	High deposition and cellular uptake in rat lungs	60
Paclitaxel and doxorubicin	Compritol® 888 ATO, oleic acid, soybean phosphatidylcholine, and N-[1-(2,3-dioleyloxy) propyl]-N, N, N-trimethyl-ammonium chloride	Non-small cell lung carcinoma	Intravenous	Ninefold greater cytotoxicity against NCI-H460 cells	Achieved 84% tumour inhibition rates compared to free drugs, with only 26% inhibition	61
	Soya lecithin, Oleic acid, Tween 80, Tween 20, and Cremophor® EL	Lung cancer	Dry powder inhalers	Significantly enhanced cellular uptake compared to solution	High drug accumulation in the lungs, no histopathological changes	62
Pimozide	Stearic acid, Oleic acid, Poloxamer 407, PEG 400, and HPMC-E5	Lung cancer	Dry powder inhalers	The optimised pimozide-loaded NLCs demonstrated the most potent anti-proliferative effect against A549 lung cancer cells with an IC_{50} of 7.52±1.20 μM, significantly lower than free pimozide at 13.27±0.41 μM	-	63
Pirfenidone	Stearic acid, Oleic acid, and Tween 20	Idiopathic pulmonary fibrosis	Inhalation	Demonstrated significantly higher cytotoxicity against HepG2 and Caco-2 cells, more than 95% sustained drug release, and maintained stability post-freeze-drying	NLCs achieved 15.94-fold higher lung retention, 1.44-fold higher maximum concentration, 2.98-fold longer half-life, and 4.21-fold higher AUC than aqueous solution, confirming improved bioavailability and prolonged lung exposure in rats	64
Rosuvastatin	Lauric acid, Capryol®-90, and Cremophor® RH40	Chronic obstructive pulmonary disease	Dry powder inhalers	High entrapment efficiency (more than 95%) with appropriate median mass aerodynamic diameter (2.56–3.14 μm), and high fine particle fraction (81.34–91.25%)	High drug deposition and targeted to the lungs, with a 35.38-fold increase compared to the solution	65

(Cont'd...)

Table 1. *(Continued)*

Drug	Composition	Disease	Dosage forms	*In vitro* results	*In vivo* results	References
Sodium colistimethate	Precirol® ATO 5, Miglyol® 812, polysorbate 80, and Poloxamer® 188	Cystic fibrosis	Dry powder inhalers	NLCs can enhance antimicrobial activity with a minimum inhibitory concentration of 1 μg/mL compared to solid lipid nanoparticles of 2 μg/mL. There was less toxicity of NLCs of 160-fold and 28-fold against H441 cells and A549 cells, respectively, compared to the free drug	-	66
	Precirol® ATO 5, Miglyol® 812, Tween 80, and Poloxamer® 188	Cystic fibrosis	Dry powder inhalers	Good stability with a minimum inhibition concentration of<16 μg/mL for 12 months of stability study	-	67
	Precirol® ATO 5, Miglyol® 812, Tween 80, and Poloxamer® 188	Cystic fibrosis	Dry powder inhalers	More effective antimicrobial activity with a minimum inhibitory concentration (MIC) of 0.125 μg/mL compared to the free drug of 2 μg/mL	Higher efficacy of antimicrobial activity with a 140-μg dose equivalent to 3,400 μg using the intramuscular route in Balb/c female mice, with high deposition into the lungs	68
Tobramycin	Precirol® ATO 5, Compritol® 888 ATO, Miglyol® 812, Tween 80, and Poloxamer® 188	Cystic fibrosis	Dry powder inhalers	Cell viability is more than 80% against the A549 cell lines, 73% penetration through artificial mucus compared to 30% free drug	High deposition and concentration in the lungs of BALB/c OlaHsd female mice	69
Trans-resveratrol	Capryol 90, Dynasan 116, Tween 80, and SPC-75	Lung cancer	Solutions for medical nebulisers	The air jet nebuliser combined with the NLCs formulation demonstrated a significantly high emitted dose of 87.44±3.36% and fine particle fraction of 36.25±4.26%	-	70
	Miglyol 810, Dynasan 116, Tween 80, and SPC-75	Lung cancer	Solutions for medical nebulisers	NLCs exhibited superior aerosolisation through an air-jet nebuliser with an emitted dose of 80% and a fine particle fraction of 24%	-	71

Abbreviations: A549: Human lung carcinoma cell line; BALB/c: Inbred strain of laboratory mouse; Caco-2: Human epithelial colorectal adenocarcinoma cell line; H441: Human lung adenocarcinoma cell line; IC_{50}: Half maximal inhibitory concentration; MIC: Minimum inhibitory concentration; NCI-H460: Human non-small-cell lung cancer cell line; NLCs: Nanostructured lipid carriers.

such as *Staphylococcus aureus* and *Bacillus* spp., in non-cystic fibrosis bronchiectasis. Ciprofloxacin-loaded NLCs could significantly inhibit bacterial growth for longer than free drugs. This may be attributed to the presence of lipids in NLCs and their smaller particle size, which could enhance the cellular uptake of NLCs into the bacterial membrane.[50] In addition, colistin-loaded NLCs also showed high antimicrobial activity against *Pseudomonas aeruginosa* in cystic fibrosis, with only 3% resistant clinical strains, and enhanced biofilm eradication efficiency.[51] Furthermore, NLCs could enhance antimicrobial activity against *P. aeruginosa,* with a minimum inhibition concentration (MIC) of 1 μg/mL, compared with 2 μg/mL of solid lipid nanoparticles.[66] Moreover, sodium colistimethate-loaded NLCs have good stability, with a MIC of <16 μg/mL against *P. aeruginosa* for up to 12 months of stability, compared with solid lipid nanoparticles, which could maintain antimicrobial activity for only 3 months.[67] In another study, incorporating copper (II) complexes into NLCs decreased the MIC against *Mycobacterium tuberculosis* by 27.3–55.4 times compared to free complexes. This result suggested that encapsulating copper (II) complexes into NLCs could significantly increase antimicrobial activity due to the interaction of the positive charge of the NLCs' surface and the anionic cell membranes of *M. tuberculosis.* This interaction could lead to longer retention times and enhance cellular drug penetration into the bacterial membrane.[52]

3.2.4. Escalated in vitro cytotoxicity

Nanostructured lipid carriers have emerged as promising nanocarriers for pulmonary drug delivery due to their biocompatibility and ability to enhance drug bioavailability in the lung tissues. Numerous *in vitro* studies have demonstrated that NLCs can escalate cytotoxic effects on target pulmonary cells by improving drug cellular uptake and facilitating sustained release of therapeutic agents directly to the lung epithelium. The biocompatible lipid composition of NLCs may facilitate fusion with cellular membranes, further enhancing the cytotoxic potential against diseased lung cells.

An *in vitro* cytotoxicity study of celecoxib-loaded NLCs revealed a correlation between *in vitro* release and half-maximal inhibitory concentration (IC_{50}) values. The higher concentration and longer exposure time of celecoxib could decrease the IC_{50} values, which strongly indicates that celecoxib could be controlled for an extended period and has enhanced effectiveness for pulmonary administration.[48] Another study revealed a similar correlation between longer exposure times and lower IC_{50} values of celecoxib-loaded NLCs, which were 252.02, 102.31, and 27.36 μg/mL at 24, 48, and 72 h, respectively.[49] Itraconazole-loaded NLCs exhibited no toxic effect, with 97.4% viability against A549 cells.[57] Sodium colistimethate-loaded NLCs were 160-fold and 28-fold less toxic than free drugs in H441 cells and A549 cells, respectively.[66] This result suggested that NLCs could reduce systemic toxicity through their controlled release properties. Moreover, the cytotoxicity assay of paclitaxel and doxorubicin-loaded NLCs was ninefold greater than that of free drug solutions.[61] This finding implies that NLCs could decrease systemic toxicity. In addition, a cytotoxicity assay with 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide against the A549 cell line of montelukast-loaded NLCs revealed that NLCs have significantly lower toxicity than pure drugs. This may be related to the sustained release of montelukast from the NLCs matrix.[58] In another study, oleuropein-loaded NLCs were shown to have no toxic effect at concentrations up to 462 μM and significantly greater antioxidant activity against A549 cell lines than pure drugs due to greater cellular uptake and cell permeability.[59] However, the *in vitro* cytotoxicity study in A549 cells revealed that gefitinib-loaded NLCs had a higher IC_{50} value than pure drugs at 24 h, and there were no significant differences in the IC_{50} values after 48 and 72 h. This could be caused by the sustained release properties of NLCs, which could prevent initial release and enhance release in targeted cells with reduced systemic toxicity.[55]

3.2.5. Enhanced in vitro cellular uptake and penetration

Nanostructured lipid carriers can improve cellular uptake and internalisation into cells due to their relatively small particle size, which facilitates easier interaction with cell membranes and promotes endocytosis.[78] Their small size allows NLCs to navigate biological barriers efficiently, especially within the pulmonary environment, where tight epithelial junctions and mucus layers can hinder drug delivery. The enhanced internalisation of NLCs by pulmonary cells leads to higher intracellular drug concentrations directly at the target site, improving the efficacy and effectiveness of drug delivery into the lungs.

Compared with the conventional emulsion, the uptake of curcumin-loaded NLCs after 2 h was significantly greater. In addition, NLCs can be internalised into the cell through caveolae uptake through endocytosis. This can be proven by the lower cellular uptake of curcumin-loaded NLCs in caveolae-devoid mice than in normal cells.[53] Paclitaxel- and doxorubicin-loaded NLCs exhibited the ability to enhance cellular uptake by A549 cells compared to free drugs. This could be caused by the high surface area and wettability, leading to membrane integrity loss and improved permeability.[62] Furthermore, the artificial mucus penetration study revealed that 73% of the NLCs loaded with tobramycin penetrated, whereas only 30% of the free drug permeated. This strongly indicates that NLCs could enhance the penetration of drugs.[69]

3.2.6. Improved in vivo efficacy

Besides *in vitro* studies, NLCs can significantly improve the efficacy of *in vivo* lung delivery by enhancing drug bioavailability and retention within the pulmonary delivery system. Their unique lipid matrix enables high drug loading and controlled release, leading to sustained therapeutic concentrations directly at the lung tissue. Numerous *in vivo* studies demonstrate that NLCs effectively penetrate lung epithelial cells and alveolar macrophages, ensuring efficient intracellular delivery and drug protection from enzymatic degradation. These advantages translate into superior therapeutic outcomes in animal models of lung diseases, confirming that NLCs provide enhanced clinical benefits beyond *in vitro* observations.

Compared to the celecoxib solution, the celecoxib-loaded NLCs could increase the retention time in the lungs of mice for up to 12 h, resulting in a slower elimination rate.[48] In another study, montelukast-loaded NLCs increased drug deposition in the bronchoalveolar lavage fluid of male Wistar rats by twofold for an hour. Another study also revealed that paclitaxel-loaded NLC dry powder inhalers have greater lung uptake than free drug suspensions. This could be caused by the longer retention time of NLCs at the targeted site, prolonging release and slowing drug clearance from the organ.[60] In addition, paclitaxel and doxorubicin-loaded NLC dry powder inhalers could maintain drug accumulation at the lung site. This could be influenced by the aerodynamic particle size, leading to the specific localisation of particles and the sustained release properties of NLCs.[62]

Furthermore, high deposition of technetium-99 m-hexamethyl propylene amine oxime-labelled NLCs (^{99m}Tc-HMPAO-NLCs) in the deeper lungs was detected through gamma scintigraphy on falcons as an animal model.[57] IR-783 dye-labelled NLCs were utilised to examine the biodistribution of NLCs in the mice after inhalation. The *in vivo* biodistribution revealed that NLCs could accumulate and be deposited into the lungs for 48 h.[66] Another study revealed high distribution in the lungs after inhalation of sodium colistimethate-loaded NLCs, which could be maintained for up to 48 h after administration.[68] Similarly, high deposition and concentrations of tobramycin were detected in the lungs of female BALB/c OlaHsd mice.[69] This may be attributed to the prolonged release of NLCs, which could lead to high accumulation in the lungs and reduced systemic toxicity.

Montelukast-loaded NLCs could also enhance the targeting factor by increasing the area under the curve $(AUC)_{0-\infty}$ to 11.76 times greater than the montelukast solution. This might be caused by the smaller particle sizes that could escape through macrophage clearance and the sustained release properties of montelukast-loaded NLCs.[58] Similarly, rosuvastatin-loaded NLCs presented a 35-fold greater $AUC_{0-\infty}$ and a 1.14-fold greater C_{max} than the pure drugs. Moreover, the t_{max} of rosuvastatin-loaded NLCs was sixfold greater than that of the pure solution, which indicates the sustained release properties of NLCs.[65] The greater bioavailability of rosuvastatin-loaded NLCs is due to the improvement in residence time and the ability to escape from mucociliary and macrophage clearance.

Compared to the control, the combination of celecoxib-loaded NLCs and docetaxel significantly inhibited the growth rate of A549 metastatic tumours in mice, with a 67% decrease in tumour volume.[49] In another study, paclitaxel- and doxorubicin-loaded NLCs presented the highest tumour inhibition rate of 84%, which was 3.23-fold greater than free drugs. This finding suggested that the combination of paclitaxel and doxorubicin encapsulated in the NLC matrix has a synergistic effect and reduces systemic toxicity in the treatment of lung cancer.[61]

3.2.7. Reduced in vivo toxicity

Nanostructured lipid carriers show excellent biocompatibility with minimal inflammatory responses, which can reduce the toxicity effect. This is primarily due to their composition of physiological lipids, which are well tolerated by biological systems, minimizing irritation or cytotoxicity on administration.[79] This biocompatible profile is crucial for pulmonary delivery, as it lowers the potential for adverse effects commonly associated with other drug delivery systems that may provoke lung inflammation or oxidative stress.

An acute toxicity study of copper (II) complex-loaded NLCs was conducted in mice over 14 days. The results revealed that 100% of the mice were lost when administered with free copper (II) complex type 1 at 300 mg/kg, 500 mg/kg, or 1,000 mg/kg. On the other hand, incorporating copper (II) complex type 1-loaded NLCs at a dose of 1,000 mg/kg increased the survival rate by 67%. Furthermore, copper (II) complex-loaded NLCs did not significantly increase alanine aminotransferase or aspartate aminotransferase levels in mice, which strongly indicates that the formulation did not induce hepatotoxicity.[52]

Compared to the free drug, sodium colistimethate-loaded NLCs at a lower CFU/g lung against *P. aeruginosa* could be achieved with a lower dose in BALB/c female mice. For example, a dose of encapsulated NLCs equivalent to 70 or 140 μg of sodium colistimethate could be similar to 648 μg or 3,400 μg of free sodium colistimethate, respectively. This indicated that a lower dose was required to achieve similar antimicrobial activity against *P. aeruginosa,* which could reduce toxicity. Histopathological analysis confirmed no significant toxicological effects in the lungs, spleen, liver, or kidney on day 5.[68] Another study revealed no histopathological changes in tissue damage or inflammatory reactions in Wistar rats after administering paclitaxel- and doxorubicin-loaded NLCs dry powder inhalers.[62]

3.3. Efficacy comparisons with traditional inhalation formulations

Nanostructured lipid carriers demonstrate transformative potential in pulmonary drug delivery by consistently outperforming conventional inhalation formulations in deposition efficiency, sustained release, and therapeutic outcomes across diverse clinical settings. NLCs can enhance drug bioavailability, reduce systemic side effects, and improve therapeutic precision. Preclinical studies consistently demonstrate superior outcomes, including higher tumour inhibition and antimicrobial efficacy. Therefore, NLCs can address key limitations of conventional inhalation therapies, including poor drug solubility, short pulmonary residence time, suboptimal therapeutic efficacy, systemic side effects, and lack of cellular targeting.

For instance, beclomethasone dipropionate-loaded NLCs have demonstrated excellent aerodynamic properties, with studies reporting MMAD values between 1.15 and 1.62 μm and respirable fractions ranging from 54.36% to 69.56%,[47] compared to conventional extra fine-particle hydrofluoroalkane-pressurised metered-dose inhalers, which have an MMAD of approximately 1.2 μm with a fine particle fraction of 24.1–28.7%.[80] Beclomethasone dipropionate-loaded NLCs not only match deep-lung deposition efficiency but also introduce a sustained-release profile governed by Higuchi kinetics, offering the potential for extended dosing intervals and

reduced systemic exposure. Moreover, ciprofloxacin-loaded NLCs demonstrate superior pulmonary delivery performance compared to ciprofloxacin spray-dried powders for non-cystic fibrosis bronchiectasis treatment. The NLC formulation achieved a fine particle fraction of 49.2% and a MMAD in the ideal range of 3.9–5.1 μm, facilitating effective deep lung deposition.[50] In contrast, the spray-dried ciprofloxacin showed a lower fine particle fraction of 23.8% with an MMAD of 7.62 μm.[81] In another study, ciprofloxacin dry powder inhalers also exhibited a fine particle fraction of 31.68 ± 1.4% and a MMAD of 7.23 ± 0.01 μm, indicating suboptimal pulmonary deposition characteristics.[82] Furthermore, the co-delivery of paclitaxel and doxorubicin through NLCs resulted in the highest lung tumour inhibition rate of 84% in the human NSCLC xenograft model using NCL-H460 cells in BALB/c nude mice. In contrast, the free drugs were significantly less effective, with free paclitaxel and free doxorubicin achieving tumour inhibition rates of only about 26%.[61] In addition, a comparison of the rifampicin dry powder inhalers and the mannosylated NLCs highlights a difference between a conventional deposition-focused strategy and an advanced cellular-targeting approach. The dry powder inhaler formulation exhibited a MMAD of 4.3–5.8 μm and achieved a low fine particle fraction of 28.9%. However, the dry powder inhalers showed a 1.5-fold higher maximum drug concentration in rat lungs compared to the oral formulation.[83] In contrast, the mannosylated NLCs are engineered for active targeting, which was demonstrated *in vitro* by a 14.5-fold increase in uptake by macrophages compared to non-mannosylated NLCs, leading to a more pronounced antimycobacterial effect at the cellular level. Thus, while the dry powder inhalers enhance overall drug load in the lungs, the mannosylated NLCs were designed for superior precision in delivering the drug directly into infected cells.[84]

Despite these demonstrated advantages over traditional formulations, conventional NLCs suffer from a fundamental limitation of low cellular selectivity. This drawback reduces their effectiveness in delivering drugs specifically to target cells. Therefore, advanced targeting strategies are essential to overcome this challenge, as discussed in Section 4.

