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Review

Drug/polymer nanoparticles prepared using unique spray nozzles and recent progress of inhaled formulation

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ABSTRACT

Inhaled formulations are promising for pulmonary and systemic non-pulmonary diseases. Functional engineered particles including drugs and drug-loaded nanocarriers have been anticipated because they can improve drug delivery efficacy against target sites in the lungs or blood. In this review, unique spray nozzles (e.g., four-fluid spray nozzle and two-solution mixing type nozzle) for the preparation of nanocomposite particles which mean microparticles containing drug nanoparticles are described. These nozzles can produce nanocomposite particles in one-step and their spray drying system is suitable for scaling-up. Nanocomposite particles are useful in improving drug absorption and delivery efficacy against alveolar macrophages. In addition, recent studies on several pulmonary diseases (tuberculosis, lung cancer, cystic fibrosis, pneumonia, vaccine and others) and related inhaled formulations were also reviewed.

1. Introduction

Pulmonary drug administration has the potential of a non-invasive and easy administration method. It has attracted much attention in the field of pulmonary and non-pulmonary diseases for decades because of the specific structure and function of lung tissue; the lungs have large surface area, thin mucosal cell membrane, and blood vasculature. Alveoli, which are the site of oxygen and carbon dioxide exchange, contain blood capillaries at high density. In addition, drugs can easily penetrate the thin layer of alveolar endothelial cells and enter the blood. Compared with the oral administration, pulmonary administration avoids the hepatic metabolism, which is known as first-pass effect. Moreover, the lungs contain lymph nodes and present immune competent cells. Therefore, various drugs and vaccine delivery systems targeting the lungs have been developed.

Currently, both liquid aerosol and dried powder formulations have been used in pulmonary administration and some

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devices for inhalation have been recently reviewed [1]. Additionally, the recently approved products of inhaled formulation was summarized in Table 1. Although the administration of aerosol by nebulizer has been established, dried formulation is superior to liquid aerosol from the stability perspective. However, the diameter of the dried powder should be controlled. The optimization of aerodynamic diameter is necessary to achieve delivery into deeper regions such as the alveoli [2]. In addition, the change in the surface morphology can affect the aerodynamic diameter, and porous and wrinkled particles have been developed [3].

In this review, two special spray nozzles were introduced. Spray nozzles have been established to prepare drug "nanocomposite particles", which are microparticles containing drug nanoparticles. These composites are useful for the preparation of inhaled formulations against pulmonary and systemic non-pulmonary diseases. Moreover, recent studies focusing on inhaled drug formulations with or without drug carriers were reviewed.

2. One-step preparation of nanocomposite particles by spray dryer equipped with special spray nozzles

The pulmonary administration has recently been anticipated to improve the absorption of poor water-soluble drugs. Approximately 40% of potential therapeutic compounds are water-insoluble compounds that are classified as biopharmaceutics classification system (BCS) class 2 or 4. A common method for improving drug absorption is the size reduction of bulk drugs (e.g., nanoparticles drugs), which could enhance their solubility because of the increase in their surface area. Furthermore, nanoparticle drugs could be more easily absorbed into the blood.

However, the beneficial properties of nanoparticles are often easily lost by self-agglutination and their redispersion is very difficult. The prevention of self-agglutination is an important issue and some solutions have been developed, such as reducing their dimensions to the order of microns, which means the preparation of nanocomposite particles. In our group, nanocomposite particles have been prepared using a spray dryer equipped with a unique spray nozzle.

Table 1 – Recent product of inhaled formulations in the market.

Name	Drug	Category	Device
Alvesco®	Ciclesonide	Steroid	pMDI
Asmanex®	Mometasone	Steroid	DPI
Adoair®	Fluticasone and Salmeterol	Combination	pMDI
Symbicort®	Budesonide and Formoterol	Combination	DPI
Oxis®	Formoterol	LABA	DPI
Onbrez®	Indacaterol	LABA	DPI
Spiriva®	Tiotropium	LAMA	SMI
Seebri®	Glycopyrronium	LAMA	DPI
Afrezza®	Insulin	Diabetes	DPI

Abbreviation: pMDI: pressurized metered-dose inhaler, DPI: dry powder inhaler, SMI: soft mist inhaler, LABA: long-acting beta agonist, LAMA: long-acting muscarinic antagonists.

