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Pulmonary drug delivery: a review on nanocarriers for antibacterial chemotherapy

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As the WHO stated, lower respiratory infections are the third leading cause of death. In addition, it is remarkable that antimicrobial resistance represents a huge threat. Thus, new therapeutic weapons are required. Among the possible alternatives, antibiotic encapsulation in nanoparticles has gained much attention in terms of improved tolerability, activity and ability to combat the resistance mechanisms of bacteria. In this regard, this review article focuses on the latest nanocarrier approaches for inhalatory therapy of antibiotics. First, the technology related to lung disposition will be reviewed. Then, nanocarrier systems will be introduced and the challenges required to perform adequate pulmonary deposition analysed. In the following part, drug delivery systems (DDSs) on the market or in clinical trials are described and, finally, new approaches of nanoparticles that have reached pre-clinical stage are enumerated. Altogether, this review aims at gathering together the novel nanosystems for anti-infectious therapy, underlining the potential of DDSs to improve and optimize currently available antibiotic therapies in the context of lung infections.

Introduction

According to the WHO, lower respiratory infection is the third leading cause of death, giving rise to 3.2 million deaths per year. Major contributors are the 1 million deaths per year caused by TB and the augmented risk of life-threatening pulmonary infections in chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF) patients.[1,2] Antimicrobial resistance (AMR) is a key issue to take into account regarding the therapy of infectious diseases. For example, hospital infections due to multiresistant bacteria, such as MRSA[3,4] or Gram-negative multiresistant bacteria, are currently serious threats.[5,6] Although bacterial evolution and resistance are natural phenomena, the misuse of antimicrobial drugs has accelerated the development of resistance.[7] In this context, there is an urgent need to optimize currently available anti-infectious therapies to overcome drug resistance.[8]

Nanotechnology has emerged as a promising approach to encapsulate antibiotics in order to avoid drug toxicity and reduce AMR. Drug delivery systems (DDSs) administered by the pulmonary route have gained increasing attention for the treatment of several pathologies, including asthma and COPD, since the inhalation process gives more direct access to the drug target than traditional routes.

Bearing this in mind, the aim of this review is to analyse the latest nanosystems to treat lung infections by the pulmonary route. First, factors affecting the lung disposition of various DDSs will be assessed and then nanotechnology advances and challenges for infectious pulmonary diseases will be discussed. However, pulmonary TB will be set aside from the scope of this review as the literature on anti-TB therapy is prolific and could be reviewed separately.[9–12]

Drug delivery to the lung

Pulmonary deposition

The lungs are constituted of two functional parts: the tracheobronchial region, from the larynx to the terminal bronchioles; and the alveolar region, comprising the respiratory bronchioles and alveoli.

The respiratory tract is highly bifurcated and >95% of the total surface area of the lungs is composed of the alveolar area (~90–100 m^2) and a thin (0.1–0.2 μm) alveolar–vascular epithelium with a large capillary network. One of the factors influencing the efficacy of pulmonary drug delivery is the dose able to reach targets in the lung. The most important mechanisms of particle deposition in the respiratory tract are inertial impaction, gravitational sedimentation and diffusion (Brownian motion) (Figure 1).[13]

Larger particles (>10 μm) are retained in the oropharyngeal region and the larynx by inertial impaction. Particles having a size between 2 and 10 μm are usually deposited in the tracheobronchial region.[14] The deposition of particles, mainly of small