4. Functionalised nanostructured lipid carriers for pulmonary-targeting delivery systems

Although NLCs have numerous advantages in delivering drugs into the lungs, they have low selectivity for target cells, which could lead to off-target drug delivery and decreased safety and efficacy of drugs. To target NLCs in specific cells in the lungs, several types of NLCs integrated through surface modification could be applied, including hydrophilic polymers, polysaccharides, peptides and proteins, small molecules, surfactants, genes, antibodies, and pH-sensitive polymers (**Figure 2**). Each surface modifier has distinct characteristics to target specific cells in the lungs. The targeting mechanisms of various functionalised NLCs are described in **Table 2**. In addition, **Table 2** also discusses the advantages and disadvantages of various surface modifiers in functionalised NLCs.

The surface modification of NLCs could be engineered through pre-assembly and post-assembly techniques. In the pre-assembly process, a specific ligand was added and mixed in the fabrication of NLCs, whereas the coating and chemical conjugation techniques of NLCs were utilised in the post-assembly process. Various methods could be utilised for ligands to attach to the surface of NLCs, such as chemical conjugation through covalent or non-covalent binding, electrostatic interactions through attractive force charges, physical interactions, and coating.[110,111] Moreover, the post-assembly process has numerous advantages, such as a high concentration of ligands attached to the surface of NLCs, which could lead to high cellular uptake and internalisation compared with the pre-assembly process.[96] For instance, bombesin has been used to fabricate doxorubicin-loaded NLCs through pre-assembly and post-assembly processes. Compared to pre-assembly, post-assembly bombesin fabrication enhanced receptor-mediated targeting and improved the cellular uptake of NLCs. The post-assembly method also preserved the structural integrity of the NLCs, which could lead to increased drug and gene delivery efficiency with reduced off-target effects.[96]

To evaluate the safety and efficacy of functionalised NLCs for pulmonary-targeted delivery systems, numerous studies have been conducted through *in vitro* and *in vivo* assessments. *In vitro* evaluations are described in **Table 3**, whereas *in vivo* assessments are shown in **Table 4**.

4.1. Hydrophilic polymers

PEGylation is a surface modification of hydrophilic polymers, such as polyethylene glycol (PEG). PEGylated particles could also be called stealthy nanoparticles, which stabilise surface nanoparticles with inert polymers.[85] PEG is a hydrophilic molecule that consists of repeated ethylene glycol units. The addition of PEG on the surface of nanoparticles could be achieved by covalent bonding between PEG chains and lipid nanoparticles.[86] The introduction of engineered PEG into the surface of nanoparticles could increase release and promote sustained release, leading to prolonged retention and circulation times. The higher molecular weight of PEG could increase the diffusion barrier of lipid nanoparticle matrix systems so that drug release could be extended. In addition, PEGylation could escape protein opsonin adsorption in the systemic circulation and mononuclear phagocytic cells or the reticuloendothelial system in the liver and spleen.[140,141] Due to the hydrophilic properties of PEG, surface modification with PEG could result in a hydrophilic surface and increased water solubility. Furthermore, PEGylation could also improve EPR effects and reduce the toxicity of drugs encapsulated in lipid nanoparticles.[142-144] The increase in the EPR effect of PEGylated NLCs is shown in **Figure 3**.

Surface modification with PEGylation can provide a stealth effect by creating a physical and energetic barrier against immune recognition.[145] The highly hydrophilic and flexible PEG chains at the molecular level attract and organise a substantial layer of water molecules around the nanoparticle, forming a dense hydration shell. This shell sterically hinders the approach and adsorption of opsonin proteins from the

Table 2. Mechanism of targeting functionalised nanostructured lipid carriers for pulmonary-targeted delivery systems

Functionalised category	Example	Targeting ligand	Targeting mechanism	Advantages	Disadvantages	References
Unmodified NLCs	-	-	• Escape from mucociliary clearance and the alveolar macrophage • Enhanced permeability and retention (EPR) effects • Sustained release properties by a controlled-diffusion mechanism • Passive direct targeting	• High stability and biocompatibility • Can enhance drug deposition, bioavailability, and efficacy • Provides sustained and controlled drug release • Can be targeted passively through the EPR effect	• Low selectivity for target cells • Non-selective uptake can lead to side effects and toxicity • May lead to reduced effectiveness and multidrug resistance	40,41
Hydrophilic polymers	PEG	Cell membrane	• Hydrophilic properties on the surface • Escape from RES uptake • Enhance cellular uptake and internalisation • Enhanced permeability and retention (EPR) effects • Sustained release properties	• Creates a "stealth" effect, escaping immune recognition and prolonging circulation time • Enhances the EPR effect for tumour accumulation • Improves water solubility and provides a sustained release profile	• Can trigger immune responses and production of anti-PEG antibodies, leading to accelerated blood clearance • May cause hypersensitivity reactions by activating the complement system • The PEG shell can hinder cellular uptake and interaction with target receptors • High molecular weight PEGs are not easily biodegradable and may accumulate, causing long-term toxicity	85,86
Polysaccharides	Mannose	CD206 (Macrophage mannose receptor)	• Enhance cellular uptake and internalisation • Targeted to macrophage mannose receptors • pH-release dependent • Sustained release properties • Electrostatic interaction	• Specifically targets mannose receptors on alveolar macrophages, ideal for treating tuberculosis • Enables pH-dependent drug release in the acidic environment of phagolysosomes, increasing specificity	• Can be mistaken for a pathogen, triggering unwanted immune responses • Cationic lipids used in conjugation can increase cytotoxicity • Synthesis is complex, often suffering from low yields and batch-to-batch variability	87,88
	Hyaluronic acid	CD44 receptor (cancer cells)	• Enhance cellular uptake and internalisation • Targeted to the CD44 receptor • pH-release dependent • Sustained release properties • Electrostatic interaction	• Targets CD44 receptors that are overexpressed on many cancer cells • Biocompatible and biodegradable • Mucoadhesive properties can improve drug retention in the lungs	• Poor penetration into solid tumours due to the dense extracellular matrix • Non-specific targeting can occur as CD44 receptors are also on healthy cells, causing off-target toxicity	89
Small molecules	Folic acid	Folate receptor	• Enhance cellular uptake and internalisation • Targeted to the specific overexpression of folate receptors in lung cancer cells • Folate receptor-mediated endocytosis	• Targets folate receptors, which are abundant on lung cancer cells but limited in healthy tissues • Triggers rapid internalisation through receptor-mediated endocytosis	• Folate receptor expression can be heterogeneous across tumours • Competition with endogenous folate in the body can reduce targeting efficiency • May inadvertently promote tumour growth by supplying a necessary nutrient	90,91

(Cont'd...)

Table 2. *(Continued)*

Functionalised category	Example	Targeting ligand	Targeting mechanism	Advantages	Disadvantages	References
	Biotin	Biotin receptor	• Enhance cellular uptake and internalisation • Targeted to the specific overexpression of biotin receptors in lung cancer cells • Biotin receptor-mediated endocytosis	• Targets receptors like the sodium-dependent multivitamin transporter, which are overexpressed on cancer cells	• Cellular uptake mechanism is ambiguous because conjugation modifies the part of biotin required for transporter recognition • Widespread expression of biotin transporters on normal tissues reduces tumour specificity and increases off-target risk	92
	N-acetyl-D-glucosamine	Glucose receptor	• Enhance cellular uptake and internalisation • Targeted to the specific overexpression of glucose receptors in lung cancer cells • Glucose receptor-mediated endocytosis	• Targets overexpressed glucose receptors in lung cancer cells, exploiting their high metabolic activity	• Carries a high risk of severe off-target toxicity because glucose transporters are widely expressed in vital organs such as the brain and heart	93
Surfactants	Tocopherol polyethylene glycol succinate	Epidermal growth factor receptor (EGFR)	• Improve bioactivity • Efflux inhibitor • Active targeting to EGFR • Enhance cellular uptake and internalisation	• Can inhibit P-glycoprotein and other efflux pumps to overcome multidrug resistance • Enhances cellular uptake, drug solubility, and bioavailability • Biocompatible and enhances permeation	• Can be inherently cytotoxic by disrupting cell membranes • May interfere with the endogenous pulmonary surfactant layer, leading to unpredictable drug deposition and absorption	94,95
Peptides and proteins	Bombesin	Bombesin receptor	• Targeted to the specific overexpression of bombesin receptors on cancer cells • Electrostatic interaction • Enhance cellular uptake and internalisation through endocytosis	• Function as high-affinity ligands for specific cell surface receptors, offering high specificity • Can enhance cellular uptake through receptor-mediated endocytosis	• Highly susceptible to enzymatic degradation by proteases, leading to loss of function • Complex structures are sensitive to pH and temperature, which can cause denaturation. • Large size and hydrophilic nature hinder their ability to penetrate biological barriers like mucus.	96
	Tuftsin	Phagolysosome (Macrophages)	• Sustained release at acidic pH • Enhance cellular uptake and internalisation			97
	AEYLR	EGFR	• Specific binding to EGFR overexpressed on cancer cells			98
	TISWPPR	CD133^{+}receptor	• Passive targeting by the EPR effect • Active targeting to the CD133^{+}receptor			99
	Polyarginine	Cell membrane (A549 cells)	• Electrostatic interaction • Enhance cellular uptake and internalisation through Clathrin and Caveolin-mediated endocytosis			100
	Wheat germ agglutinin	Macrophages	• Mucoadhesion, enhances endocytosis and phagocytosis			101
	Transferrin	Transferrin receptor	• Active targeting • Targeted to the specific overexpression of transferrin receptors in lung cancer cells • Enhance cellular uptake and internalization			102,103

(Cont'd...)

Table 2. *(Continued)*

Functionalised category	Example	Targeting ligand	Targeting mechanism	Advantages	Disadvantages	References
Gene	Si-RNA	Specific gene	• Enhance cellular uptake and internalisation • Targeted to specific overexpression genes in lung cancer cells • Inhibit specific gene sequence expression through RNA interference • Receptor-mediated endocytosis	• Offers a highly specific therapeutic approach by modulating cellular processes at the genetic level • Can be used to silence genes responsible for multidrug resistance (e.g., *MRP1* and *BCL2*)	• Highly unstable in the extracellular environment and rapidly degraded by nucleases • Subject to rapid renal clearance, leading to negligible bioavailability • Must escape the endosome to be effective, but often undergoes lysosomal degradation • Can cause off-target gene silencing and trigger innate immune responses	104
Antibody	ICAM-1	Intracellular adhesion molecule-1	• Targeted to a specific immune receptor (ICAM-1) • Growth signalling pathways are interfered with by altering the activation status of cell membrane receptors or deactivating essential cytokines involved in cell growth and proliferation • Enhance cellular uptake and internalisation	• Represents highly specific targeting, capable of recognizing a single epitope with high affinity • Can stimulate the immune system to eliminate cancer cells • Can prolong retention time and enhance cellular uptake while minimizing off-target effects	• Conjugation chemistry is complex and can lead to variable efficacy and immunogenicity • Large size can limit penetration into solid tumours • On-target, off-tumour toxicity can occur if the target antigen is also on normal tissues • Tumours can develop resistance by downregulating the target antigen •	105,106
	Cetuximab	EGFR	• Targeted to EGFR • Enhance cellular uptake and internalisation			107,108
pH-sensitive	mPEG-Hyd-DSPE	-	• Conformational changes due to pH alteration in the environment of tumour cells • Enhance cellular uptake and internalisation.	• Enables triggered drug release specifically in the acidic microenvironment of tumours or endosomes • Restricts drug release at the normal physiological pH of healthy tissues, potentially reducing systemic toxicity	• Requires a delicate balance; linkers may not be perfectly stable at physiological pH, causing premature release • Lacks absolute specificity, as other sites such as inflammation or normal lysosomes are also acidic • Chemical and manufacturing challenges, as not all drugs have functional groups for conjugation, and linker byproducts may be toxic	109

Abbreviations: AEYLR: Epidermal growth factor receptor-binding peptide; A549: Human lung carcinoma cell line; CD133: Cluster of differentiation 133; CD206: Macrophage mannose receptor; CD44: Cluster of differentiation 44; DSPE: 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine; EGFR: Epidermal growth factor receptor; EPR: Enhanced permeability and retention; ICAM-1: Intercellular adhesion molecule-1; mPEG: Methoxy polyethylene glycol; mPEG-Hyd-DSPE: Methoxy polyethylene glycol–hydrazone–1,2-distearoyl-sn-glycero-3-phosphoethanolamine; MRP1: Multidrug resistance-associated protein 1; NLCs: Nanostructured lipid carriers; PEG: Polyethylene glycol; RES: Reticuloendothelial system; siRNA: Small interfering RNA; TISWPPR: $CD133^{+}$receptor-targeting peptide.

Table 3. *In vitro* evaluation of functionalised nanostructured lipid carriers for pulmonary-targeted delivery systems

Functionalised agent	Drug	Disease/ Function	Cell lines	Key findings	Referecnes
Hydrophilic polymers					
Polyethylene glycol (PEG)	10-hydroxy camptothecin	Lung cancer	A549	PEGylated NLCs can enhance cellular uptake of A549 cell lines, with higher uptake of PEG-40 compared to PEG-100	112
	Etoposide and cisplatin	Lung cancer	A549/DDP cells	High cellular uptake efficiency for 55.1–57.4% with a synergistic effect. PEGylated NLCs can significantly enhance the activation of caspase 3 in A549/DPP cells compared to unmodified NLCs	113
	Fluticasone	Lung disease-induced cigarette smoke	16-human bronchial epithelial cells	Pegylated NLCs showed no toxicity or necrosis or cell apoptosis. Pegylated NLCs can decrease oxidative stress and more effectively reduce TLR4 expression-induced cigarette smoke extracts compared to unmodified NLCs	114
	Itraconazole	Pulmonary aspergillosis	A549	There was no toxicity up to 0.5 μg/mL for 72 h	115
	lumacaftor and ivacaftor	Cystic fibrosis	CFBE41o– cells	The complete cellular uptake and internalisation of PEG-NLCs was achieved in 24 h	116
Polysaccharides					
Mannose	Blank (no active)	Tuberculosis	Triple cells: MDMs (top), A549, MDDCs (basal)	There was no toxicity and pro-inflammatory response after 24 h of exposure to both mannosylated and non-mannosylated NLCs. High internalisation efficiency of mannosylated NLCs by A549 compared to non-mannosylated NLCs	117
	Clofazimine	Tuberculosis	Macrophage J774 cells	Mannosylated NLCs have higher cell viability compared to the drug dispersion and similar non-toxic properties to blank NLCs	118
	Rifabutin	Tuberculosis	Calu-3, A529, RAW 264.7 cells	The IC_{50} measured using the MTT assay for mannosylated rifabutin-loaded NLCs showed 238.9 μg/mL for Calu-3, 185.7 μg/mL for A549, and 108.7 μg/mL for RAW cells.	119
	Rifampicin	Tuberculosis	Bone marrow-derived macrophages (BMDM)	Mannosylated rifampicin-loaded NLCs have high cell viability and lower IC_{50} compared to free drug and unmodified NLCs. The internalisation of mannosylated rifampicin NLCs measured by mean fluorescence intensity with flow cytometry was 14.5 times higher compared to unmodified NLCs. Sustained release properties at pH 5.0 and lower release at pH 7.4	84
	Rifampicin	Tuberculosis	NR8383 AMs cells	The mean fluorescence intensity of mannosylated rifampicin-loaded NLCs was significantly higher by 2.6-fold than unmodified NLCs. This strongly indicates that mannosylated rifampicin-NLCs can enhance cellular uptake in NR8383 AM cells. There was no increase in pro-inflammatory response (IL-6) after administration of NLCs. Sustained release properties at pH 5.0 and lower release at pH 7.4	120
Hyaluronic acid	pDNA	Lung cancer	A549 cells	Hyaluronic acid-decorated NLCs exhibited higher cell viability compared to unmodified NLCs. Hyaluronic acid pDNA NLCs exhibited significantly better gene transfection (approximately 40% for 36 h and 72 h) compared to unmodified NLCs, with only 30%	121
	Kaempferol	Non-small cell lung cancer	A549 cells	The IC_{50} value of hyaluronic acid with a molecular weight of 200–400 and 1,300–1,600 kDa decorated kaempferol NLCs was significantly lower compared to unmodified NLCs. Hyaluronic acid-decorated NLCs have significantly enhanced cell proliferation inhibition and cell apoptosis. Hyaluronic acid with a molecular weight of 200-400 kDa can significantly enhance cellular uptake, targeted and selective to NSCLC cells, and internalisation by the endocytosis mechanism compared to unmodified NLCs	122

(Cont'd...)