Four-fluid spray nozzle is a unique spray nozzle containing two liquid passages and two air passages (Fig. 1) (Fujisaki Electric Co.; http://www.fujisaki-hest.com). Compressed air is flown from the air passages to transform solutions into mist. The solution flown in the passages is mixed with compressed air and the solution is accelerated. The collision of the compressed air at the end of the nozzle generates a shockwave that transforms the solution into single micron mist. The single micron mist is quickly and efficiently dried because of its reduced size and increased surface area. For example, poor water-soluble drugs are dissolved in an organic solvent and the solution is flown in one passage. Then, mannitol (MAN) aqueous solution is flown into the other passage. The drug-organic mist and MAN-aqueous mist are mixed at the end of the nozzle, where the drug starts crystallizing by anti-solvent effect, which is the phenomenon in which solubility change induces the precipitation of the drug. The drug/MAN mist is immediately spray-dried until the drug crystallization has been completely progressed. Thus, relatively small drug nanoparticles are produced in MAN microparticles. Because one-step preparation and large amount of nanocomposite particles can be obtained by spray drying, the method is suitable for scaling-up. In previous studies, composites of flurbiprofen/salicylate [4], polymer/MAN [5], rifampicin/MAN [6], and rifampicin/polymer/MAN [7] were developed using the spray dryer equipped with a four-fluid spray nozzle.

Although the four-fluid spray nozzle is useful in the one-step preparation of nanocomposite particles, optimization of spray drying conditions has been sometimes necessary, and it has been unclear if the anti-solvent effect occurred efficiently. Therefore, a two-solution mixing type nozzle has been developed to prepare nanocomposite particles (Fig. 2) (Ohkawara Kakohki Co.; http://www.oc-sd.co.jp/english/index.html). In this device, similar to the four-fluid spray nozzle, the two solutions are separately flown in different passages and are mixed in the mixing chamber in the nozzle. The drug solution dissolved in organic solvent is added into the MAN-aqueous solution in the mixing parts and the mixture is immediately spray-dried. Anti-solvent effect properly occurs in the mixing parts of the nozzle, and we speculate that the preparation of the nanocomposite particles is achieved in suitable conditions. The spray nozzle is customizable and some parts of it can adjust the mixing condition. Ethylcellulose (EC) is a poor water-soluble polymer, which was used as the model compound; EC/MAN composite was prepared using a spray dryer equipped with the two-solution mixing type spray nozzle [8]. The composite particles prepared with this method exhibited relatively small diameter compared with those prepared using the four-fluid spray nozzle. To extend our study, microparticles containing solid dispersion drug nanoparticles were prepared [9]. Solid dispersion is a technique used to increase drug dissolution and absorption. Solid dispersion of nanoscale drugs showed enhanced intestinal absorption compared with composites containing drug nanoparticles and original drug powders. Although a detailed investigation about on the inner structure, crystallinity, and physical stability of microparticles is required in the study, these unique nanocomposite particles are promising formulations to enhance the absorption of poor water-soluble drugs.

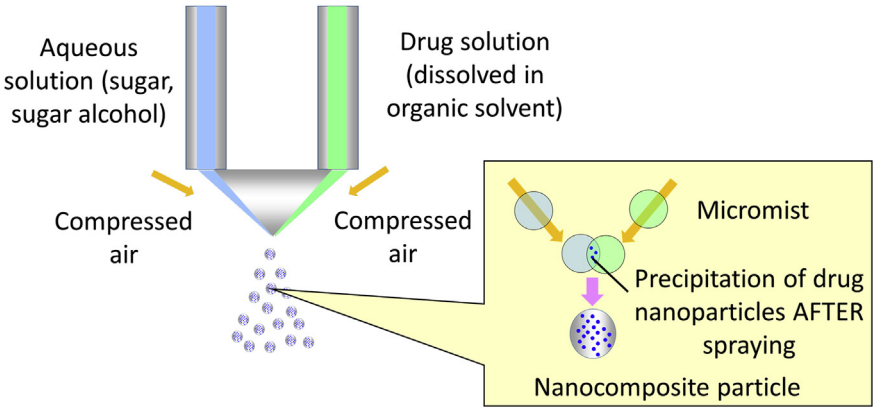

Fig. 1 – A four-fluid spray nozzle (simplified image) and preparation of nanocomposite particles.