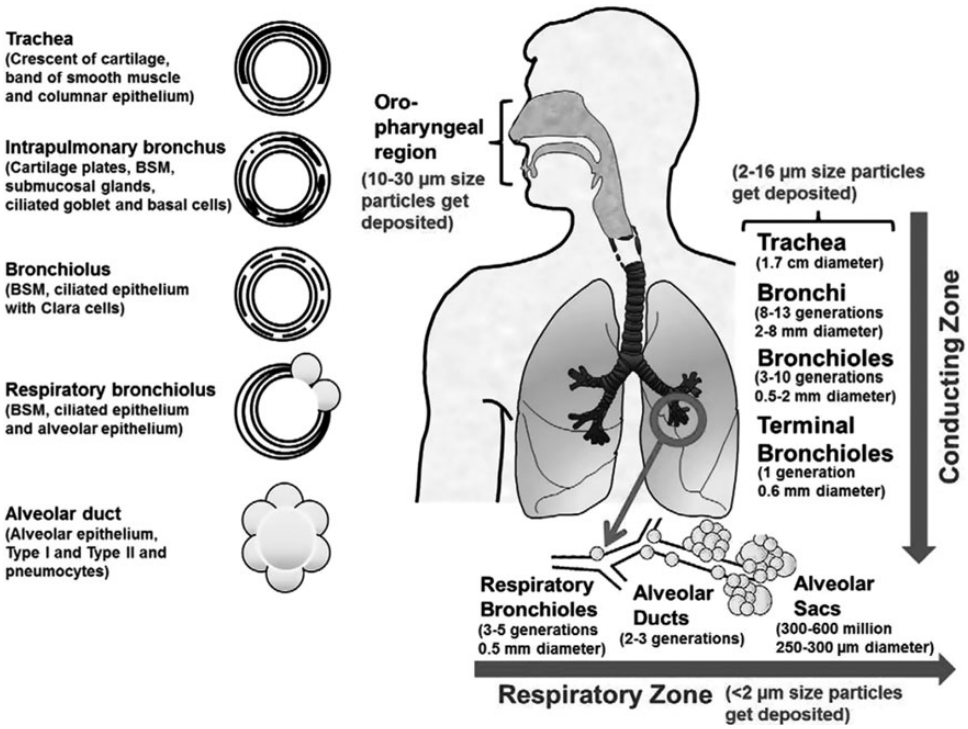

Figure 1. Representation of particle deposition in the lungs according to different mechanisms related to particle size. BSM, bronchial smooth muscle. Reproduced with permission from Nahar *et al*.[22]

size (0.5–2 μm), in the small conducting airways and alveoli is the result of gravitational sedimentation.[15] Particles having a size <0.5 μm, as a consequence of Brownian diffusion, are generally not deposited and are expelled upon exhalation.[16]

Up to 80% of small aerosol particles (<1 μm) can be exhaled during breathing; however, nanoparticles (NPs) ~100 nm are able to deposit in the alveolar region in acceptable amounts.[17,18] Drug NPs usually deposit by sedimentation after being released from the aerosol device due to an agglomeration process in the lung. These agglomerated NPs are able to sediment for longer periods in the tracheobronchial section, thereby improving the biological activity of the delivered therapeutic agent.

Models for studying deposition patterns of inhaled therapeutics

The deposition of inhaled particles in the respiratory airways depends on a number of parameters related to the particles, including size, charge, density, shape, solubility and lipophilicity, together with many physiological and anatomical factors of the respiratory system.[19,20]

One of the key issues for studying deposition and evaluating aerosol characteristics is the determination of the aerodynamic behaviour of the particles. Several methods are used for this purpose with the following equipment: (i) twin-stage impinger (TSI); (ii) multistage liquid impinger (MSLI); (iii) Andersen cascade impactor (ACI); and (iv) next-generation impactor (NGI). The TSI is relatively easy to use as it operates on the principle of liquid impingement to divide the dose emitted from the inhaler into non-respirable (stage 1) and respirable (stage 2) fractions. More recently developed equipment such as the MSLI, ACI and NGI consist of an administration device coupled to a spacer simulating the throat followed by stages 1–8 where the particles are deposited according to their size. Each stage of the impactor comprises a series of nozzles (progressively reducing jet diameters through which the sample-laden air is drawn) and a collection plate. At the end of the test, particles are removed from each plate using a suitable solvent and then analysed, usually by HPLC, to quantify the amount of drug actually present at each stage. Mathematical programs can be applied to calculate the emitted dose (ED, the total mass of drug emitted from the inhaler), fine particle dose (FPD, the mass of drug deposited in the cascade), fine particle fraction (FPF, the mass fraction of particles smaller than 5 μm) and, subsequently, mass median aerodynamic diameter (MMAD, the median of the distribution of airborne particle mass with respect to the aerodynamic diameter). The interpretation of these parameters predicts the deposition patterns of particulate drug carriers in the respiratory tract. In order to reach the alveolar region of the lung, particles must present a high FPF and an adequate MMAD, ranging from ~1 to 5 μm.[21]

After the *in vitro* characterization, *in vivo* studies should be carried out. In this regard, different approaches have been proposed in order to reach animal lungs, i.e. aerosol inhalation by means of a nebulization chamber or intratracheal instillation by different syringes such as the Penn-Century® device.[22]

Delivery devices

Aerosols are an effective method to deliver therapeutic agents to the lung. There are different kinds of devices available on the market useful for pulmonary administration. Depending on the type of

Table 1. Features of the devices currently use for pulmonary delivery

Device	Mechanism	Characteristics	Inconveniences
Nebulizer	air-jet or ultrasonic nebulization	vibrating mesh technology generates aerosol droplets from liquids	long inhalation times cleaning times frequent administration
pMDI	generates aerosol droplets from a drug suspension in volatile liquids (propellant)	unit dosing inexpensive correct size to deposit in the lung	propellant requirement lung deposition <60% coordination difficulties
DPI	dry powder	store drug in dry state: stability and sterility small portable devices short administration avoids coordination problems	requires high inspiratory effort

formulation, the most commonly used are nebulizers, pressurized metered-dose inhalers (pMDIs) and dry powder inhalers (DPIs), whose main characteristics are summarized in Table 1. Drug delivery by means of DPIs is considered the most convenient, as it is free of propellant and is chemically stable and patient friendly. Usually, however, drug or nanocarriers have to undergo additional formulation steps in order to be suitable for DPI administration.[23]