Table 3. *(Continued)*

Functionalised agent	Drug	Disease/ Function	Cell lines	Key findings	Referecnes
Polysaccharides					
	Apigenin	NSCLC	A549 cells	Sustained release properties at acidic pH (5.5). Hyaluronic acid functionalised NLCs have a 1.5-fold lower IC_{50} value and enhanced cellular uptake against A549 cell lines than unmodified NLCs. The tumour cell apoptosis rate against A549 cells of hyaluronic acid functionalised NLCs was 36.64% higher than unmodified NLCs	123
Hyaluronic acid-conjugated DSPE-PEG2000	Vinorelbine	Lung cancer	A549 cells	The *in vitro* cytotoxicity of vinorelbine-loaded hyaluronic acid-functionalised NLCs showed a significantly lower IC_{50} (35.7 μg/mL) against A549 lung cancer cells compared to conventional NLCs (57.29 μg/mL) and marketed vinorelbine (45.65 μg/mL), with a cumulative drug release of 85.23% within 48 h	124
Small molecules					
Folic acid	Paclitaxel	NSCLC	A549 cells	Folate-functionalised NLCs exhibited a significantly higher cellular uptake and internalization	125
	Paclitaxel	Lung squamous carcinoma	NCI-H226 cells	Folate-functionalised NLCs demonstrated superior performance with a concentration needed to inhibit 50% of cell growth of 5.84 μM (compared to 9.72 μM for unmodified-NLCs and 18.51 μM for PTX).	126
	Docetaxel and curcumin	NSCLC	NCI-H460 cells	IC_{50} values of folate-functionalised NLCs were significantly lower than unmodified NLCs. The cellular uptake of folate-functionalised NLCs against NCI-H460 cells was significantly higher than unmodified NLCs	127
Biotin	Sunitinib	Lung cancer	A549 cells	The *in vitro* cytotoxicity of biotin-functionalised sunitinib NLCs was higher, with the lowest IC_{50} of 1.66 μg/mL compared to unmodified NLCs (2.17 μg/mL) and free drugs (3.14 μg/mL). Biotin-decorated NLCs displayed significantly increased fluorescence intensity and cellular uptake into the targeted cells compared to unmodified NLCs	128
N-acetyl-D-glucosamine	Gemcitabine and paclitaxel	NSCLC	A549, NCI-H1299, LTEPa2, L929 cells	The decorated NLCs showed higher cytotoxicity with the lowest IC_{50} compared to the unmodified NLCs and pure drugs. The decorated NLCs also showed the lowest combination index, which indicates the greatest synergistic properties. The cellular uptake of decorated NLCs was enhanced and rapidly internalised through endocytosis. The mean fluorescence intensity of functionalised NLCs was significantly higher compared to the unmodified NLCs	93
Surfactants					
Tocopheryl polyethylene glycol succinate (TPGS)	Gefitinib	Metastatic lung cancer	A549 cells	The functionalised TPGS NLCs exhibited the lowest IC_{50} (7.01 μg/mL) compared to unmodified NLCs (15.05 μg/mL) and pure drug (11.16 μg/mL) after 24 h. In addition, decorated NLCs can enhance apoptotic activity approximately 3.3-fold compared to pure drugs	129
Peptides and proteins					
Bombesin	Doxorubicin and DNA	Lung cancer	NCI-H460 cells	The IC_{50} value of bombesin-decorated doxorubicin and DNA NLCs was threefold and sixfold more effective than unmodified NLCs and free drug, respectively. *In vitro*, the gene transfection level of bombesin-fabricated doxorubicin and DNA NLCs was 67.9% at 72 h, which is significantly greater than unmodified NLCs and free drug	96
Tuftsin	Rifampicin	Tuberculosis	J774 A.1 macrophages	The cellular uptake of tuftsin-integrated NLCs was significantly 2.5-fold higher compared to unmodified NLCs, with a mean fluorescence intensity of 93.4 and 37.3 for decorated and undecorated NLCs at 30 min, respectively. The cellular uptake of tuftsin-integrated NLCs was significantly increased and reached 3.6-fold higher compared to unmodified NLCs at 24 h	130
EGFR targeting peptide (AEYLR)	Biotin	NSCLC	NCI-H1299 cells	AEYLR small peptide integrated NLCs have higher cytotoxicity and internalisation into the cells compared to the unmodified NLCs	98

(Cont'd...)

Table 3. *(Continued)*

Functionalised agent	Drug	Disease/ Function	Cell lines	Key findings	Referecnes
Peptides and proteins					
$CD133^{+}$targeting peptide TISWPPR	Paclitaxel and salinomycin	NSCLC	NCI-H1299 cells	Functionalised NLCs can enhance the specific targeting and cellular internalisation into the cancer stem cells compared to the unmodified NLCs. The *in vitro* tumour inhibition of decorated NLCs exhibited a significantly higher rate compared to the undecorated NLCs	99
Poly-arginine	Paclitaxel	NSCLC	A549 cells	The *in vitro* cellular uptake study against A549 cells showed that polyarginine-functionalised NLCs can elevate cellular internalisation fourfold higher than unmodified NLCs	131
Wheat germ agglutinin	Quercetin	Pulmonary intracellular drug intake	J774.A1 cells	The functionalisation with wheat germ agglutinin can increase the internalisation measured by the fluorescent intensity	101
Transferrin	pDNA	Lung cancer	A549 cells	Transferrin-integrated pDNA NLCs exhibited higher cell viability compared to unmodified NLCs. Transferrin pDNA NLCs showed significantly better *in vitro* gene transfection efficiency on A549 cells for approximately 40% and 50% at 36 and 72 h, respectively, compared to unmodified NLCs for just 30%	121
	Paclitaxel and DNA	NSCLC	NCI-H460 cells	The IC_{50} value of transferrin-integrated paclitaxel and DNA was fourfold more effective compared to the solution. The *in vitro* gene transfection of transferrin-decorated paclitaxel and DNA was significantly higher compared to undecorated NLCs at 36 and 72 h	132
	DNA	Lung cancer	A549 cells	The *in vitro* gene transfection efficiency of transferrin-integrated NLCs was significantly higher compared to unmodified NLCs at 48 and 72 h	133
	DNA and doxorubicin	Lung cancer	A549 cells	The IC_{50} value of transferrin-decorated NLCs was lower than unmodified NLCs and free drugs. The *in vitro* transfection efficiency of transferrin-functionalised NLCs was significantly higher than unmodified NLCs.	134
Gene					
siRNA	Prostaglandin E	Idiopathic pulmonary fibrosis	A549 cells	Three siRNA sequences (*MMP3*, *CCL12*, and *HIF1A*) can inhibit protein synthesis due to fibrotic lung damage, with lower gene expression measured by quantitative polymerase chain reaction, which can be utilised to target drugs into the lung cells	135
	Doxorubicin or paclitaxel	Lung cancer	A549 cells	The functionalised siRNA NLCs can significantly decrease *BCL2* and *MRP1* gene expression and suppress targeted mRNA compared to free drugs. The functionalised siRNA NLCs can also enhance cellular uptake and internalisation into A549 lung cancer cells	136
DNase	Levofloxacin	Cystic fibrosis	*Pseudomonas aeruginosa* in soft nutrient agar	Decorated NLCs can enhance antimicrobial activity by reducing biofilm formation and have greater potency to destroy bacterial membrane integrity	137
Antibodies					
ICAM-1 antibody	Simvastatin	Acute lung injury	EAhy926 cells	The ICAM-1 antibody-functionalised simvastatin NLCs showed enhancement in cellular uptake ability, low cytotoxicity, and improvement in cellular internalisation compared to unmodified NLCs due to specific targeting into the ICAM-1 epitope	138
	Dexamethasone	Acute lung injury	EAhy926 cells	ICAM functionalised NLCs showed a higher mean fluorescence intensity of 96.8% compared to unmodified NLCs, which is 90.5% at 8 h, due to overexpression of ICAM-1 epitope on EAhy926 surface cells. This result suggested that ICAM modification can enhance cellular internalisation and lead to high deposition of drugs into inflammatory endothelium cells through ICAM-mediated endocytosis	105
Cetuximab	Paclitaxel and 5-Demethylnobiletin	NSCLC	A549 cells	Cetuximab functionalised NLCs have a higher cellular internalisation of approximately 1.85-fold than unmodified NLCs	108

(Cont'd...)

Table 3. *(Continued)*

Functionalised agent	Drug	Disease/ Function	Cell lines	Key findings	Referecnes
pH-sensitive					
Methoxy (polyethylene glycol) 2000- hydrazone-1,2-stearoyl-sn-glycerol-3-phospho ethanolamine (mPEG-Hyd-DSPE)	Doxorubicin and β-elemene	Lung cancer	A549, A549/ ADR, and MRC-5 cells	The pH-sensitive functionalised NLCs have a faster release at acidic pH (5.5) and a slower release at basic conditions (pH 7.4). The uptake efficiency and cytotoxicity of pH-sensitive functionalised NLCs were greater than unmodified NLCs. The functionalised NLCs had a higher cellular inhibition effect against lung cancer cells compared to the unmodified NLCs	139

Abbreviations: AEYLR: Epidermal growth factor receptor-binding peptide; A549: Human lung carcinoma cell line; ADR: Adriamycin-resistant; *BCL2*: B-cell lymphoma 2 (anti-apoptotic gene); BMDM: Bone marrow-derived macrophages; CCL12: Chemokine (C-C motif) ligand 12; CD133: Cluster of differentiation 133; CFBE41o-: Cystic fibrosis bronchial epithelial cell line; DNA: Deoxyribonucleic acid; DNase: Deoxyribonuclease; DSPE: 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine; EAhy926: Human endothelial cell line; EGFR: Epidermal growth factor receptor; HIF1A: Hypoxia-inducible factor 1-alpha; IC_{50}: Half maximal inhibitory concentration; ICAM-1: Intercellular adhesion molecule 1; J774: Murine macrophage cell line; L929: Mouse fibroblast cell line; LTEP-a2: Human lung epithelial cell line; MDDC: Monocyte-derived dendritic cells; MDM: Monocyte-derived macrophages; MMP3: Matrix metalloproteinase 3; MRP1: Multidrug resistance-associated protein 1; mPEG: Methoxy polyethylene glycol; mPEG-Hyd-DSPE: Methoxy polyethylene glycol–hydrazone–1,2-distearoyl-sn-glycero-3-phosphoethanolamine; MRC-5: Normal human fetal lung fibroblast cell line; NCI-H1299/NCI-H460: Non-small cell lung cancer cell lines; NLCs: Nanostructured lipid carriers; NSCLC: Non-small cell lung cancer; pDNA: Plasmid DNA; PEG: Polyethylene glycol; RES: Reticuloendothelial system; RNAi: RNA interference; siRNA: Small interfering RNA; TPGS: Tocopheryl polyethylene glycol succinate; TLR4: Toll-like receptor 4; TISWPPR: $CD133^+$receptor-targeting peptide.

bloodstream, a process required for recognition and clearance by the mononuclear phagocyte system.[146] The effectiveness of this steric shield is critically dependent on the conformation of the PEG chains, which is governed by their grafting density. At low densities, PEG adopts a coiled mushroom-like conformation that offers minimal protection due to more interaction at the surface of particles. At higher densities, however, steric repulsion forces the chains to extend outwards, forming a dense brush-like conformation that creates a much more robust and impenetrable barrier, significantly prolonging circulation time and enhancing the potential for tumour accumulation.[147]

Improvements in drug efficacy at lung sites can be achieved through surface modification with PEGylated NLCs. Du and Yin[112] investigated the combination of etoposide- and cisplatin-loaded NLCs with PEG modification for lung cancer treatment. Compared to free drugs, the PEG-functionalised NLCs significantly inhibited tumour growth *in vivo*, resulting in greater lung cancer deposition. This result suggested that PEGylated NLCs have improved efficacy for lung cancer therapy due to the EPR effect.[113] In another study, lumacaftor- and ivacaftor-loaded PEGylated NLCs were internalised through CFBE41o-cell uptake. Hence, imaging with magnetic resonance imaging and computed tomography scans of mouse lungs revealed that cystic fibrosis and oedema fully disappeared within 4 weeks. This could be influenced by the enhancement of mucus penetration and cellular uptake through CFBE41o- bronchial epithelial cells due to their high loading capacity and prolonged release properties.[116]

Bondì *et al.*[114] also studied the effects of fluticasone propionate-loaded NLCs with PEG surface modifications on corticosteroid resistance induced by cigarette smoke. The results showed that drug release was sustained for 72 h, with an initial burst release of 15% in the first few hours. Furthermore, PEG-functionalised NLCs did not induce cell necrosis or apoptosis in 16-human bronchial epithelial cells, which indicates that the nanoparticles have good biocompatibility. Moreover, fluticasone-loaded PEGylated NLCs can significantly decrease the reactive oxygen species and toll-like receptor 4 expression induced by cigarette smoke extract and increase the glutathione concentration compared with unencapsulated drugs. Therefore, PEGylated NLCs can enhance drug efficacy against COPD-induced cigarette smoke effects. This can be attributed to the greater cellular uptake of fluticasone in 16-human bronchial epithelial cells than in free drugs and unmodified NLCs.[114]

The molecular weight of the PEG also plays a crucial role. Higher-molecular-weight PEG chains create a thicker steric barrier, enhancing the stealth effect. Zhang *et al.*[112] studied two types of PEGs for functionalizing NLCs: PEG-40, with a molecular weight of 2,000 Da, and PEG-100, with a molecular weight of 5,000 Da. The release kinetics of 10-hydroxy camptothecin from NLCs through erosion and diffusion depend on the molecular weight and chain length of PEG. The longer chains of PEG can lead to a slower release of drugs due to the thicker diffusion layer of the matrix system, with 56.5% and 43.3% of the drugs released at 24 h for the PEG-40- and PEG-100-functionalised NLCs, respectively. The *in vitro*

Table 4. *In vivo* evaluation of functionalised nanostructured lipid carriers for pulmonary-targeted delivery systems

Functionalised agent	Drug	Disease/ Function	Animal model	Key findings	References
Hydrophilic polymers					
Polyethylene glycol (PEG)	10-hydroxy camptothecin	Lung cancer	A549-bearing nude mice	PEG-40 decorated NLCs can significantly reduce tumour growth rate with a 40-fold higher maximum concentration compared to the solution	112
	Etoposide and cisplatin	Lung cancer	A549/DPP cells injected into BALB/c nude mice	High distribution in tumour cells compared to unmodified NLCs and free drugs. Pegylated NLCs can increase antitumour activity with a higher tumour inhibition rate compared to free drugs	113
	Lumacaftor and ivacaftor	Cystic fibrosis	Cftrtm1Unc Tg (FABPCFTR) 1Jaw/J homozygote/ homozygote bitransgenic mice	The drug combination-loaded NLCs can completely treat lung cystic fibrosis. The lesions of cystic fibrosis had completely disappeared within 4 weeks of treatment	116
Polysaccharides					
Mannose	Clofazimine	Tuberculosis	Male Wistar rats	There was no toxicity and histopathological changes (lung, spleen, and liver) in rats after inhalation for 14 days. Mannosylated NLCs can enhance the AUC value twofold compared to the drug dispersion. The highest concentration of the drug is deposited into the lungs compared to unmodified NLCs and drug dispersion	118
	Rifampicin	Tuberculosis	Male Wistar rats	Mannosylated rifampicin NLCs have higher drug deposition in the lungs, 10.48-fold compared to the drug solution at 30 mins, and significantly higher compared to unmodified NLCs	120
	Isoniazid	Tuberculosis	Dunkin Hartley male guinea pigs	Mannosylated isoniazid NLCs were sustained for 48 h compared to unmodified NLCs for 24 h and the drug solution for 12 h. The AUC was increased approximately 1.17-fold compared to unmodified NLCs	34
Hyaluronic acid	pDNA	Lung cancer	Lung cancer-bearing BALB/c nude mice	Hyaluronic acid-functionalised pDNA has a better cellular uptake and transfection efficiency compared to unmodified NLCs	121
Hyaluronic acid-conjugated DSPE-PEG2000	Vinorelbine	Lung cancer	Kunming mice bearing the LL2 lung cancer	Hyaluronic acid-functionalised NLCs decreased tumour volume by 1.67-fold compared to marketed vinorelbine and 1.82-fold compared to non-activated NLC, increased body weight, and elevated the BAX/BCL2 apoptosis ratio by 4.5-fold	124
Small molecules					
Folic acid	Docetaxel and curcumin	NSCLC	Sprague Dawley rats	Folate-functionalised NLCs can improve the bioavailability of docetaxel by 12.39-fold and 2.61-fold compared to the free drugs and unmodified NLCs, respectively. Folate-functionalised NLCs can also enhance the tumour inhibition rate, reduce the immune expression of mutated p53 and cell proliferation Ki67 compared to the unmodified NLCs and free drugs	127
	Paclitaxel	Lung squamous carcinoma	Wistar rats with benzo-alpha-pyrene-induced lung tumours	Folate-functionalised NLCs achieved 25.86±0.39% lung tumour uptake and an extended half-life of 39.50 h, thereby significantly improving bioavailability	126

(Cont'd...)