3. Inhaled formulations against pulmonary and non-pulmonary diseases

Various types of inhaled formulation have been recently developed. These formulations target various pulmonary diseases such as asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, lung cancer, pneumonia derived from bacterial infection, and tuberculosis. In this review, recent studies about inhaled formulations specific for several pulmonary diseases were reviewed. The summary of articles in this review was shown in Table 2.

3.1. *Tuberculosis*

Tuberculosis is an infectious disease spread worldwide and primarily occurs in south Saharan regions. Patients with HIV are easily infected with TB and the co-infection yields complications [10]. The infection mechanism has been well investigated [11–13]. After the invasion in the lungs by the airway, the bacterium is digested by alveolar macrophages. Although several parts of the bacterium are sterilized by macrophages, other components escape from digestion, thereby residing for a long term in macrophages and allowing the bacteria to grow after host immunity decreases. Inhaled drug formulations have been adopted for tuberculosis and formulations targeting the alveolar macrophages have been successfully tested.

The dry powder formulation of nitroimidazopyran, which is a promising drug against TB, was prepared using spray drying [14]. The prepared particles were porous with modified aerodynamic diameters and contained 5% dipalmitoylphosphatidylcholine (DPPC), 20% L-leucine, and 75% drug. The blood concentration profile showed that the concentration of the drug through pulmonary route was significantly higher than that of the drug through oral route, which implies that its pulmonary bioavailability is higher than its oral bioavailability. The same research group evaluated the therapeutic effect and tissue damage in infected guinea pigs by comparison with a positive control (i.e., drug/cyclodextrin suspension) [15].

Poly-(DL-lactide-co-glycolide) (PLGA) microspheres have been well studied because of their biodegradative properties and suitable particle size for pulmonary delivery. PLGA has the advantage of having a long half-life, which can vary from one week to one year. The Trojan approach (as shown in Fig. 3), which targets alveolar macrophages containing bacteria, has been proposed because PLGA particles, which are

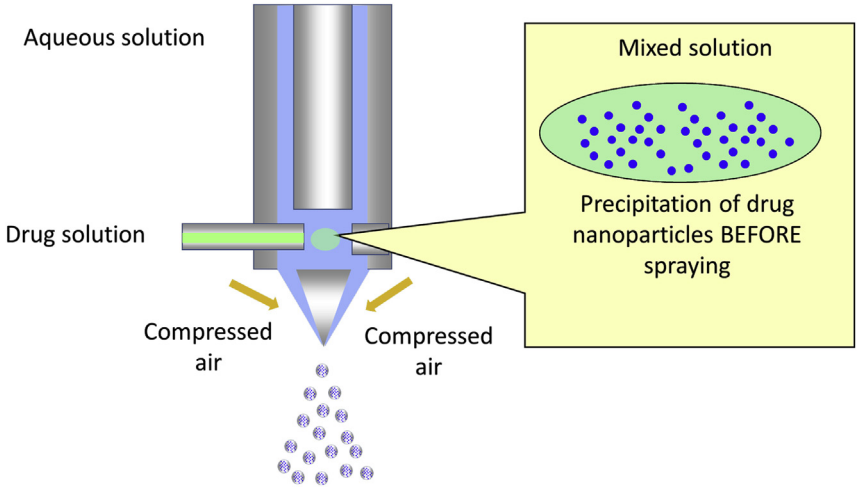

Fig. 2 – A two-solution mixing type spray nozzle (simplified image) and preparation of nanocomposite particles.

Table 2 – Inhaled formulation for various types of pulmonary diseases in recent articles.