Nanosystems

Definition and types

NPs are solid colloidal particles ranging in size between 1 and 100 nm, but depending on the context, most of the NPs described in the literature are 50–500 nm in diameter. They can be made of biodegradable and biocompatible materials where active compounds such as antibiotics can be adsorbed, attached to their surface or entrapped into the matrix. Several methods for the elaboration of nanoparticulate systems have been reported, e.g. the emulsion–solvent evaporation technique, the high-pressure homogenization technique or nanoprecipitation.[24,25]

Among nanoparticulate DDSs, liposomes have deserved special attention. Liposomes are sphere-shaped vesicles consisting of one or more phospholipid bilayers, which can trap both hydrophobic and lipophilic drugs; water-soluble drugs are entrapped in the aqueous core whereas oil-soluble drugs are located in the lipid bilayer.[26]

On the other hand, polymeric NPs are more stable than liposomes as they present a higher structural integrity afforded by the rigidity of the polymer matrix. However, they might poorly encapsulate water-soluble drugs due to the fast leakage of the drug from NPs during the high-energy emulsification step commonly employed during their preparation. In addition, polymeric NPs usually require the use of organic solvents to dissolve the polymers. Poly(lactic-*co*-glycolic acid) (PLGA) is an FDA-approved polymer for therapeutic use in humans and an attractive candidate for NP preparation owing to its minimal toxicity, biodegradability and biocompatibility properties.[27] Other biodegradable polymers that are currently being extensively explored are chitosan, dextran, alginates, polyvinyl alcohol (PVA), polyethylene glycol (PEG), etc.[28,29]

Solid lipid NPs (SLNs) have emerged during the last decades as an alternative approach for drug encapsulation. SLNs possess a solid lipid matrix that, due to changes in the lipid polymorphism, can leak out the drug. To overcome this limitation they have been modified, leading to the introduction of nanostructured lipid carriers (NLCs), which represent the second generation of lipid NPs. The main difference between them is the configuration of the lipid matrix: in NLCs it is a less ordered matrix consisting of a mixture of solid and liquid lipids, increasing drug loading and preventing leakage.[30,31]

Apart from these NPs, there are many other DDSs, including niosomes, dendrimers, nanocapsules, etc.[32,33]

Advantages and disadvantages of nanosystems

Nano-DDSs have some advantages for the treatment of lung infection compared with the free drug (Figure 2):

(i) Protection of the antibiotics from enzymatic (e.g. degradation by β-lactamases) or chemical degradation.
(ii) The possibility of achieving mucoadhesive properties to the formulation. Nanocarriers can be decorated with different molecules in order achieve target delivery to specific airway tissue/cells, e.g. penetrate the mucus barrier or remain attached to the bacterial biofilm.[34,35]
(iii) Sustained drug release. Drug is released in a controlled manner, avoiding too high drug concentrations and prolonging the residence time in lung tissue over several weeks.[36,37]
(iv) The ability to escape from alveolar macrophages.

Overall, these characteristics enhance the antimicrobial activity by decreasing the MIC, hence giving rise to an improved treatment. The advantages that different DDSs might present over free-drug administration depend on the nature of the DDS itself (Figure 3).[38–41]

The major disadvantage of nano-DDSs is their potential toxicity. Nanotoxicology has gained much attention in recent decades, especially in the health–pollution field, due to the prevalence of NPs in air. However, most of the NPs for drug delivery are usually made with well-tolerated materials, generally recognized as safe (GRAS), avoiding the possibility of toxicity effects.[42,43] Therefore, when designing NPs, *in vitro* and *in vivo* toxicity studies