Table 4. *(Continued)*

Functionalised agent	Drug	Disease/ Function	Animal model	Key findings	References
Peptides and proteins					
Bombesin	Doxorubicin and DNA	Lung cancer	Tumour-bearing BALB/c nude mice	The gene transfection level in mice was significantly higher for decorated NLCs compared to unmodified NLCs and free drugs. Bombesin-decorated NLCs exhibited a tumour inhibition rate of 76% which is 2.1-fold and 5.6-fold for unmodified NLCs (36%) and free drug (13%), respectively	96
Transferrin	pDNA	Lung cancer	Lung cancer-bearing BALB/c nude mice	Transferrin-decorated pDNA has a better cellular uptake and transfection efficiency compared to unmodified NLCs	121
	Paclitaxel and DNA	NSCLC	Male BALB/c mice	The *in vivo* gene transfection of transferrin-decorated paclitaxel and DNA was significantly higher compared to undecorated NLCs at 36 and 72 h. The decorated NLCs can significantly inhibit tumour regression and show a lower tumour growth rate of 23% compared to the unmodified NLCs of 42% at 14 days	132
	DNA	Lung cancer	Tumour-bearing BALB/c mice	The *in vivo* gene transfection efficiency of transferrin decorated NLCs was statistically significantly higher compared to unmodified NLCs at 24, 48, and 72 h	133
	DNA and doxorubicin	Lung cancer	Lung tumour-bearing C57BL/6 mice	Transferrin-decorated NLCs exhibited higher *in vivo* transfection efficiency and cellular internalisation compared to unmodified NLCs at 48 and 72 h. The functionalised NLCs can inhibit 66% tumour growth compared to unmodified NLCs, which can only inhibit 47% tumour growth	134
$CD133^+$ targeting peptide TISWPPR	Paclitaxel and salinomycin	NSCLC	S180 tumour-bearing mice	The decorated NLCs have a synergistic tumour-targeting effect, which can improve tumour targeting by enhancing cellular uptake and internalisation by tumour cells. The tumour inhibition rate of functionalised NLCs was 53.24% which was significantly greater compared to the unmodified NLCs (26.86%)	99
Gene					
siRNA	Prostaglandin E	Idiopathic pulmonary fibrosis	SKH1-hr. hairless mice	The combination of PGE2 and siRNA can reduce the hydroxyproline content and targeted gene expression in the lungs. The siRNA-functionalised NLCs can decrease lung inflammation and fibrotic tissue volume 3.8-fold compared to the control. The inhalation of PGE2 and siRNA can significantly decrease pro-fibrotic genes, which are connective tissue growth factors and TGF-β, in pulmonary fibrosis compared to the unmodified NLCs	135
	Doxorubicin or paclitaxel	Lung cancer	Athymic nu/nu mice	The drug deposited into the lungs of functionalised NLCs can be more specific to lung tumours than non-targeted NLCs. The conjugation of luteinizing hormone-releasing hormone can significantly enhance antitumour activity and reduce tumour volume within 4 weeks	136

(Cont'd...)

Table 4. *(Continued)*

Functionalised agent	Drug	Disease/ Function	Animal model	Key findings	References
Antibodies					
ICAM-1 antibody	Simvastatin	Acute lung injury	Male Balb/c mice	The pulmonary signals for anti-ICAM functionalised NLCs were significantly stronger compared to those of non-targeted NLCs due to overexpression of ICAM-1 on pulmonary endothelium. The anti-ICAM functionalised NLCs can significantly reduce the inflammatory cytokines of TNF-α and IL-6 compared to unmodified NLCs. In addition, the sustained release profile was found up to 36 h	138
	Dexamethasone	Acute lung injury	Male Balb/c mice	ICAM-functionalised NLCs exhibited more intense pulmonary fluorescence signals than unmodified NLCs due to specific binding to the lung endothelium surface through endocytosis. ICAM-functionalised NLCs can significantly inhibit TNF-α and IL-6 expressions compared to unmodified NLCs.	105
Cetuximab	Paclitaxel and 5-demethylnobiletin	NSCLC	Female BALB/c nude mice	Cetuximab functionalised NLCs have a significantly higher tumour growth inhibition compared to free drug and unmodified NLCs	108
pH-sensitive					
Methoxy (polyethylene glycol) 2000-hydrazone-1,2-stearoyl-sn-glycerol-3 -phosphoethanolamine (mPEG-Hyd-DSPE)	Doxorubicin and β-elemene	Lung cancer	Male C57BL/6 mice	The functionalised NLCs exhibited significantly greater tumour growth inhibition compared to the unmodified NLCs, with the highest tumour inhibition rate of 82.9% with a smaller tumour volume	139

Abbreviations: AUC: Area under the curve; BALB/c: Inbred strain of laboratory mice; C57BL/6: Common inbred strain of laboratory mice; CD133: Cluster of differentiation 133; Cftrtm1Unc Tg (FABPCFTR) 1Jaw/J: Bitransgenic mouse model for cystic fibrosis; DNA: Deoxyribonucleic acid; DSPE: 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine; IC_{50}: Half maximal inhibitory concentration; ICAM-1: Intercellular adhesion molecule 1; IL-6: Interleukin 6; mPEG-Hyd-DSPE: Methoxy polyethylene glycol–hydrazone–1,2-distearoyl-sn-glycero-3-phosphoethanolamine; NLCs: Nanostructured lipid carriers; NSCLC: Non-small cell lung cancer; pDNA: Plasmid DNA; PEG: Polyethylene glycol; PGE2: Prostaglandin E2; siRNA: Small interfering RNA; TGF-β: Transforming growth factor-beta; TISWPPR: $CD133^{+}$receptor-targeting peptide; TNF-α: Tumour Necrosis factor-alpha.

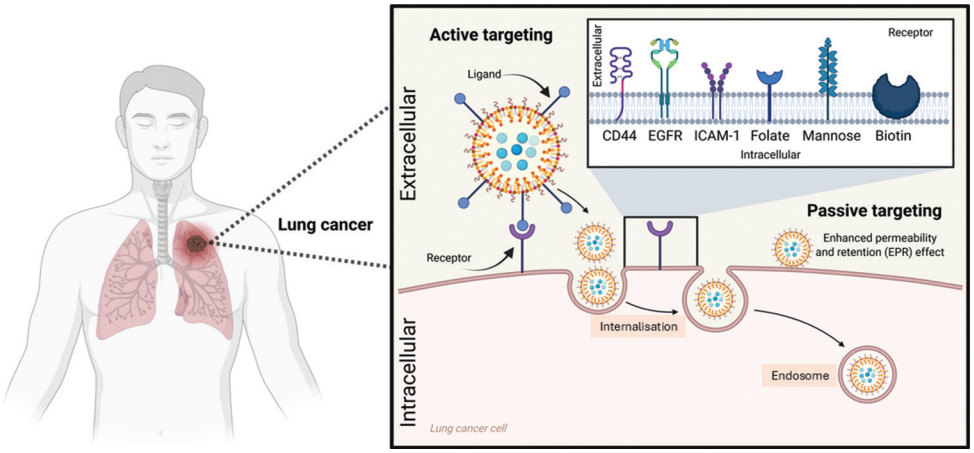

Figure 3. The targeting mechanism of functionalised nanostructured lipid carriers involves active targeting through receptor-mediated endocytosis and passive targeting through the EPR effect. Created in BioRender. Gunawan, M. (2025) https://BioRender.com/1rxsqr2.
Abbreviations: CD44: Cluster of differentiation 44; EGFR: Epidermal growth factor receptor; EPR: Enhanced permeability and retention; ICAM-1: Intercellular adhesion molecule-1; NLCs: Nanostructured lipid carriers.

cellular uptake of PEG-functionalised NLCs by A549 cells was significantly greater than that of unmodified NLCs. This can be influenced by surface charge interactions between the positive or neutral charge of PEGylated NLCs and the negative charge of the membrane cells. In contrast, unmodified NLCs have a highly negative charge, which can lead to electronic repulsion instead of attraction. Moreover, PEG-40-functionalised NLCs have greater cellular uptake than PEG-100, which strongly indicates that the higher molecular weight of NLCs can also decrease their cellular uptake. Similarly, pharmacokinetic analysis in mice revealed that compared with unmodified NLCs and solutions, PEG-functionalised NLCs have longer lung retention times and half-lives. PEG-functionalised NLCs have a significantly greater degree of deposition into the lungs and a lower distribution in the liver and spleen, whereas unmodified NLCs can be distributed into the liver and spleen due to RES uptake. This might be caused by the sustained release properties of the PEG-functionalised NLCs and the EPR effect of the PEGylated NLCs, which could lead to type I pneumocyte adsorption and enhanced cellular uptake in the lungs. However, the molecular weight also influenced the RES uptake of the PEG-functionalised NLCs. The number of drugs distributed in the liver and spleen in the PEG-100 group was significantly greater than that in the PEG-40 NLCs group. In addition, the $AUC_{0\text{-}24h}$ values in the lungs of the PEG-functionalised NLCs were 3.13-fold and 1.87-fold greater than those of the unmodified NLCs for the PEG-40- and PEG-100-functionalised NLCs, respectively. Compared to PEG-40, PEG-100-functionalised NLCs with higher molecular weights could induce and initiate phagocytosis of RES, leading to greater RES uptake and a lower distribution in the lungs. Compared with unmodified NLCs, the highest level of cellular uptake of PEG-40-functionalised NLCs could lead to more efficient treatment for antitumour activity *in vivo* in A549-bearing nude mice. This might be influenced by sustained release properties and the ability to escape RES uptake, which could result in EPR effects.[112]

However, PEGylation is now associated with several clinical challenges. PEG can trigger immune responses, including the production of anti-PEG antibodies, which leads to the accelerated blood clearance phenomenon.[148] This results in rapid elimination of repeated doses, reduced therapeutic efficacy, and accumulation in organs such as the liver and spleen. These issues concern humans, who often possess pre-existing anti-PEG antibodies due to widespread exposure.[149] In addition, PEGylated nanoparticles can also activate the complement system, which can cause acute hypersensitivity reactions and serious adverse events.[150] Moreover, the dense hydrophilic shell created by PEG can hinder cellular uptake by forming a steric barrier that prevents receptor interaction and intracellular delivery, especially for therapies that require entry into the cell, such as gene-based treatments. This presents a trade-off where increased PEG coverage enhances circulation time but reduces drug delivery efficiency at the target site. Furthermore, PEG is not easily biodegradable, and high molecular weight (20–50 kDa) may accumulate in tissues, leading to long-term toxicity.[148]

4.2. Polysaccharides

Polysaccharides, such as biopolymers, can be utilised in targeted delivery systems due to their biocompatibility and high targeting selectivity for polysaccharide receptors such as mannose receptors, toll-like receptor 4, scavenger receptors, and other receptors on the surface of cells.[151] Mannose and hyaluronic acid are commonly used polysaccharides in lung delivery because they offer excellent biocompatibility and biodegradability while enabling pulmonary-targeted drug delivery. Mannose specifically binds to mannose receptors on alveolar macrophages, facilitating enhanced uptake by these immune cells, which is beneficial for treating respiratory infections and inflammation. Hyaluronic acid interacts with CD44 receptors on lung epithelial and inflammatory cells, providing mucoadhesive properties that improve drug retention in lung tissues. Therefore, mannose and hyaluronic acid are effective ligands for improving the specificity, efficiency, and safety of pulmonary drug delivery systems.

4.2.1. Mannose-functionalised nanostructured lipid carriers

In lung-targeted delivery, mannose receptors can be presented on the surface of macrophages and can be utilised to target various antituberculosis drugs.[152,153] Functionalised NLCs with mannose facilitate their recognition by the mannose receptor (CD206), which is highly expressed on alveolar macrophages. The mannose receptor can mediate macrophage phagocytosis and endocytosis, which is the primary infection site for *M. tuberculosis*.[154,155] This interaction can facilitate the efficient internalisation of the NLCs by intracellular pathogens. This pathway can bypass certain bactericidal responses, preserving the encapsulated drug, which can then be released in the acidic environment of the phagolysosome, where the bacteria reside. Therefore, the use of mannosylated lipid nanocarriers could be a promising strategy to increase the efficacy of antituberculosis drugs with enhanced selectivity for the mannose receptor of alveolar macrophages and reduced toxicity in other lung sites.[87,88,156] The results of the macrophage-targeted delivery of mannosylated NLCs are shown in **Figure 4**.

The surface modification of NLCs with mannose could be a potential strategy for targeting specific alveolar macrophages, which have mannose receptors, in tuberculosis infections. Patil and Deshpande[118] developed mannosylated NLCs to improve the safety and bioavailability of clofazimine through inhalation, for tuberculosis treatment. The release kinetics of mannosylated NLCs were lower than those of non-mannosylated NLCs due to the coating and protective properties of mannose on the surface of NLCs. In addition, the *in vitro* release results revealed that drugs released from the matrix system could be influenced by the pH of the medium. The highest dissolution rate was observed at pH 5.0, which has controlled release properties, whereas the lowest dissolution rate was observed at pH 7.4.[118] Another study revealed that the pH-dependent rifabutin-loaded mannosylated NLCs could be released at pH 5.0, whereas only 20% could be released at pH 7.4 during the 25-h study. These results suggested that the drug could be released in the acidic environments of *M. tuberculosis* residents and enhance uptake in acidified phagosomes and phagolysosomes while preventing drug release at non-infected sites (**Figure 4**).[119] Moreover, rifampicin-loaded NLCs resulted in greater drug release at pH 7.4 than mannosylated NLCs, which indicates that mannosylated NLCs could improve the specificity of macrophages in acidic environments.[84]

Vieira *et al.*[84] studied rifampicin-loaded NLCs coated with mannose for targeted macrophage delivery. Interestingly, non-mannosylated NLCs have a negative charge on the surface of lipid nanoparticles. However, the addition of a positive charge lipid, such as stearyl amine, in the mannose-functionalised NLCs can reverse the surface charge from a negative charge of −34 mV (unmodified) into a positive charge (+47 mV), which can enhance the electrostatic interaction between mannosylated NLCs and the surface of the negatively charged cell membrane. During the mannosylation process, mannose is conjugated through Schiff base formation (-N=CH-) between the aldehyde group of mannose and the amine groups of stearyl amine-functionalised NLCs. This change to a positive charge can enhance the electrostatic interactions between the positively charged mannosylated-NLCs and the negatively charged cell membranes, which can facilitate the improvement of cellular uptake by macrophages. In addition, mannosylated NLCs can also improve macrophage recognition through specific macrophage mannose receptors. These dual strategies can improve cellular uptake and internalisation into macrophages. However, the positive charge of rifampicin-loaded mannosylated NLCs could induce greater cytotoxicity than unmodified NLCs.[84] Nevertheless, using cationic lipids, such as stearyl amine, in mannose-functionalised NLCs can significantly elevate cytotoxicity.[157] This increased toxicity

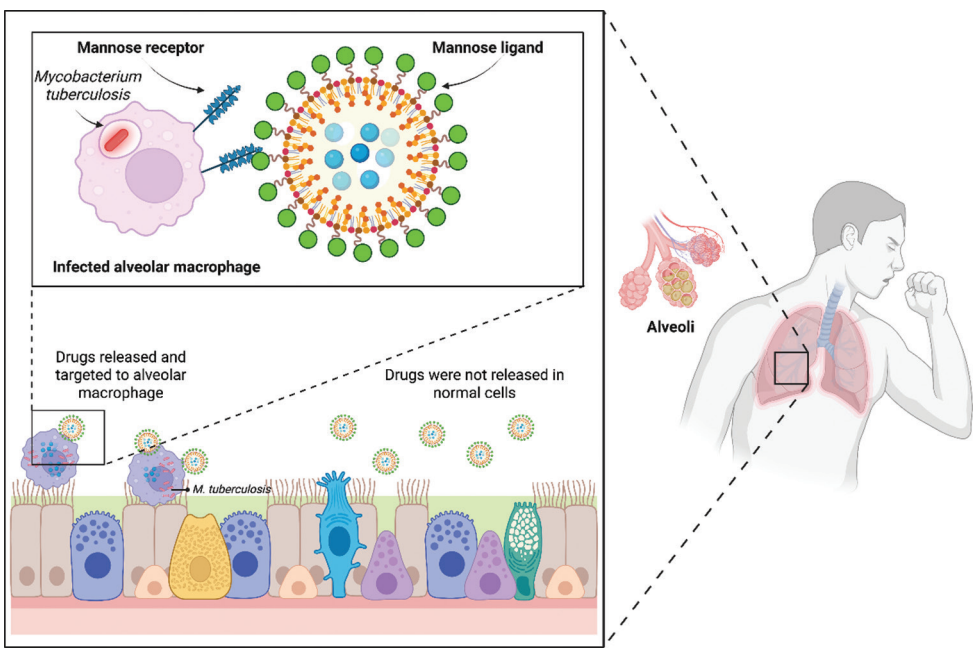

Figure 4. Mannose-functionalised nanostructured lipid carriers for targeted tuberculosis treatment in alveolar macrophages. Created in BioRender. Gunawan, M. (2025) https://BioRender.com/elegvfo.

can be due to the strong electrostatic interactions between the positively charged lipid molecules and the negatively charged components of the cell membrane.[158] These interactions can compromise membrane integrity, leading to membrane destabilisation, increased permeability, and ultimately, cell lysis or programmed cell death.[159] Although this mechanism may facilitate improved cellular uptake, it also introduces significant safety concerns, especially regarding potential toxicity in non-target tissues. The non-specific disruption of cell membranes can lead to unintended cytotoxic effects; hence, the lipid composition, surface charges, and targeting specificity should be optimised.