Diseases	Drug	Formulation (Carrier)	References
Tuberculosis	Nitroimidazopyran	DPPC/leucine porous microparticle, Aerosol	[14,15]
	Rifampicin	PLA microparticles	[17]
Lung cancer	Cisplatin	EGF-modified gelatin nanoparticles	[19]
	GEM	Drug alone	[20]
	GEM	Drug alone	[21]
	GEM	Drug alone	[22]
	Paclitaxel	DPPC/DPPE-PEG microparticles	[23,24]
	DOX	Effervescent powder	[25,26]
	DOX	Drug alone	[27,28]
	DOX, Oligonucleotide	Liposomes	[29]
	Celecoxib	PLGA microparticles	[31]
Cystic fibrosis	Corticosteroids	Drug alone	[32]
	Tobramycin, Clarithromycin	Drug nanoparticles	[33]
	Ketoprofen	Drug/leucine powder	[34]
	Meloxicam	Drug/MAN microparticles	[35]
	Naringin, Gentamicin	Drug/leucine powder microparticles	[36,37]
Infection disease	Tobramycin	PVA or chitosan-coated PLGA nanoparticles	[38]
	Acyl-homoserine lactone acylase	Drug/sugar powder	[39]
	ITZ	Drug/TPGS/MAN composite	[40]
	Vancomycin	Liposomes	[43]
Vaccine	ESAT-6 antigen	Virus-like particles	[47]
	Bacteriophage	Drug/sugar powder	[48]
	Bacteria	Bacteria-leucine nano-and micro- structure	[49]
	85-B antigen	PLGA microparticles	[50]
	DNA encoding Rv1733c antigen	PLGA-PEI	[51]

large porous particles, are preferably digested by alveolar macrophages [16]. Various methods to prepare homogenous PLGA particles have been investigated. In a recent study, rifampicin-loaded PLGA microspheres were prepared by exploiting the supercritical anti-solvent process [17]. The average particle size was less than 5 μm (>60%) and this formulation was inhalable.

3.2. *Lung cancer*

Lung cancer is one of the major types of cancers whose onset is related to various factors, including tobacco and asbestos. These continuous stimulations against the squamous epithelium, alveolar epithelium, and other types of cells in the lungs can damage genes and induce malignant alterations. Various anti-cancer drugs alone or in combination are currently used for the treatment of lung cancer. However, drug resistance is a challenging issue. Transporters such as those of the ATP-binding cassette transporter family, including multidrug resistance proteins, are expressed in the endothelial cells and other types of cells in the lung tissues and most types of lung cancer cells overexpress P-glycoproteins [18]. One of the strategies to overcome the drug resistance is the use of drug carriers and lipid-based drug carriers such as liposomes. These drug-loaded carriers are endocytosed by cancer cells and the drugs are then released into the cytoplasm, thereby escaping from the pumping of P-glycoproteins at the cell membrane.

In a recent study, Tseng et al. prepared cisplatin-loaded EGF-modified gelatin nanoparticles for targeting cancerous cells in the lung [19]. This formulation showed higher Pt concentration on the highly EGFR-expressing A549 cells and showed lower Pt concentration on the low EGFR-expressing HFL1 cells, resulting in significant cytotoxicity for A549 cells.

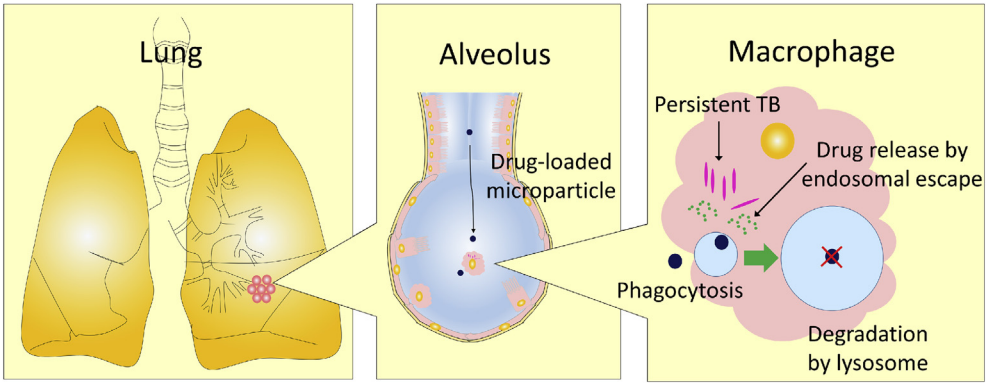

Fig. 3 – Trojan approach: the smart drug delivery for persistent bacterium in alveolar macrophage.

In addition, the nanoparticles accumulated in EGFR-overexpressing cells in murine lung tissue after the administration of the aerosol formulation, indicating that this formulation is useful for EGFR-expressing cancer cells.