Magalhães *et al.*[117] developed a three-dimensional (3D) human lung model that consists of three layers of a coculture model, including monocyte-derived macrophages, A549 cells, and monocyte-derived dendritic cells, for upper, middle, and lower lung cell models, respectively. This 3D human lung model can mimic the human alveolar epithelial tissue barrier in the lungs. The results showed that the functionalised and unmodified NLCs were biocompatible without destroying the integrity of the cell membrane and did not induce a proinflammatory response in cytokines, such as interleukin (IL)-1β, tumour necrosis factor alpha, and IL-8. Furthermore, a higher concentration of coumarin-labelled mannosylated NLCs was detected in A549 cells than in non-mannosylated NLCs. These findings strongly indicated that NLCs' surface modification with mannose could enhance the cellular uptake and internalisation of NLCs into targeted alveolar macrophages.[117] Similarly, rifampicin-loaded mannosylated NLCs could increase the cellular uptake of bone marrow-derived macrophages 14.5 times greater than unmodified NLCs. These effects could significantly increase the antimycobacterial activity of rifampicin for mannosylated NLCs.[84] Another study reported that rifampicin-loaded mannosylated NLCs could improve the cellular uptake of NLCs by NR8383 alveolar macrophages 2.6-fold greater than that of non-mannosylated NLCs.[120] Song *et al.*[120] studied alveolar macrophage uptake *in vivo* in mice for rifampicin-loaded NLCs with and without a mannose coating. The results revealed that compared with unmodified NLCs, mannosylated NLCs could increase cellular uptake threefold *in vivo*.[120] However, non-mannosylated NLCs could also be internalised into A549 cells through non-specific endocytosis.[117]

Pharmacokinetic analysis revealed that compared to the free drug, mannosylated NLCs could increase lung deposition and $AUC_{0-\infty}$ by twofold.[118] Another study investigated mannosylated isoniazid NLCs with sustained release properties for 48 h. The $AUC_{0-\infty}$ increased approximately 1.17-fold compared to that of unmodified NLCs.[34] This could be affected by sustained release properties, leading to an extended half-life and lung retention time. In addition, surface modification of NLCs with mannose could induce adhesion to lung epithelial cells and decrease mucociliary clearance while enhancing cellular uptake and internalisation. As expected, the concentration of clofazimine found in the nontargeted tissue of non-mannosylated NLCs was greater than that in the mannosylated NLCs after 48 h. These results strongly indicated that compared to non-mannosylated NLCs, mannosylated NLCs could be more specific and deposited into the lungs.[118] Moreover, an *in vivo* acute inhalation toxicity study revealed no significant changes in histopathological examinations of lung, liver, or spleen rat organs after 14 days[118] or 4 weeks of study.[34] These results indicated that mannosylated NLCs could increase the safety and efficacy of targeted lung delivery for tuberculosis treatment.

However, mannose-functionalised NLCs still have several drawbacks. The mannose receptor can mistakenly interpret mannosylated NLCs as pathogens, triggering unwanted immune responses and pro-inflammatory cytokine release.[160] Furthermore, the cationic lipids used in mannose conjugation can increase cytotoxicity by disrupting cell membranes and inducing cell death.[161] Moreover, synthesizing structurally well-defined, multivalent mannosylated NLCs present significant chemical and engineering challenges. Precise control over ligand density and surface distribution is required to ensure optimal receptor binding and biological performance. Nevertheless, current conjugation strategies often suffer from low yields, heterogeneous surface modification, and batch-to-batch variability, limiting scalability, reproducibility, and translational feasibility in clinical applications.

4.2.2. Hyaluronic acid-functionalised nanostructured lipid carriers

Another polysaccharide that can be used to target lung cancer is hyaluronic acid. Hyaluronic acid is a polysaccharide that consists of D-glucuronic acid and *N*-acetylglucosamine.[162] Hyaluronic acid has been utilised as a natural ligand to target the CD44 receptor, which is overexpressed in numerous cancer cells.[89,163,164] The hyaluronic acid CD44 receptor was overexpressed in many tumour cells, while its low expression was found on the epithelial surface, as shown in **Figure 3**.[163,164] The binding of hyaluronic acid-functionalised NLCs to CD44 initiates cellular uptake through a clathrin-independent, lipid raft-mediated endocytic pathway.[165] Hyaluronic acid can be internalised after enzymatic reactions with hyaluronidase in cells. Chemical reactions can lead to the breakdown of hyaluronic acid chains and the protonation of ammonia groups in polyethylene imine. The positive charge of polyethyleneimine could enhance its interaction with the cell membrane and disrupt the lysosomal membrane.[166] Therefore, hyaluronic acid could be engineered on the surface of lipid nanoparticles to target lung cancer cells.

Hyaluronic acid can be utilised as a negatively charged polysaccharide that can target the CD44 protein because it is overexpressed in NSCLC. Mahmoudi *et al.*[123] studied the targeting of lung cancer cells by apigenin-loaded NLCs with hyaluronic acid as a surface modifier. The hyaluronic acid-functionalised NLCs retain a slightly positive charge due to cationic lipids in their formulation, which can provide electrostatic attraction with the negatively charged cell membrane. In addition, hyaluronic acid can bind to CD44 receptors, which are overexpressed in lung cancer cells and facilitate active targeting of the drug-loaded nanoparticles to tumour cells. Furthermore, the sustained release properties of apigenin-loaded hyaluronic acid-functionalised NLCs were

lower at pH 7.4 than at pH 5.5. These results suggested that apigenin could be released in the specific acidic environment of lung cancer cells rather than under basic or neutral conditions. Moreover, compared with unmodified NLCs, functionalised NLCs have a 1.5-fold lower IC_{50} value and greater cellular uptake in A549 cells. In addition, the tumour cell apoptosis rate of A549 cells in the hyaluronic acid-functionalised NLCs group was 36.64% greater than that in the unmodified NLCs group. This could be influenced by the high affinity of hyaluronic acid on the surface of NLCs for CD44 receptors in lung cancer cells.[123] Another study revealed that compared with unmodified NLCs, hyaluronic acid-decorated NLCs resulted in greater cell viability. In addition, hyaluronic acid pDNA NLCs resulted in significantly better gene transfection (approximately 40% at 72 h), with a value of only 30%, compared with unmodified NLCs.[121] Therefore, surface modification of NLCs with hyaluronic acid may be an appropriate method for targeted delivery in NSCLC.

Ma *et al.*[122] investigated the effects of different molecular weights of hyaluronic acid, which are 200–400 kDa and 1,300–1,600 kDa, on the *in vitro* tumour activity of kaempferol-loaded hyaluronic acid-functionalised NLCs (**Figure 5A**). Both shorter and longer chains of hyaluronic acid could enhance the sustained release properties due to an increased diffusion barrier, leading to prolonged tumour cell exposure. Unlike unmodified NLCs, surface modification with hyaluronic acid could decrease IC_{50} values. Interestingly, the lower molecular weight of hyaluronic acid could significantly decrease the IC_{50} values compared with those of the higher molecular weight (**Figure 5B**). In addition, an evaluation of the percentage of A549 cells that were positive using a 5-ethynyl-2'-deoxyuridine staining assay revealed that the lowest percentage of positive cells was associated with the shorter hyaluronic acid-functionalised NLCs, with a 16.20% higher percentage than the percentages related to the longer hyaluronic acid-functionalised NLCs (23.75%) and unmodified NLCs (40.68%). Furthermore, the shorter hyaluronic acid-functionalised NLCs also increased cellular uptake compared to the longer hyaluronic acid-functionalised NLCs and unmodified NLCs at 4 h (**Figure 5C**). These results suggest that hyaluronic acid,

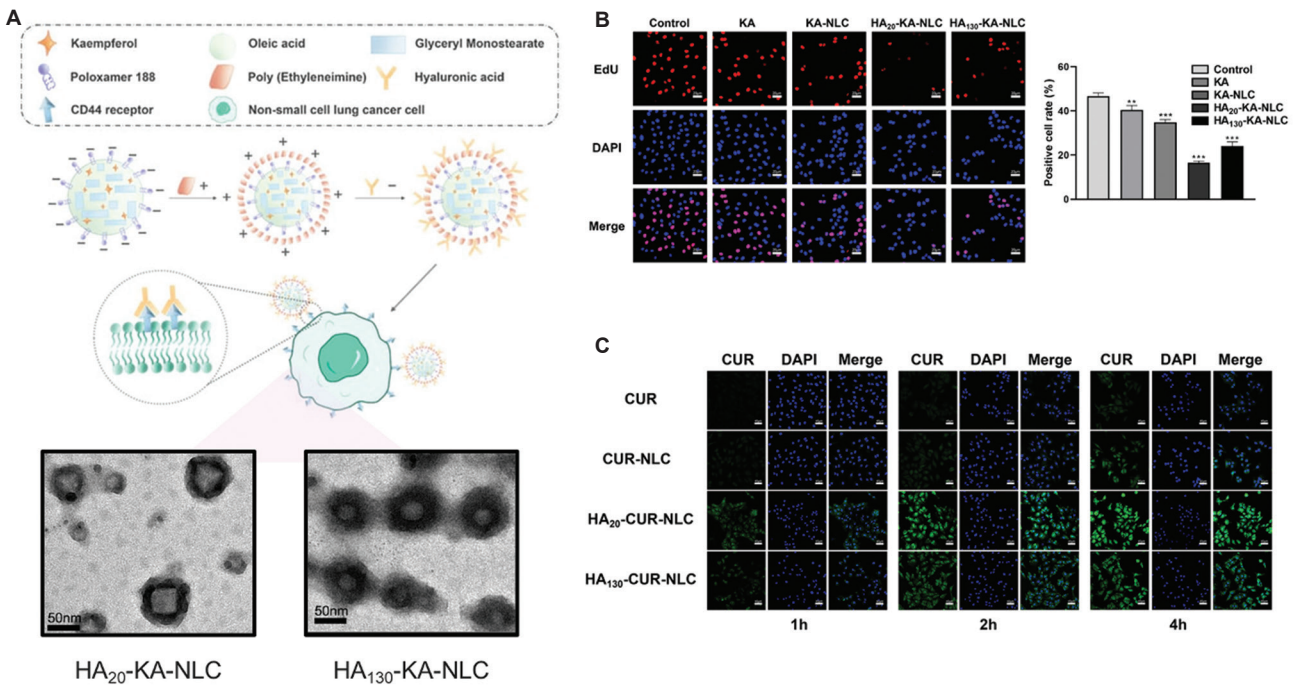

Figure 5. Characterisation and anticancer effects of hyaluronic acid-functionalised nanostructured lipid carriers loaded with kaempferol. (A) Schematic representation of kaempferol-loaded hyaluronic acid-functionalised nanostructured lipid carriers with transmission electron microscope images indicating nanostructured lipid carriers functionalised with 20 kDa hyaluronic acid (HA_{20}-KA-NLC) and 130 kDa hyaluronic acid (HA_{130}-KA-NLC). Scale bar: 50 nm. (B) The results of the EdU assay revealed that, compared with pure kaempferol, kaempferol-loaded nanostructured lipid carriers significantly reduced the cell proliferation rate, with further enhancement in inhibition when functionalised with hyaluronic acid. Among the treatments, hyaluronic acid 20 kDa-functionalised kaempferol-loaded nanostructured lipid carriers demonstrated the most potent antitumour activity, with the lowest positive cell rate in A549 cells. Scale bar: 20 μm(C) The stronger fluorescence in the hyaluronic acid 20 kDa-functionalised curcumin nanostructured lipid carrier (HA_{20}-CUR-NLC) and 130 kDa-functionalised curcumin nanostructured lipid carrier (HA_{130}-CUR-NLC) groups highlights the enhanced cellular uptake due to hyaluronic acid, confirming its key role in tumour targeting and penetration through CD44 receptor-mediated endocytosis in non-small cell lung cancer cells. Scale bar: 40 μm. Reprinted with permission from Ma *et al.*[122] Copyright, 2022 MDPI.

Abbreviations: A549: human lung carcinoma cell line; CD44: cluster of differentiation 44; CUR: Curcumin; DAPI: 4',6-diamidino-2-phenylindole; EdU: 5-ethynyl-2'-deoxyuridine; HA_{20}-CUR-NLC: Hyaluronic acid 20 kDa-functionalised curcumin nanostructured lipid carriers; HA_{20}-KA-NLC: Hyaluronic acid 20 kDa-functionalised kaempferol-loaded nanostructured lipid carriers; HA_{130}-CUR-NLC: Hyaluronic acid 130 kDa-functionalised curcumin nanostructured lipid carriers; HA_{130}-KA-NLC: Hyaluronic acid 130 kDa-functionalised kaempferol-loaded nanostructured lipid carriers; KA: Kaempferol; NLCs: Nanostructured lipid carriers.

which has a relatively low molecular weight, had the best antitumour activity. This might be caused by the shorter chains of hyaluronic acid, which CD44 receptors in lung cancer cells could recognise more than the longer chains, leading to greater intracellular accumulation and internalization.[122]

Despite the presence of the targeting ligand, hyaluronic acid-functionalised NLCs often exhibit poor accumulation and penetration into solid tumours. This is partly due to the dense extracellular matrix of tumours, which acts as a physical barrier. Moreover, non-specific targeting remains a concern due to the widespread presence of CD44 and other hyaluronic acid receptors on healthy cells. Although these receptors are overexpressed in lung cancer cells, their broad expression in normal tissues can compromise targeting specificity. This can lead to premature binding of NLCs to circulating blood cells, significantly hindering their systemic transport and reducing delivery efficiency to the tumour site. In addition, this non-specific interaction contributes to off-target accumulation in healthy organs, raising concerns about unintended toxicity and reduced therapeutic selectivity.[167]

4.3. Small molecules

Numerous small molecules, such as folic acid and biotin, can be decorated onto the surface of lipid nanoparticles due to the overexpression of receptors in cancer cells. This strategy exploits the upregulated metabolic activity of cancer cells, which often overexpress receptors for essential vitamins. Folic acid or folate is a high-affinity ligand for the folate receptor-α, which is abundant in lung cancers but restricted in healthy tissues. On binding, the folate-NLC conjugate is rapidly internalised through receptor-mediated endocytosis into an endosome (**Figure 3**).[90,91] As the endosome matures, its internal pH drops to approximately 5.0, inducing a conformational change in the folate receptor-α that releases the drug from the folate-NLC. This allows the drug to escape into the cytoplasm, while the empty receptor is recycled back to the cell surface.[168] Similarly, biotin or Vitamin B7 targets receptors like the sodium-dependent multivitamin transporter, which are also overexpressed on cancer cells to meet their high metabolic demands. The binding of biotin-functionalised NLCs triggers an analogous receptor-mediated endocytic process, leading to the selective and efficient accumulation of the therapeutic payload within malignant cells (**Figure 3**).[169] These properties can lead to sustained release, high cellular uptake, and internalisation through vitamin receptor-mediated endocytosis.[92,128,170]

4.3.1. Folic acid-based nanostructured lipid carriers

Folate decorated on the surface of docetaxel- and curcumin-loaded NLCs was developed to improve their bioavailability and anticancer activity against NSCLC. The *in vitro* release kinetics revealed that the folate-functionalised NLCs had sustained release properties, with 90% of the drug released at 120 h. In addition, the IC_{50} values of folate-functionalised NLCs were significantly lower than those of unmodified NLCs, which strongly indicate that surface modification with folate could improve their cytotoxicity. Moreover, the cellular uptake of folate-functionalised NLCs by NCI-H460 cells was significantly greater than that of unmodified NLCs. The increased cellular uptake and internalisation could be caused by the binding affinity of folate with overexpressed folate receptors on NCI-H460 cells.[127] Similarly, paclitaxel-loaded folate-functionalised NLCs exhibited a significantly greater fluorescence intensity than A549 cells, which signifies the highest cellular uptake of folate-functionalised NLCs.[125] Moreover, the *in vivo* pharmacokinetics study revealed that folate-functionalised NLCs could improve the bioavailability of docetaxel by 12.39-fold compared to free drugs and 2.61-fold compared with unmodified NLCs. Unlike unmodified NLCs and free drugs, folate-functionalised NLCs could also increase the tumour inhibition rate and reduce the immune expression of mutated p53 and the cell proliferation marker Ki67. This could be influenced by enhanced cellular uptake and internalisation through folate receptor-mediated endocytosis due to the overexpression of folate receptors in lung cancer cells.[127]

The main drawbacks of folate-functionalised NLCs include heterogeneous and non-exclusive receptor expression, as folate receptor is variably expressed across tumours and also found on healthy tissues, including kidneys and activated macrophages, leading to limited patient eligibility and off-target effects.[171] In addition, endogenous circulating folates compete with folate-functionalised NLCs for receptor binding, which can reduce the targeting efficiency.[172] Moreover, folic acid may promote tumour growth by supplying nutrients to cancer cells. Folic acid is an essential nutrient required for nucleotide synthesis and DNA repair in proliferating cancer cells, and it may promote tumour growth by supplying these critical nutrients to the cancer cells.[173] Furthermore, variability in both ligand density on the nanoparticle and receptor density on the cell can lead to insufficient receptor-mediated internalisation and prevent effective intracellular drug delivery.[174]

4.3.2. Biotin-based nanostructured lipid carriers

Taymouri *et al.*[128] engineered sunitinib-loaded NLCs with biotin as a surface modification for lung cancer-targeted treatment. The *in vitro* cytotoxicity results revealed that the IC_{50} values of biotin-decorated NLCs were lower than those of undecorated NLCs and free drugs. Furthermore, compared with undecorated NLCs, biotin-decorated NLCs resulted in greater cellular uptake by A549 cells. These results suggested that biotin-decorated NLCs were positively charged and could attach to negatively charged cell membranes. Therefore, the retention time could be prolonged, and cellular uptake could be enhanced through biotin receptor-mediated endocytosis.[128]

Biotin-conjugated nanoparticles encounter significant challenges primarily due to an ambiguous cellular uptake mechanism. The essential carboxylic acid group on biotin required for recognition by the sodium-dependent multivitamin transporter is typically chemically modified during conjugation. However, the process of conjugating biotin to NLCs involves the modification of this carboxylic acid group, which normally forms an amide or ester bond.[175] Moreover, clinical translation remains limited despite encouraging preclinical data due to these mechanistic uncertainties and difficulties in developing

stable, reproducible formulations that retain *in vivo* targeting functionality.[169] In addition, the widespread expression of biotin transporters on normal tissues can reduce tumour specificity, which can increase the risk of off-target delivery and associated toxicities.[92,175]

4.3.3. N-acetyl-D-glucosamine-based nanostructured lipid carriers

Gemcitabine- and paclitaxel-loaded NLCs were decorated with *N*-acetyl-D-glucosamine (NAG) to increase cellular uptake and internalisation in NSCLC cells. The *in vitro* cellular uptake of NLCs by A549 cells overexpressing glucose receptors showed that NAG-decorated NLCs could enhance cellular internalisation through glucose receptor-mediated endocytosis. NAG could be utilised as a ligand to target overexpressed glucose receptors in lung cancer cells.[93] However, glucose transporter-targeting carries a high risk of severe off-target toxicity, as glucose transporters are expressed in vital organs dependent on glucose metabolism, such as the brain and heart.[176] In addition, the complexity and tissue-specific expression of multiple glucose transporter isoforms can limit the tumour selectivity.[177,178]

4.4. Surfactants

Surfactants can play a key role in pulmonary drug delivery by acting as ligands that enhance drug interaction with lung biological membranes, improving absorption, targeting, and overcoming physiological barriers, such as mucus and cellular defences. Their amphiphilic nature facilitates better drug dispersion, stability, and cellular uptake within the respiratory tract, which is especially important for effective treatment. In addition, surfactants can help address multidrug resistance by inhibiting drug efflux mechanisms and improving intracellular drug accumulation, making them promising carriers for inhalation therapies against resistant respiratory diseases and tumours.[179,180] The primary mechanism involves the inhibition of adenosine triphosphate-binding cassette efflux transporters, such as P-glycoprotein, which are overexpressed on resistant tumour cells and actively pump chemotherapeutic agents out of the cytoplasm.[181]

A prominent example is d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS), a non-ionic surfactant derived from Vitamin E with biocompatibility and permeation-enhancing properties.[95] This surfactant can enhance cellular uptake and internalisation through endocytosis and efflux inhibitor effects.[94,182] TGPS primarily targets cellular membranes by enhancing drug uptake through stimulation of endocytosis. TPGS targets and destabilises the mitochondria within the cancer cell, which can lead to mitochondrial dysfunction. In addition, TPGS acts as an inhibitor of efflux transporters such as P-glycoprotein, which normally pump drugs out of cells and reduce their intracellular concentration. By inhibiting these efflux pumps, TPGS increases the retention, intracellular accumulation, enhanced cytotoxicity, and bioavailability of therapeutic agents within cells. In addition, TPGS can also increase drug solubility and control the release of drugs as well as drug safety and efficacy, which can be utilised in the case of multidrug resistance in various tumour cells.[183]

Sherif *et al.*[129] studied gefitinib-loaded NLCs engineered with TPGS for the lymphatic metastasis of lung cancer. TPGS-decorated NLCs showed sustained release properties for up to 12 h. The IC_{50} value of TGPS-functionalised NLCs against A549 cells decreased to 7.01 μg/mL, whereas the values for the unmodified NLCs and pure drug were 15.05 μg/mL and 11.16 μg/mL, respectively, after 24 h of incubation. Furthermore, compared to the free drug, TGPS-functionalised NLCs could also enhance cytotoxicity and induce cell apoptosis, significantly decreasing the number of living cells by 58–67% (approximately threefold). TGPS, as surfactant-decorated NLCs, could enhance the bioactivity of gefitinib and its cellular uptake through endocytosis with efflux inhibitor characteristics.[129]

A major drawback of surfactant-functionalised NLCs is their inherent cytotoxicity, as their ability to interact with and disrupt lipid bilayers can damage cell membranes, resulting in leakage and cell death.[184] Moreover, exogenous surfactants can significantly interfere with the endogenous pulmonary surfactant layer, leading to unpredictable spreading and deposition of drug carriers within the airways. These effects are highly variable between patients, depending on individual differences in surfactant composition, which can be further influenced by disease conditions such as asthma or cystic fibrosis.[185] As a result, the amount of drug released and absorbed in the lungs becomes unpredictable and inconsistent.

4.5. Peptides and proteins

Peptide and protein-functionalised lipid nanoparticles can target ligands to specific sites. They function as high-affinity ligands for specific cell surface receptors that are dysregulated in disease. Peptides and proteins can enter the cell without interrupting membrane integrity, enhancing receptor-mediated endocytosis and direct translocation to improve cellular uptake and internalization.[186,187] To date, various peptides and proteins, such as bombesin, tuftsin, polyarginine, wheat germ agglutinin, and transferrin, can be decorated on the surface of lipid nanoparticles.

Bombesin is a linear tetra-decapeptide with EQRLGNQWAVGHLM that can be overexpressed in several types of tumour cells, such as lung cancer, prostate cancer, colon cancer, and breast cancer.[188,189] Hence, bombesin can be a target ligand for lung cancer cells. Bombesin receptor-targeting peptides can bind to the gastrin-releasing peptide receptor, which is overexpressed in non-small and small cell lung carcinoma. Tuftsin is a tetrapeptide with a sequence of L-threonine, L-lysine, L-proline, and L-arginine that can bind and stimulate various immune cells, such as macrophages and dendritic cells. Therefore, tuftsin has been utilised as a surface modifier to target macrophages.[97,190] Synthetic epidermal growth factor receptor (EGFR)-binding peptides specifically recognise and bind to EGFRs, which are overexpressed on lung cancer cells, triggering receptor-mediated endocytosis to enable targeted drug delivery. Arginine-rich peptides or polyarginine can be cell-penetrating peptides in various cancer cells, including lung cancer cells.[100] This peptide can escape the RES and enhance cellular uptake with specific targeting

into cells so that systemic toxicity can be reduced.[191-193] Furthermore, the positively charged guanidinium groups on arginine residues strongly interact with negatively charged cell membrane components, such as proteoglycans and phospholipids. This interaction can destabilise the membrane for direct NLC entry into the cytoplasm or activate endocytic pathways, thereby greatly improving intracellular delivery of the therapeutic cargo. Moreover, transferrin can be used to target transferrin receptors that are overexpressed in tumour cells.[103] This receptor can be used to target and facilitate the penetration of numerous chemotherapy drugs into cells through endocytosis.[102]

Peptides and proteins, while offering high specificity in targeted drug delivery, present several critical disadvantages. Their inherent instability makes them highly susceptible to enzymatic degradation by proteases in biological fluids, leading to rapid loss of structure, function, and *in vivo* activity.[194,195] In addition, their complex 3D structures are sensitive to physical and chemical environments, such as pH changes, temperature fluctuations, and mechanical forces, which can cause denaturation or aggregation, reduce efficacy, and trigger immune responses.[196] Furthermore, the large size and hydrophilic nature of peptides and proteins hinder their ability to penetrate biological barriers such as mucus layers and tight epithelial junctions, which can limit their bioavailability at the target site.[197,198]

4.5.1. Bombesin-based nanostructured lipid carriers

Bombesin receptors can be overexpressed in several types of tumour cells, including lung cancer cells. Du and Li[96] investigated the effects of decoration methods incorporating doxorubicin and DNA for lung cancer treatment, which involved pre-assembly, where nanoparticles were prepared with a target ligand, and post-assembly or surface modification with a target ligand. The *in vitro* release study revealed that post-assembly decorated NLCs were released more slowly than pre-assembly undecorated NLCs. The IC_{50} values of post-assembly were twofold, threefold, and sixfold better than those of pre-assembly, undecorated NLCs, and free drugs, respectively. In addition, *in vitro* and *in vivo* gene transfection studies revealed that post-assembly had the highest transfection level at 72 h. Similarly, the *in vivo* antitumour activity also revealed that post-assembly NLCs had the highest tumour inhibition rate of approximately 76%, which was 1.5-fold, 2.1-fold, and 5.6-fold greater than that of pre-assembly, undecorated NLCs, and free drugs, respectively. These results showed that compared to pre-assembly and undecorated NLCs, post-assembly NLCs were more effective at treating lung cancers. This could be influenced by the electrostatic interaction between the positive charge on the NLC's surface and the negative charge of the cell membrane, which enhances the targeting affinity of bombesin-decorated NLCs and facilitates their cellular uptake through endocytosis. However, pre-assembly bombesin-decorated NLCs were not significantly different from undecorated NLCs because the ligands could be encapsulated in the NLCs' matrix system, reducing their effectiveness and selectivity for the target ligands.[96]

4.5.2. Tuftsin-based functionalised peptide

Rifampicin-loaded tuftsin-decorated NLCs were developed by Carneiro *et al.*[130] to improve macrophage uptake and antimycobacterial activity. *In vitro* release showed that lower drug release was achieved at pH 7.4 for 72 h, with only 18% drug release. This result suggested that release could be affected by the lower pH of the phagolysosome, with minimum release at non-targeted sites. Furthermore, the *in vitro* uptake measurements of the mean fluorescence intensity revealed that, compared to unmodified NLCs, peptide-functionalised NLCs improved cellular uptake and internalisation by 2.5- and 3.6-fold, respectively, after 30 min and 24 h of incubation. Due to its high degree of internalisation, the efficacy of peptide-functionalised NLCs could also be enhanced twofold compared with free drugs against *M. tuberculosis*. Moreover, compared with rifampicin solution, peptide-functionalised NLCs could also improve cell viability.[130]

4.5.3. Epidermal growth factor-targeting peptide

AEYLR is a small peptide with the highest binding activity to EGFR, which is overexpressed in NCI-H1299 cells, with a 93.09% binding rate. Therefore, the AEYLR peptide can be utilised for targeted and selective binding due to its high affinity for EGFR. *In vitro* flow cytometric analysis revealed that, compared to unmodified NLCs, peptide-functionalised NLCs had a greater fluorescence response. This could be influenced by the active targeting of NLCs due to surface modification with small peptides, which could lead to increased affinity, enhanced cellular uptake, and stimulated cell proliferation.[98]

4.5.4. CD133+ targeting peptide

The $CD133^+$ targeting peptide with the TISWPPR sequence was attached to the surface of the salinomycin-loaded NLCs, whereas the AEYLR peptide was decorated on the PEG-functionalised paclitaxel-loaded NLCs. An *in vitro* targeting assay revealed that the targeting peptide could increase the affinity of NLCs for $CD133^+$ cancer cells, resulting in increased cellular uptake and internalisation against NCI-H1299 and S180 cells. Furthermore, the ability of the peptide-functionalised NLCs to inhibit cell proliferation was also improved twofold compared with that of unmodified NLCs and fourfold greater than that of free drugs. Moreover, an *in vivo* fluorescence assay revealed that, compared to unmodified NLCs, the targeting peptide-functionalised NLCs highly accumulated in lung cancer cells, resulting in significantly reduced tumour volume. This targeting mechanism could involve passive and active targeting of lung cancer cells. For passive targeting, NLCs could be targeted into leaky tumour vasculature and enhance cellular uptake through EPR effects, whereas targeting peptides could specifically bind with $CD133^+$ receptors on cancer cells and enhance their internalisation into tumour cells.[99]

4.5.5. Polyarginine

Polyarginine (R8), cell-penetrating peptide-functionalised NLCs, was designed to enhance the cellular uptake and cytotoxicity of paclitaxel-loaded NLCs. An *in vitro* cellular uptake study in A549 cells revealed that compared to

unmodified NLCs, polyarginine-functionalised NLCs could increase cellular internalisation by fourfold. This may be attributed to the rich arginine structure with a highly positive charge, which could be strongly attached to the negatively charged cell membrane. In addition, the cellular uptake mechanism of polyarginine-functionalised NLCs was mainly endocytosis with energy, clathrin, and caveolin mediation.[131]

4.5.6. Wheat germ agglutinin-based nanostructured lipid carriers

Hädrich *et al.*[101] developed wheat germ agglutinin (WGA)-decorated NLCs to improve the cellular uptake and internalisation of quercetin. The *in vitro* cellular uptake study in J774. A1 cells revealed that WGA-functionalised NLCs improved the cellular internalisation of unmodified NLCs and the free drug by 3.1-fold and 14.6-fold, respectively. In addition, the fluorescence intensity increased with increasing concentrations of drugs. There were several potential mechanisms for cellular uptake of WGA, including mucoadhesion followed by endocytosis.[101]

4.5.7. Transferrin-functionalised nanostructured lipid carriers

Han *et al.*[133] investigated transferrin linked to PEG-phosphatidyl-ethanolamine conjugates as a surface modification of NLCs to deliver gene therapy. Compared to unmodified NLCs, transferrin-functionalised NLCs had sustained release at 72 h and greater transfection efficiency at 48 and 72 h *in vitro* against A549 cells and *in vivo* against A549 tumours in mice. These results suggested that transferrin could enhance active cell targeting to overexpress transferrin receptors in A549 solid tumour cells.[133] Other studies also reported that transferrin-functionalised doxorubicin and DNA exhibited increased transfection efficiency *in vivo* in mice and increased tumour inhibition rates against A549 tumour cells.[134] Moreover, compared to unmodified NLCs, transferrin-decorated pDNA-loaded NLCs had significantly greater *in vitro* gene transfection efficiency in A549 cells (approximately 40% and 50% at 36 and 72 h, respectively) than unmodified NLCs (only 30%). In addition, the dual-target ligand of transferrin and hyaluronic acid could significantly improve gene transfection efficiency *in vitro* in A549 cells and *in vivo* in mice.[121]

Transferrin-decorated paclitaxel and DNA-loaded NLCs can enhance active targeting properties, improving antitumour activity and gene transfection efficiency. Shao *et al.*[132] studied the effects of transferrin types with different molecular weights, which are 5,000 and 10,000 Da. Transferrin-functionalised NLCs had a positive charge on their surface, which could improve their binding affinity to the cell membrane and prolong their residence time. However, the lower molecular weight of transferrin has a significantly greater zeta potential and faster release than the higher molecular weight. The IC_{50} values of the shorter transferrin-functionalised NLCs were fourfold greater than those of the free drug and approximately twofold greater than those of the longer transferrin-functionalised NLCs and unmodified NLCs. Furthermore, the *in vivo* antitumour efficacy study in NCl-H460 cell-bearing mice also revealed that the tumour regression rate of 5,000 Da transferrin-functionalised NLCs was slower than that of 10,000 Da transferrin-functionalised NLCs and unmodified NLCs. Similarly, *in vitro* and *in vivo* transfection revealed that the highest transfection efficiency was associated with the lower-molecular-weight of transferrin-functionalised NLCs. This might be because the longer chain of transferrin could block and restrict drug release.[132]

4.6. Genes

Gene-targeted therapy can also be utilised in pulmonary-targeted drug delivery systems to cure various defective genes. Gene-based functionalisation can modulate cellular processes at the genetic level, offering a highly specific therapeutic approach for diseases driven by defective gene expression, such as cancer or fibrosis. Various gene therapies, including small interfering (si)RNAs and DNases, can mediate cell apoptosis and gene replacement, which can enhance the drug efficacy and restore normal gene growth regulation.[104,199] For example, the *MMP3*, *CCL12*, and *HIF1A* mRNAs could be used to inhibit lung fibrosis damage-induced proteins.[135]

Small interfering RNA-NLC complexes harness the RNA interference pathway. Once inside the cell, the siRNA is released into the cytoplasm, where it associates with the RNA-induced silencing complex, which uses one strand of the siRNA to locate and degrade the matching target mRNA, blocking protein production.[200] In lung cancer therapy, this strategy is used to overcome multidrug resistance by co-delivering siRNAs with chemotherapy to silence genes such as *MRP1* and *BCL2*, thereby restoring the sensitivity of cancer cells to treatment.[31,201] DNase-modified NLCs use an enzymatic approach to bypass physical barriers, especially in treating bacterial infections in cystic fibrosis.[202,203] Pathogens, including *P. aeruginosa*, form biofilms rich in extracellular DNA, which protect them from antibiotics and immune defences. By attaching DNase to the surface of antibiotic-loaded NLCs, the system can break down the extracellular DNA, disrupting the biofilm structure and enabling the NLCs to penetrate and deliver antibiotics directly to the bacteria, greatly improving antimicrobial effectiveness.[137]

However, gene-functionalised NLCs have numerous challenges that significantly limit their efficacy and safety. In the extracellular environment, siRNA is highly unstable and is rapidly degraded by nucleases, resulting in a serum half-life of only minutes. Its small size, negative charge, and hydrophilicity also make it prone to rapid clearance by renal filtration and the reticuloendothelial system, leading to negligible bioavailability.[204] Although NLC carriers can enhance cellular uptake through endocytosis, siRNA must still overcome the crucial barrier of escaping the endosome to access the RNA-induced silencing complex in the cytoplasm. Failure to achieve endosomal escape exposes siRNA to lysosomal compartments, undergoing enzymatic degradation. This degradation compromises the integrity of the siRNA and results in a loss of therapeutic activity.[205,206] In addition, siRNA poses safety concerns due to off-target gene silencing caused by partial sequence complementarity, as well as innate immune activation through recognition by pattern recognition receptors like toll-like receptors, potentially triggering systemic inflammation and toxicity.[204]