Gemcitabine (GEM), which is a nucleoside analog, is frequently used for the treatment of lung cancer by intravenous infusion. Studies investigating the side effects of pulmonary administration of GEM have been recently reported. In a recent report, GEM was administered by aerosol [20] to four patients and no changes in hematologic toxicity were observed after the treatment with aerosolized GEM (4 mg/kg); however, one patient showed pulmonary toxicity. Plasma concentration of GEM was very low after pulmonary administration and the side effects were expected to be decreased. Animal studies on GEM administration by different pulmonary routes were also conducted [21]. Intratracheal instillation by tracheotomy (i.t.t.) or orotracheal route (i.t.o.) was performed in rats. Then, pulmonary toxicity was investigated by checking lung morphology, histopathology, coefficient, wet/dry weight ratio, cells related with inflammation, and inflammatory cytokines. Both i.t.t. and i.t.o. administrations showed good absolute bioavailability and similar acute lung injury compared with intravenous route. Preclinical studies on the use of GEM aerosol in osteosarcoma-bearing dogs were conducted by translational research [22]. GEM aerosol formulation induced increased apoptotic effect with enhanced Fas expression against lung metastatic foci. These studies indicated that GEM aerosol is an effective treatment for lung cancer.

Lung surfactant-mimetic microparticles composed of DPPC and PEG-lipid conjugate of different lengths were also prepared using spray dryer [23]. Phospholipid microparticles, which are the major component of lung surfactants, can have a fate similar to native lipids. Conjugation of PEG on the surface of these particles helps to prevent the recognition of immune competent cells, including alveolar macrophages, and enhance the mucus penetration of these particles. The same research group prepared similar lung surfactant-mimic particles containing paclitaxel [24].

Doxorubicin (DOX) is used as an anti-cancer drug against lung cancer. DOX-loaded nanoparticles were incorporated into inhalable effervescent powder [25]. Lung cancer mouse model treated with this formulation had its survival significantly prolonged compared with mice treated with intravenous injection of DOX. Inhalable nanoparticles remained for longer duration in the lung tissue, and the radioactivity of the labeled drug-loaded nanoparticles was not found in the heart [26]. These results indicated that the side effects of DOX released from those nanoparticles was minimal. A clinical trial employing inhaled DOX was also commenced for non-small cell lung cancer patients [27]. A novel type of inhalation device (OncoMyst Model CDD-2a) was introduced in a previous study [28] and the combined effect of inhaled DOX with cisplatin was investigated. The authors concluded that the combination was safe, even though small side effects were observed in several patients. As a rational approach, it is important to overcome drug resistance. Liposomal formulation containing DOX and designed antisense oligonucleotide (ASO) was used for inhalation [29]. ASO can inhibit the target mRNA level by designing complementary sequences, and in that study, ASO against multidrug resistance-associated protein and Bcl-2, which plays the key role in apoptosis, were used. This formulation showed high antitumor activity with fewer side effects than the combination of DOX with cisplatin.

In the context of administration of inhaled anti-cancer drug formulations, the safety and protection of researchers and medical staff is important. Zarogoulidis et al. reviewed the protection methods used [30] and observed that several strategies including the use of HEPA filters, protected rooms (negative-pressure room), hoods and cages were mentioned, but uniform and global rules are required. In another report, large porous celecoxib-PLGA microparticles that were prepared using the supercritical fluid method were assessed from the safety aspect [31]. The microparticles prepared did not alter the lactate dehydrogenase (LDH), total protein, cell number in the bronchoalveolar lavage fluid (BAL), collagen level, and histological analysis.