4.6.1. Small interfering RNA-nanostructured lipid carrier complexes

Prostaglandin E combined with siRNA and luteinizing hormone-releasing hormone encapsulated in NLCs can be utilised to treat fibrosis and inflammatory lung damage. Garbuzenko *et al.*[135] studied three potential sequences of siRNAs to inhibit protein synthesis in fibrotic lung damage: the *MMP3*, *CCL12*, and *HIF1A* mRNAs. The prostaglandin E and siRNA combination could significantly decrease targeted gene expression and induce profibrotic gene expression in mice. This could decrease lung inflammation and reduce the 3.8-fold volume of fibrotic lung tissue in mice after 3 weeks of inhalation. The possible mechanism of the anti-inflammatory effect of the combination of prostaglandin E and siRNA is the use of a transforming growth factor beta inhibitor.[135]

Small interfering RNAs can also be complexed with anticancer drugs, such as doxorubicin or paclitaxel, in the NLC matrix system. The siRNA complexes were engineered to target *MRP1* and *BCL2* mRNAs for pump and non-pump drug resistance suppressors in lung cancer treatment. The *in vitro* cytotoxicity results revealed that the effects of the siRNA complexes were 120-fold and 16-fold greater than those of the free drug and unloaded siRNA, respectively. Furthermore, after 3 h of incubation, the green fluorescence dye-labelled siRNA complexes were highly internalised into the cytoplasm of A549 lung cancer cells. Moreover, the siRNA complexes significantly decreased the expression of the *MRP1* and *BCL2* genes, whereas the unloaded siRNA increased the expression of *MRP1* and *BCL2*. The *in vivo* antitumour activity of siRNA complexes also revealed that the smallest volume or almost completely absent tumours were found after 24 days of inhalation treatment.[136]

4.6.2. DNase-functionalised nanostructured lipid carriers

DNase was incorporated into levofloxacin-loaded NLCs to enhance levofloxacin delivery to specific sites of lung cystic fibrosis. DNase-decorated NLCs can reduce and disrupt the biofilm formation of *S. aureus* and *P. aeruginosa,* leading to bacterial membrane damage and integrity loss. The incorporation of DNase can be utilised to improve the antimicrobial activity of levofloxacin to overcome the mucus barrier in cystic fibrosis patients.[137]

4.7. Antibodies

Monoclonal antibodies can be used for targeted delivery systems in various cancer treatments.[207] Monoclonal antibodies represent the highly specific targeting capable of recognizing a single and unique epitope on a tumour-associated antigen with exceptionally high affinity. Antibodies can enhance the binding affinity to specific ligands, leading to prolonged retention time and enhancing cellular uptake while minimizing off-target sites and immunogenicity.[106] Antibodies can stimulate the immune response to eliminate cancer cells without damaging normal cells.[208] Furthermore, antibodies can also increase the specificity of overexpressed tumour-associated antigens.[209] Several pathways are involved in the cellular internalisation of antibodies, such as endocytosis, transcytosis, and lysosomal degradation.[210] When functionalised to an NLC, the antibody specifically targets cancer cells by binding to its corresponding antigen. This triggers receptor-mediated endocytosis, allowing the entire nanoparticle complex to be internalised. Numerous antibodies, such as intercellular adhesion molecule 1 (ICAM-1) and cetuximab, have been engineered to bind to the surface of lipid nanoparticles. This can be a specific antibody that can bind to specific overexpressed receptors in lung cancer cells, such as the epithelial cell adhesion molecule receptor and EGFR.[107] **Figure 3** shows the mechanism of receptor-mediated endocytosis.

The development of antibody-functionalised NLCs still possesses significant challenges, including complex and heterogeneous conjugation processes that affect drug-to-antibody ratios and antibody orientation, leading to variable efficacy and increased immunogenicity.[211] The large size of monoclonal antibodies can limit tumour penetration due to restricted extravasation and the binding site barrier, which can cause uneven drug distribution and low accumulation within solid tumours.[212] In addition, many tumour antigens targeted by these conjugates are also expressed on normal tissues, resulting in on-target, off-tumour toxicity and a narrow therapeutic window.[213] Furthermore, tumours can acquire resistance through antigen downregulation, mutation, or altered intracellular processing, which reduces treatment effectiveness over time.[214,215]

4.7.1. ICAM 1 antibody-functionalised nanostructured lipid carriers

ICAM 1 antibodies have been utilised to target simvastatin-loaded NLCs for acute lung injury treatment. The *in vitro* cellular uptake of ICAM-1 antibody-functionalised NLCs by EAhy926 cells was greater than that of non-functionalised NLCs. Furthermore, an *in vivo* study in mice revealed that the fluorescence intensity of ICAM-1 antibody-functionalised NLCs had a higher signal in the ICAM-1 overexpressed mice than in healthy mice. Moreover, the highest drug accumulation was found in the pulmonary region rather than in non-target sites.[138] Similarly, ICAM-1 antibody-functionalised dexamethasone-loaded NLCs presented a greater mean fluorescence intensity than IgG-functionalised NLCs did.[105] This may be influenced by the overexpression of ICAM-1 on the cell surface, which could facilitate the active targeting of ICAM-1 epitopes and lead to ICAM-mediated endocytosis.[138]

4.7.2. Cetuximab-functionalised nanostructured lipid carriers

Guo *et al.*[108] functionalised cetuximab with paclitaxel and 5-demethylnobiletin co-loaded NLCs for synergistic treatment of NSCLC. The *in vitro* cellular uptake in A549 cells revealed that, compared to unmodified NLCs, cetuximab-functionalised NLCs had a greater cellular internalisation efficiency of 65.8%, with a 35.5% greater cellular uptake efficiency. Moreover, compared with the free drugs (approximately 0.6) and unmodified NLCs (approximately 0.5), the cetuximab-functionalised NLCs also presented the smallest combination index values of approximately 0.4. Furthermore, the *in vivo* antitumour activity of the cetuximab-functionalised NLCs was significantly greater than that of the free drug and unmodified NLCs. These results may be caused by the active targeting of cetuximab to cancer cells, especially EGFR, which could induce lung cancer cell apoptosis without destroying normal cells.[108]

4.8. pH-sensitive nanostructured lipid carriers

pH-sensitive drug delivery systems can be considered to target lung cancer cells due to the acidic microenvironment of tumour cells.[216] Several chemical bonds can be specifically attached to the surface of lipid nanoparticles, such as imines, hydrazones, oximes, amides, and acetals.[109] This chemical reaction can be responsive because it triggers acidic conditions. In other words, the drug can be released at a lower pH of the tumour microenvironment but is restricted to the basic or normal pH of non-target cells.[217,218] pH-responsive NLCs utilise the acidic tumour microenvironment for triggered drug release through two primary molecular mechanisms. The first involves acid-sensitive linkers, such as hydrazones or acetals, which attach the drug to the carrier and break down under low pH conditions to release the drug. A hydrazone bond undergoes acid-catalyzed hydrolysis initiated by the protonation of its imine nitrogen, followed by a nucleophilic attack from water, leading to bond cleavage and release of the free drug.[219,220] Similarly, an acetal linkage is stable at neutral pH but rapidly hydrolysed in acid. The mechanism involves protonation of an ether oxygen to create a resonance-stabilised oxonium ion, and subsequent attack by water to break the bond.[221,222] The second mechanism incorporates pH-responsive polymers containing ionizable groups, such as tertiary amines. At physiological pH (7.4), these polymers are neutral and hydrophobic. However, on entering the acidic environment of an endosome (pH < 6.5), the amine groups become protonated. The resulting accumulation of positive charges creates strong electrostatic repulsion along the polymer backbone, causing it to rapidly swell or dissolve, physically disrupting the structure of NLCs and triggering a burst release of its entire payload.[216,223,224]

Doxorubicin and β-elemene were encapsulated in NLCs with a pH-sensitive polymer, methoxy (polyethylene glycol) 2000- hydrazone- 1,2-distearoyl-sn-glycero-3-phosphoethanolamine. Surface modification with pH-sensitive polymers can increase the dissolution rate under acidic conditions (pH 5.5) and decrease the dissolution rate under basic conditions (pH 6.8). This might be caused by conformational changes due to pH alterations in the acidic environment of tumour cells. An *in vitro* uptake and cytotoxicity study in A549/ADR cells revealed that pH-sensitive NLCs had greater cellular uptake and synergistic effects than non-pH-sensitive NLCs. Furthermore, the *in vivo* study of A549/ADR cell-induced mice revealed high accumulation of drugs in lung cancer tumours and a high tumour inhibition rate of 82.9% on day 18 after pH-sensitive NLCs administration. pH sensitivity could enhance cellular uptake and antitumour activity due to the acidic environment of lung cancer cells, which could enhance the EPR effects for tumour targeting and cellular internalisation through endocytosis.[139]

Designing effective pH-sensitive drug delivery systems requires a careful balance where the linker must remain stable at the physiological pH of 7.4 to prevent premature drug release during circulation, yet cleave rapidly in acidic environments such as tumours.[225] Furthermore, pH-based targeting lacks specificity because acidity is not unique to tumours but is also found in sites of inflammation, infection, and within normal cellular compartments, including endosomes and lysosomes. This widespread acidity can cause off-target drug release and reduce therapeutic precision.[226,227] In addition, chemical and manufacturing limitations restrict the use of pH-sensitive linkers because many drugs lack the necessary functional groups for conjugation, and degradation products from these linkers may introduce toxicity concerns, complicating clinical development.

5. Challenges of nanostructured lipid carriers for pulmonary-targeting delivery systems

5.1. Pulmonary-specific targeting

To reach the lateral stage of the lung, the particle size must be small enough to reach the alveoli but not so small that they are easily removed by the pulmonary defence system, such as alveolar macrophages. Most pulmonary delivery systems require devices to deliver drugs into the deeper lung region, such as nebulisers, metered-dose inhalers, and dry powder inhalers. The optimal particle size is 1–5 μm to reach the alveoli. Large particles are deposited in the upper respiratory tract, whereas small particles may be lost during exhalation. Conversely, the macrophage as a lung defence system can also be a target for delivery; for example, *M. tuberculosis*, the bacteria reside inside alveolar macrophages. Hence, delivering drugs directly to macrophages can ensure the medication reaches the infection site more effectively.[84] Alveolar macrophages can defend against inhaled microbes and foreign particulates with a 1–3 μm diameter. Macrophages can remove insoluble particles accumulated in the alveoli by phagocytosis.[228]

A critical challenge specific to pulmonary delivery is the need to formulate the NLCs into a dosage form compatible with inhalation devices, such as nebulisers, metered-dose inhalers, or dry powder inhalers, while maintaining the desired aerodynamic properties for deep lung deposition.[229] NLCs must be engineered to be compatible with the chosen device and remain stable during aerosolisation. The formulation must withstand the mechanical stress and potential temperature changes for nebulisers without causing the nanoparticles to aggregate or degrade. In metered-dose inhalers, NLCs must be compatible with the propellant to prevent coalescence or changes in particle size. The NLCs must be formulated into a stable solid-state powder with suitable aerodynamic properties for dry powder inhalers to ensure efficient aerosolisation and deep lung deposition on inhalation. The instability during these processes can compromise the effectiveness and safety of the therapy.[4]

Furthermore, a major drawback of conventional NLCs is their low selectivity for target cells, which can lead to off-target effects, increased toxicity, and reduced therapeutic efficacy. The non-selective uptake by various cell types can decrease drug deposition at the intended site and shorten the half-life, potentially leading to ineffectiveness and the development of multidrug resistance. Functionalisation with specific ligands aims to overcome the non-selectivity, but it has further complexity. A critical challenge for active targeting is the variable level of receptor overexpression on

target cells. For instance, lung cancer often exhibits significant tumour heterogeneity between patients, which complicates the effectiveness of targeted therapy. The expression of a target receptor can differ significantly between patients, between metastatic sites in the same patient, and even within a single tumour. This variability presents a major challenge in designing universally effective targeted NLCs across different patient populations. Since therapeutic success relies on the consistent expression of target receptors, inconsistent or low receptor presence can limit efficacy.[230]

To address these challenges, combining functionalisation agents presents a promising strategy to enhance the selectivity of pulmonary-targeted delivery. For instance, to address the limitations of conventional EGFR-targeted therapies for NSCLC, which are often hindered by toxicity and resistance, dual active and passive-targeted inhalation NLCs were developed incorporating five essential elements. This proposed system integrates passive lung targeting through inhalation delivery, active tumour targeting through luteinizing hormone-releasing hormone receptors, broad suppression of EGFR-tyrosine kinases by the siRNA pool, induction of apoptosis and necrosis by paclitaxel, and enhanced stability, solubility, and cellular penetration provided by the NLCs system. The inhalation delivery of luteinizing hormone-releasing hormone-targeted NLCs with siRNA combined with paclitaxel synergistically induced NSCLC cell apoptosis and tumour growth suppression, demonstrating therapeutic efficacy superior to either gene or chemotherapy alone. This enhanced targeting suggests improved anticancer efficacy and reduced side effects from the treatment.[231]

5.2. Toxicity of nanostructured lipid carriers in the lungs

Nanostructured lipid carriers consist of solid lipids, liquid lipids, and surfactants that are biodegradable and biocompatible, mostly categorised as "Generally Recognised as Safe." However, the specific choice of components and their arrangement within the NLCs structure are primary determinants of the cytotoxicity of formulations.[232] NLCs, particularly NLCs with positive charges, can induce cytotoxicity through direct membrane disruption and the induction of oxidative stress, which can subsequently lead to apoptosis and other forms of cell death.[233] Cationic NLCs can compromise membrane integrity and trigger harmful calcium influx through strong electrostatic interactions with cell membranes.[37] The oxidative stress from increased intracellular reactive oxygen species can damage cellular macromolecules and may cause mitochondrial disruption. Moreover, NLCs can also improve therapeutic safety by localizing drugs to the lungs and minimizing systemic toxicity, demonstrated by reduced hepatotoxicity and improved survival in animal models. However, this localised delivery also increases the risk of concentrated toxicity within the lung tissue.[76]

5.3. Immunogenicity of nanostructured lipid carriers in the lungs

The interaction between NLCs and the immune system is a critical factor in their therapeutic success, particularly within the immunologically active environment of the lungs. Inhaled NLCs can initiate biological interactions immediately on deposition on the respiratory mucosa. The recognition of the immune system for NLCs can trigger a rapid clearance by alveolar macrophages, which limits the efficacy of inflammatory responses that cause adverse effects.[229,234]

The PEGylation has been designed as stealth nanoparticles that can reduce immune recognition and prolong circulation time. PEGylation is intended to help NLCs penetrate the mucus barrier and escape uptake by the RES in the liver and spleen if they reach systemic circulation.[145] However, repeated administration of PEGylated NLCs may lead to the development of anti-PEG antibodies, primarily of the IgM isotype, from stimulated B-cells in the spleen. These anti-PEG antibodies can trigger immune responses that accelerate clearance and reduce therapeutic efficacy.[149] Therefore, the widespread preclinical investigation of PEGylated NLCs has not translated into numerous clinical trials for pulmonary delivery, which is likely due to the now well-understood immunogenicity concerns.

Nanostructured lipid carriers with their surface charges can also function as active immunomodulators, directly influencing the behaviour of immune cells within the lung. The interaction of NLCs with antigen-presenting cells, including alveolar macrophages and dendritic cells, can initiate this immunomodulation.[235] Cationic NLCs can activate macrophages, leading to a more responsive state to later inflammatory signals and a significant increase in pro-inflammatory cytokines such as IL-6 and tumour necrosis factor-alpha. This functional activation involves significant metabolic reprogramming of the macrophages towards a pro-inflammatory M1 phenotype.[236]

Surface modifications with specific ligands, such as mannose, can also actively target immune cells. Adding a cationic lipid, such as stearyl amine, can provide a positive charge that enhances electrostatic interactions with cell membranes, improving cellular uptake before mannose directs binding to specific receptors on macrophages.[237] Mannose-functionalised cationic NLCs have been developed to target mannose receptors on alveolar macrophages, the primary site of *M. tuberculosis* infection. This active targeting strategy enhances uptake by the target immune cells, increasing the efficacy of antituberculosis drugs while reducing toxicity in other lung sites.[84]

5.4. Production and scalability challenges

While the functionalisation of NLCs holds promise for enhancing the effectiveness of pulmonary-targeting delivery systems, several challenges still need to be addressed. The quality of functionalised NLCs should be monitored during production and storage. Most fabrication techniques in functionalised NLCs require complex and multi-step processes, which should be precisely controlled. NLCs can undergo aggregation, crystallisation, or degradation during storage and use, affecting drug delivery effectiveness.

Another challenge in the fabrication of functionalised NLCs is that the production method must be consistently reproducible

and can be scaled up to an industrial scale without losing the functional characteristics of the NLCs. The main challenge in the scale-up of NLCs production for pulmonary targeting drug delivery systems involves the reproducibility and stability of functionalised NLCs. In the laboratory scale, the manufacturing process may be able to produce particles with uniform size, but in the industrial scale, parameters such as pressure, temperature, and process duration must be tightly controlled to maintain the optimal particle size distribution without aggregation or coalescence. In addition, the physical and chemical stability of NLCs is a major challenge because processing in large batches can cause changes in lipid structure, decreased encapsulation efficiency, and drug degradation, especially during storage. Furthermore, the functionalisation method should be controlled and monitored to ensure that NLCs have been functionalised in a homogenous and reproducible manner. The optimisation of the functionalisation process involves selecting appropriate ligands, optimising reaction conditions, and minimising potential side effects associated with surface modifications. Batch-to-batch reproducibility and scalability of the functionalised NLCs are essential for their successful application in pulmonary targeting delivery systems.

6. Case studies in clinical translation: Nanostructured lipid carriers and related formulations in human trials

The progression of NLCs and their lipid nanoparticle groups, from preclinical models to human studies, marks a critical milestone for the clinical translation of nanomedicine-based drug delivery systems. While numerous research studies explore their potential, only a few have initiated the rigorous clinical evaluation process. An examination of these pioneering trials reveals key trends in therapeutic payloads, disease indications, and formulation strategies that are currently defining the translational landscape.