3.3. *Cystic fibrosis*

Cystic fibrosis is a disease caused by a mutation in the cystic fibrosis transmembrane conductance regulator gene and the immune response to airway infection is the most common cause of death. The viscosity of mucus derived from the dysfunction of mucus clearance enhances because of the infection. Various antibiotics and anti-inflammatory agents are used for the treatment. Corticosteroids have been used to reduce lung damages from inflammation. Inhaled corticosteroids have been used in children and adults to investigate the adverse effects [32]. Tobramycin nanoparticles containing amorphous clarithromycin were prepared using spray drying [33]. These nanoparticles were classified as dispersible and porous agglomerates by evaluating the fine particle fraction of the drug deposition profiles in the lungs. The anti-inflammatory drug was spray-dried in combination with leucine, a dispersibility enhancer [34] and showed good *in vitro* aerosol performance. A study to understand the drug dissolution properties in the lungs using artificial cystic fibrosis mucus was investigated and the cytotoxicity was evaluated. Meloxicam/MAN composites were prepared using spray drying [35]. The microparticles contained polymers (PVP and PVA) that could prevent aggregation. The particles were spherical and the size was 3–5 μm, which was appropriate for pulmonary delivery. Prota et al. prepared leucine-based naringin particles as dispersibility enhancer, which exhibited better fine particles fraction than naringin-alone particles [36]. Gentamicin/leucine powder was spray-dried to conduct an anti-microbial test [37] on artificial mucus model. Franz-type vertical diffusion cells were used and the formulation was loaded on the synthetic membrane covered by the artificial mucus layer. The mucus showed delayed permeation from the formulation.

3.4. *Infectious diseases (pneumonia)*

Pneumonia infection occurs in people whose immunity is weak. In particular, pneumonia is one of the major causes of death for elderly persons; thus, a fast therapy to treat it is important. Antibiotics-loaded PLGA nanospheres embedded in lactose powder have been prepared [38]. Several hydrophilic polymers such as PVP and chitosan were used to

achieve efficient size and surface properties, and alginate was used to entrap the drug. PVA-modified PLGA nanoparticles reached deep regions of the lungs compared with chitosan-modified PLGA nanoparticles. The drug formulation of PvdQ, which is an acyl-homoserine lactone acylase effective against *Pseudomonas aeruginosa*, was prepared using spray freeze-drying [39]. Several types of sugar such as MAN, trehalose, and inulin were tested. Only trehalose and inulin stabilized PvdG for 4 weeks at 55 °C because of their amorphous properties, whereas MAN did not stabilize PvdG because of its crystalline properties.

Duret et al. prepared itraconazole (ITZ) nanoparticles by using tocopherol polyethylene 1000 succinate (TPGS) as a stabilizer for the treatment of invasive pulmonary aspergillosis [40]. After optimization, spray-dried ITZ/MAN microparticles exhibited good fine particles fraction. This group prepared ITZ/TPGS/MAN solid dispersion microparticles [41]. The formulation showed improved drug dissolution rate and saturation solubility. In a recent study, liposomal formulation was used. Liposomal formulations have been developed because of their biocompatibility and good drug half-life [42]. Liposomal carriers can add functions on their surface modifications. For example, PEGylation of liposomes could be used to prolong the mucosal clearance. In a recent study, liposomal vancomycin was nebulized in rats for the treatment of MRSA pneumonia [43]. Liposomal vancomycin remained in the lung tissues compared with free vancomycin formulation.

3.5. *Vaccines*

Remarkably, inhaled vaccine also has been developing. The advantage of pulmonary formulation of vaccines is that the administration route is the same as that of the infection of MTB and potent immunity can be supplied [44–46] In addition, the presence of both bronchoalveolar lymphoid tissue (as lymph system) and antigen-presenting cells (as immunocompetent cells) in the lungs, enables the induction of the immune response. Hundreds of vaccines have been developed, such as recombinant BCG, BCG overexpressing native proteins, attenuated MTB, and DNA vaccines. However, in many studies, liquid formulations administered by nebulizer proved to be unstable from the standpoint of preservation.

To induce potent immune response, unique particles were prepared [47]. Influenza-like nanoparticles containing a peptide having the same sequence of the TB early secretary antigenic target 6 protein (ESAT-6) was developed. ESAT is a foreign epitope that has potent T-cell immune response. These particles exhibited high titers in serum. Spray-dried preparations of bacteriophages were also used against infectious diseases [48]. One of the main issues of that study regarded the phage titer after spray drying. In fact, phages are delicate and suffer temperature increase and shear stress during the process of spray drying. The phage titer was different among phages, indicating that the optimization of the formulation was necessary. Another group prepared a unique inhaled formulation for tuberculosis [49] consisting of dried bacterial rod-like structures (1–4 μm in length and 200–400 nm in diameter) coupled with small particles of leucine (1 μm) The unique nano-microstructure showed drug efficacy and considerably reduced the bacterial burden in guinea pigs. A carrier-based inhaled formulation was also developed and PLGA microparticles containing recombinant antigen 85-B for TB were inhaled into guinea pigs [50]. The antigen showed potent immunity and the bacterial burden in the lung and spleen was reduced. Moreover, a DNA vaccine encoding the bacterial latency antigen Rv1733c in which the DNA could bind with the PLGA-polyethyleneimine (PEI) chain by electric interaction showed enhanced T-cell immune response [51].

3.6. *siRNA delivery for pulmonary diseases*

Many pulmonary diseases including lung cancer are malignant, and therefore, gene therapy has been studied from new approaches. Functional nucleic acids such as small interfering RNA (siRNA) and plasmid DNA coding therapeutic genes received much attention in the field of pulmonary delivery [52]. In particular, the post-transcriptional gene silencing of a target gene by siRNA is potent compared with the effect induced by conventional antisense oligonucleotides. In addition, siRNA is safer than viral vectors. Therefore, siRNA is a

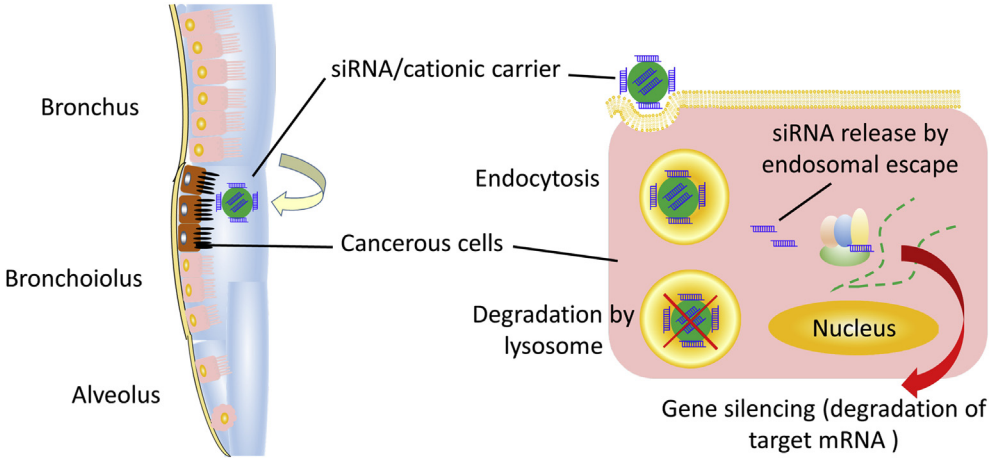

Fig. 4 – A scheme of siRNA delivery system via pulmonary route.

suitable novel therapeutic approach. siRNA are relatively large molecules (approximately 13 kDa) compared with conventional drugs and are water-soluble. However, although the penetration of siRNA into the cytoplasm of the target cells where siRNA can induce RNA interference is an important step to achieve therapeutic effects, it is difficult for siRNA to enter the cytoplasm by itself. Some reports indicate that inhaled siRNA alone can reach the targeted site, but its delivery into the cytoplasm is important for the treatment of the cells of lung tissues.

Cationic lipid-based and polymer-based carriers have been extensively used for the delivery of siRNA because these carriers can deliver the siRNA into the cytoplasm by the interaction with the negatively charged cell surfaces and by endocytosis (Fig. 4). After endocytosis, the siRNA has to avoid the endosomes before it is degraded by lysosomes. In addition, cationic carriers can entrap large amount of siRNA by electrostatic interaction, and the entrapped siRNA is protected by degradation from nucleases. On the other hand, cationic carriers influence the gene expression related with apoptosis, resulting in cytotoxicity. Additionally, it is sometimes difficult for cationic carrier-based inhaled formulations to reach the deepest regions of the lungs and the optimization of the conditions to avoid cytotoxicity and improve delivery efficiency is required.

4. Conclusion

Drug delivery technology by inhalation has been progressing and novel techniques to prepare drug formulations with ease and cost effectiveness have been developed. The use of two unique spray nozzles to prepare nanocomposite particles is a beneficial method for the preparation of water-insoluble drug nanoparticles and to enhance their preservation. Furthermore, the understanding of details of the target diseases is important to develop effective drug carriers. The recent studies reviewed here will be useful for the development of novel drug formulations.

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