Among the lipid-based nanoparticles in clinical development, the AAHI-SC2 vaccine provides the most direct example of a formulation explicitly identified as NLCs in human trials. This formulation was investigated in the THEMBA II T-CELL Vaccine study (NCT05370040), a Phase 1/2 trial sponsored by ImmunityBio, Inc. (San Diego, California, United States).[238] The active component of AAHI-SC2 is a self-amplifying RNA (saRNA) designed to encode the spike protein of the SARS-CoV-2 virus. AAHI-SC2 demonstrates significant clinical translational potential, supported by robust quantitative data from preclinical studies and advancement into human trials. Unlike conventional mRNA, saRNA contains genetic machinery derived from an alphavirus that allows it to replicate within the host cell, enabling robust antigen expression from a much lower dose.[239,240] This high-value nucleic acid payload is delivered through an NLC platform. A key feature of specific NLC formulation is its exceptional thermostability. Preclinical data demonstrated that the lyophilised (freeze-dried) saRNA-NLC vaccine maintained its immunogenicity after storage for up to 6 months at room temperature (25°C) and up to 10 months under refrigeration (4°C).[241] This represents a significant logistical advantage over the stringent ultracold chain requirements of the first-generation lipid nanoparticle-based mRNA for COVID-19 vaccines. Moreover, the saRNA-NLC vaccine through intranasal administration induces over 20% CD69$^+$ lung-resident CD8$^+$ T-cells post-boost in mice and maintains nasal mucosal IgA in hamsters for over 71 days. This mucosal immunity provides strong protection, as vaccinated hamsters had no detectable infectious SARS-CoV-2 in lung tissue, and co-housed naïve hamsters exposed to infected vaccinated animals showed minimal to non-detectable replicating virus in their airways.[239,240] This indicates that the AAHI-SC2 vaccine significantly reduced viral loads, suppressed viral replication in the respiratory tract, and protected against SARS-CoV-2-induced lung damage. The strong preclinical data in animal models exhibited that intranasal vaccination generates specialised lung-resident memory T-cell populations and protects the upper and lower respiratory tracts from viral replication and pathology, providing the crucial preclinical justification for advancing this approach into human trials to assess its potential to elicit protective pulmonary immunity.

For lung cancer, one of the most advanced clinical trials utilizing a lipid-based nanoparticle system for cancer therapy is led by Genprex, Inc. (Austin, Texas, United States). Their lead candidate, quaratusugene ozeplasmid (Reqorsa®, Genprex, Inc.), is a gene therapy delivered through the proprietary ONCOPREX® Nanoparticle Delivery System (Genprex, Inc.). Reqorsa® is designed to combat cancer by restoring the function of a critical tumour suppressor gene, tumour suppressor candidate 2 (*TUSC2*), which is frequently inactivated or absent in most lung cancers. The therapeutic payload is a DNA plasmid engineered to express the TUSC2 protein. This plasmid is encapsulated within the ONCOPREX® delivery system, a non-viral, multi-lamellar lipoplex composed of lipid molecules that impart a net positive electrical charge. This cationic nature is a key design feature, as it is intended to facilitate electrostatic targeting to the negatively charged surface of cancer cells, thereby minimizing uptake by normal tissues. A prominent example of the clinical study for this delivery system is the Acclaim-1 study (NCT04486833), a Phase 1/2 clinical trial evaluating Reqorsa® in combination with osimertinib (Tagrisso®, AstraZeneca, Cambridge, United Kingdom). Quaratusugene ozeplasmid consists of non-viral lipid nanoparticles that encapsulate a DNA plasmid containing the *TUSC2* tumour suppressor gene, representing a novel gene therapy approach for patients with late-stage NSCLC through the activation of *EGFR* mutations. Based on phase I clinical trials, this combination could be well tolerated, and no dose-limiting toxicities occurred, proceeding to phase II clinical trials, with a dose recommendation of 0.12 mg/kg.[242] In addition, the Acclaim-3 study (NCT05703971), a Phase 1/2 clinical trial, is evaluating Reqorsa® in combination with the immune checkpoint inhibitor atezolizumab (Tecentriq®, Roche, Basel, Switzerland) as a maintenance treatment for patients diagnosed with extensive-stage small cell lung cancer. This is a particularly challenging patient population with a poor prognosis.[243]

The treatment of cystic fibrosis has also seen significant advances, with the Food and Drug Administration approval

of amikacin (ARIKAYCE®, Insmed Inc., Richmond, Virginia, USA) establishing a clear regulatory path for inhaled lipid-based nanomedicines.[244] Next-generation therapies are now focused on correcting underlying genetic defects. A notable example is the Phase 2 clinical trial (NCT06747858) for ARCT-032, a nebulised therapy that uses lipid nanoparticles to deliver *CFTR* mRNA directly to the lungs.[245] This approach, supported by extensive preclinical work demonstrating restoration of *CFTR* function in patient-derived cells and animal models, aims to be a universal treatment independent of a patient's specific mutation type. Moreover, preclinical studies are demonstrating the potential of lipid-based nanoparticles to deliver clustered regularly interspaced short palindromic repeats-based gene editing tools to correct cystic fibrosis-causing mutations *in vivo*.[246,247]

The relatively small number of functionalised NLCs that have advanced to human trials, compared to the vast body of promising preclinical research, highlights a significant translational gap. This gap is largely attributable to the formidable challenges in immunogenicity, toxicity, and scalable manufacturing detailed in Section 5. It highlights the critical need for future research to address these clinical translational issues from the initial stages of formulation design to ensure that promising preclinical candidates have a viable path to the clinic. However, despite these challenges, the clinical translation of NLCs and other lipid-based nanoparticles is steadily progressing from conceptual promise to clinical application. Although still in its early stages, emerging human trial data provide strong evidence of their potential to reshape therapeutic strategies for various respiratory diseases. These early studies demonstrate the practical utility of the NLCs platform and help define the path forward by highlighting key factors such as formulation design, administration routes, immune responses, and manufacturing challenges. Addressing the remaining scientific and regulatory barriers will be essential as the field advances. Nevertheless, the progress achieved so far marks a pivotal moment, positioning inhaled NLCs and other lipid nanoparticles as a transformative and patient-focused approach in next-generation pulmonary-targeted delivery.

7. Future perspective

Numerous factors, including formulation, fabrication, surface modification, surface engineering, characterisation, *in vitro* evaluation, and *in vivo* studies, must be considered to successfully develop conventional or functionalised NLCs for pulmonary-targeted delivery systems. An Ishikawa diagram for the process development of NLCs in pulmonary targeting delivery systems is shown in **Figure 6**. Various aspects can contribute to the quality, safety, and efficacy of NLCs for pulmonary-targeted delivery systems, including formulation, fabrication, surface modifier types, functionalisation process, physicochemical characteristics, and *in vitro* and *in vivo* assessments of both unmodified and functionalised NLCs. Applying the quality by design approach to the development of NLCs for pulmonary-targeted delivery enables systematic control over product quality, safety, and efficacy. The quality target product profile defines the desired attributes, such as lung deposition efficiency, aerodynamic diameter, and biocompatibility, while critical quality attributes, such as particle size, zeta potential, and drug release, can guide formulation decisions. These essential quality attributes are influenced by critical material attributes—including lipid type and surfactant concentration—and key process parameters, such as homogenisation speed and temperature. By integrating risk assessment tools and design of experiments, the quality by design approach can systematically identify and control variability, ensuring reproducible quality and enhancing the translational potential of both conventional and functionalised NLCs.

Furthermore, integrating artificial intelligence (AI) and machine learning (ML) is set to transform this paradigm into a prediction-by-design workflow. Instead of simply optimising known parameters within a limited experimental set, ML models can analyse vast datasets from high-throughput screening (HTS) experiments to uncover complex, non-obvious, and multi-parameter relationships that govern the performance of NLCs. This process begins by transforming the chemical structures of formulation components, including lipids and surfactants, into machine-readable formats, such as molecular descriptors or fingerprints, using open-source cheminformatics tools. These data are then used to train ML algorithms to build quantitative structure-activity relationship models to predict critical quality attributes for novel and untested formulations, such as cellular transfection efficiency. Subsequently, the data performance should be validated using test sets and standardised protocols.[248] A study by Cheng *et al.*[249] utilised an ML workflow to analyse HTS data from 1,080 unique plasmid DNA lipid nanoparticle formulations across six cell types to determine design rules for cell type-preferential transfection. The ML models, particularly decision-tree-based algorithms, demonstrated high accuracy with prediction errors averaging between 5% and 10% across the different cell types. This integrated approach of HTS and ML successfully established quantitative structure-function relationships to guide the design of lipid nanoparticles for targeted cell transfection.[249] Another study by Rouco *et al.*[250] demonstrated the successful use of an AI model combined with genetic algorithms to predict the optimal formulation of NLCs. The model suggested a formulation with a particle size of 152 nm, 100% encapsulation efficiency, and 5% drug loading. Experimental validation closely matched the prediction, yielding nanoparticles with an encapsulation efficiency of 95.2% and 4.7% drug loading, which remained stable for at least 1 month during storage.[250] Therefore, AI algorithms and ML utilisation in NLCs development can generate vast virtual libraries of chemically valid and synthetically accessible lipids, screen them *in silico* for desired properties, and prioritise a small, high-potential subset for physical validation. This can dramatically reduce the experimental burden, lower costs, and accelerate the discovery of optimised NLCs.

Despite advances in manufacturing processes, the successful development of conventional or functionalised NLCs for pulmonary delivery remains a significant challenge. Transitioning from a laboratory concept to a clinically approved therapy requires a multi-stage preclinical and clinical

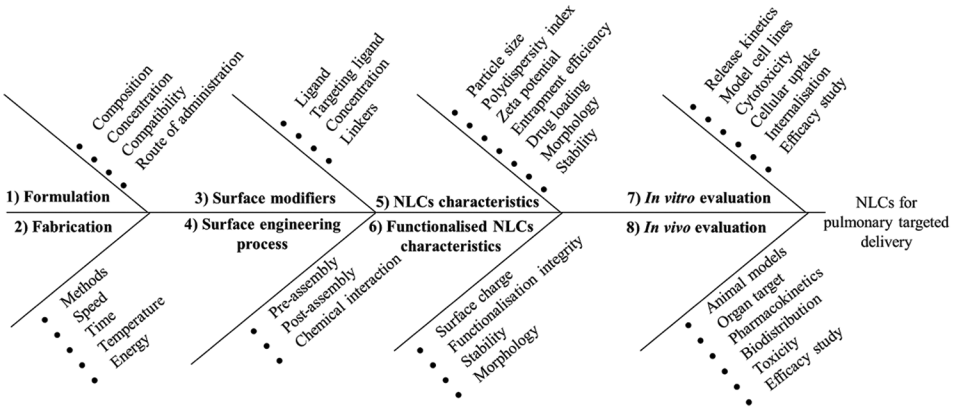

Figure 6. Ishikawa diagram of nanostructured lipid carrier process development for pulmonary-targeted delivery systems. Created using Microsoft PowerPoint 365 Education (license provided by Chulalongkorn University).
Abbreviation: NLCs: Nanostructured lipid carriers.

evaluation process designed to systematically generate a comprehensive data package on safety and efficacy. The initial stages of the preclinical pipeline rely heavily on advanced *in vitro* models, which serve as powerful, high-throughput platforms for foundational screening and formulation optimisation. For instance, most researchers have evaluated *in vitro* cellular uptake and internalisation using A549 cell lines, which are appropriate model cell lines for lung cancer cells. However, 3D cell lines have been widely developed and utilised to mimic the real environment, physiological lung tissue, and specific lung diseases.[251] A key advantage is their ability to establish an air–liquid interface, which forces cultured respiratory cells to differentiate, form tight junctions, and secrete mucus, thereby better replicating the native architecture and barrier functions of the human airway epithelium. Therefore, 3D cell lines can be considered as more appropriate model cell lines for *in vitro* cellular uptake and cytotoxicity assessment.[117] These advanced *in vitro* systems offer a more biologically relevant platform than traditional two-dimensional cultures, serving as a critical intermediate step that can refine experimental design and provide more predictive data before proceeding to *in vivo* studies.

However, despite the significant advantages of 3D cell lines, the most profound limitation is the absence of systemic circulation and large variabilities.[251,252] It has been noted that data from *in vitro* models may not always correlate well with outcomes from subsequent *in vivo* studies, due to differences in complexity, including the absence of complex biological interactions, tissue structure, and systemic effects.[253] An *in vitro* model cannot provide any data on the critical parameters that govern the fate of NLCs in the body, such as their pharmacokinetics and biodistribution. In addition, *in vitro* cytotoxicity tests only assess effects on isolated cells and cannot predict systemic toxicity in a whole organism. *In vivo* studies are crucial, as they reveal how NLCs interact with other organs, such as the liver and kidneys, which are responsible for metabolizing and clearing foreign substances. Therefore, progression to well-designed animal models is an absolute necessity to bridge the gap between promising *in vitro* data and clinical trials. As this review has extensively detailed, animal models, such as those involving mouse lungs, remain the gold standard for evaluating the systemic effects, biodistribution, efficacy, and toxicity of NLCs before they can be considered for human clinical trials.

For initial efficacy screening and pharmacokinetics/biodistribution characterisation, the orthotopic xenograft model can be used as an appropriate lung cancer model. This involves the direct implantation of human lung cancer cell lines, such as A549 or NCI-H1299, into the lung parenchyma of immunocompromised mice. These models are vastly superior to traditional subcutaneous models because placing the tumour in its native organ microenvironment enables researchers to study more relevant tumour growth patterns, vascularisation, and metastatic potential.[254,255] This is particularly crucial for testing functionalised NLCs that target receptors, such as EGFR or folate receptors, which are overexpressed on these human cancer cells. Moreover, genetically engineered mouse models can be further used to develop *de novo* tumours in response to the activation of specific oncogenes or the inactivation of tumour suppressor genes, thereby closely recapitulating the molecular drivers and progression of human lung cancer.[256,257] Although more complex and time-consuming, this model can be utilised for long-term studies of tumour evolution, the development of drug resistance, and the efficacy of NLCs in the genetic and histopathological features of the human disease.

According to the results of *in vitro* and *in vivo* studies in animals with various functionalised NLCs, numerous advantages, such as high cellular uptake and internalisation, enhanced specificity, improved efficacy in specific cells, and reduced toxicity in normal cells, can be achieved after NLCs are decorated with specific ligands through surface modification. These results are very promising for pulmonary-targeted delivery systems. Therefore, functionalised NLCs can proceed with clinical trials to obtain market approval. Several factors should be considered in clinical trials of functionalised NLCs, such as the stability of surface modifications and sufficient data for preclinical studies, including *in vitro* drug release, *in vitro* cellular uptake, *in vivo* pharmacokinetics, *in vivo* efficacy, and *in vivo* acute and chronic toxicity studies. Hence, developing and evaluating functionalised NLCs in clinical trials is challenging for researchers and pharmaceutical companies. In the case

of other major respiratory diseases, such as tuberculosis and asthma, lipid-based nanoparticle therapies are in the early stages of development but show significant promise. While specific clinical trials for NLCs are still emerging, the robust preclinical data for formulations, such as mannosylated NLCs for tuberculosis, provide a strong foundation for future clinical investigation. In asthma, advanced preclinical research is focused on engineering targeted lipid-based nanoparticles to deliver siRNA to T-cells, aiming to modulate the specific immune responses that drive the disease. The clinical success of NLCs in various pulmonary disease applications provides a strong rationale and a clear roadmap for advancing these promising candidates into human trials.

8. Limitations

This review provides a comprehensive overview of conventional and functionalised NLCs for pulmonary delivery. However, certain limitations should be acknowledged. A significant limitation in this field is the substantial reliance on preclinical data. Most evidence for the efficacy and safety of conventional and functionalised NLCs is derived from *in vitro* cell culture models and *in vivo* animal studies. Although these models are essential for initial development, their results do not always translate directly to human clinical outcomes. This limitation is reflected in the clinical translation section, which reveals that only a few functionalisation strategies discussed in the review have advanced to human trials. Therefore, the review highlights the gap between the numerous promising preclinical findings and the limited number of formulations based on NLCs that have advanced to human clinical trials.

9. Conclusions

Nanostructured lipid carriers can be potential vehicles for pulmonary-targeted drug delivery systems to treat numerous lung-related diseases, such as asthma, COPD, cystic fibrosis, tuberculosis, and acute lung injury. Conventional NLCs can be utilised to directly target the lungs through EPR effects. However, the low selectivity of unmodified NLCs can lead to ineffectiveness and high potential systemic toxicity. Therefore, surface modifications can be engineered with numerous ligands, such as hydrophilic polymers, polysaccharides, peptides and proteins, small molecules, surfactants, genes, antibodies, and pH-sensitive polymers. In general, surface modification can improve the binding affinity for specific target ligands on the surface of the cell membrane. This can lead to prolonged retention time and receptor-mediated endocytosis, enhancing cellular uptake and internalisation. Various *in vitro* and *in vivo* animal studies have shown that, compared with unmodified NLCs, functionalised NLCs can improve selectivity, safety, and efficacy through active targeting to specific lung disease sites. Despite various challenges, such as drug delivery efficiency, safety concerns, immunogenicity, and the complexity of large-scale manufacturing, which have restricted the progression of many formulations to further studies, the available preclinical data consistently demonstrate promising therapeutic potential. These early-stage findings highlight the feasibility and effectiveness of NLCs in pulmonary delivery systems, which strongly indicates their promise for future clinical translation.

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