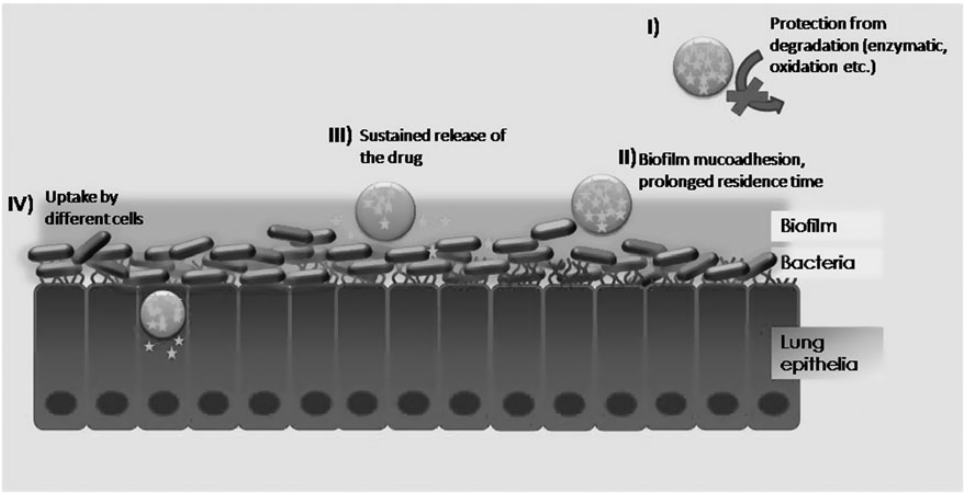

Figure 2. Advantages of nanosystems for the treatment of lung infections. (I) DDSs protect drugs from degradation, e.g. enzymatic degradation or oxidation. (II) DDSs can be tailored to present mucoadhesive properties. (III) Sustained drug release. (IV) DDS uptake by different cells enabling intracellular infection treatment.

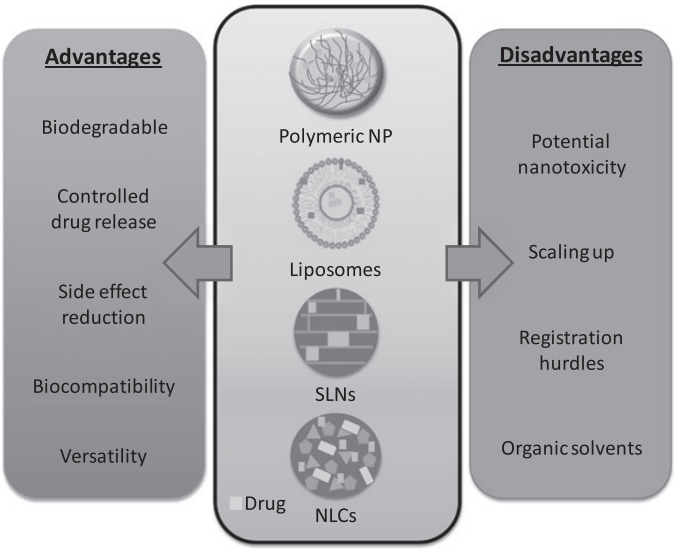

Figure 3. Main advantages and disadvantages of NPs.

should be carefully performed to ensure their safety for human health. Another issue that should be overcome before inhalable NPs reach the market is the scale-up of the preparation process. In this regard, the complexity of the NP production could be a disadvantage (Figure 3).

Challenges faced by NPs before reaching the deep lung

The pulmonary route can be approached to provide a systemic or a local effect. The enthusiasm regarding this route for local targeting is based on the following:

(i) The DDS or the drug comes into direct contact with the pulmonary epithelium, allowing a fast onset of the therapeutic effect.
(ii) High systemic drug concentrations are avoided and thus adverse effects are minimized or prevented.
(iii) Drug degradation is slowed down due to low intra- and extracellular enzymatic activity in the lung environment.[44–46]

The fate of inhaled drug after lung deposition strongly depends on its interaction with the different components of the biological environment. Among them, lung lining fluids, lung cell populations and bacterial biofilm are the most critical factors. In certain pathological conditions, the natural airway mucus can be thicker, enabling bacterial growth and hampering drug action. Another important point is the different cell populations present in the lung. For example, alveolar macrophages may phagocytose the particles, which could be interesting for TB treatment, but disadvantageous when treating other types of bacteria. Finally, overcoming bacterial biofilms also plays a major role in antibiotic therapy. Biofilms are intricate bacterial communities enclosed by an extracellular matrix composed of polymeric substances, DNA and proteins. Bacterial biofilms exhibit high resistance to antimicrobial agents that together with the complex and well-coordinated biofilm mode of growth are the main causes of chronic infections development, e.g. in CF patients, clusters of *Pseudomonas aeruginosa* are embedded in a thick, stationary mucus layer overlying airway epithelial cells.[47]

The development of inhalable pharmaceutical forms using nanocarriers represents a huge challenge. Due to their particle size, they lack suitable aerodynamic flow properties and are exhaled during breathing. In order to overcome this limitation, two main strategies have been followed: nebulization of nanocarriers as a colloidal suspension or associating the system with microsized carriers. The latter approach could be accomplished by either mixing nanocarriers along with inert carriers such as inhalable lactose or mannitol or by embedding the nanosized system into microparticles (Figure 4).[48] Furthermore, another hurdle for the formulation development step is caused by the limited number of excipients approved for inhalation therapy.[49] Carbohydrates, especially lactose and mannitol, are generally

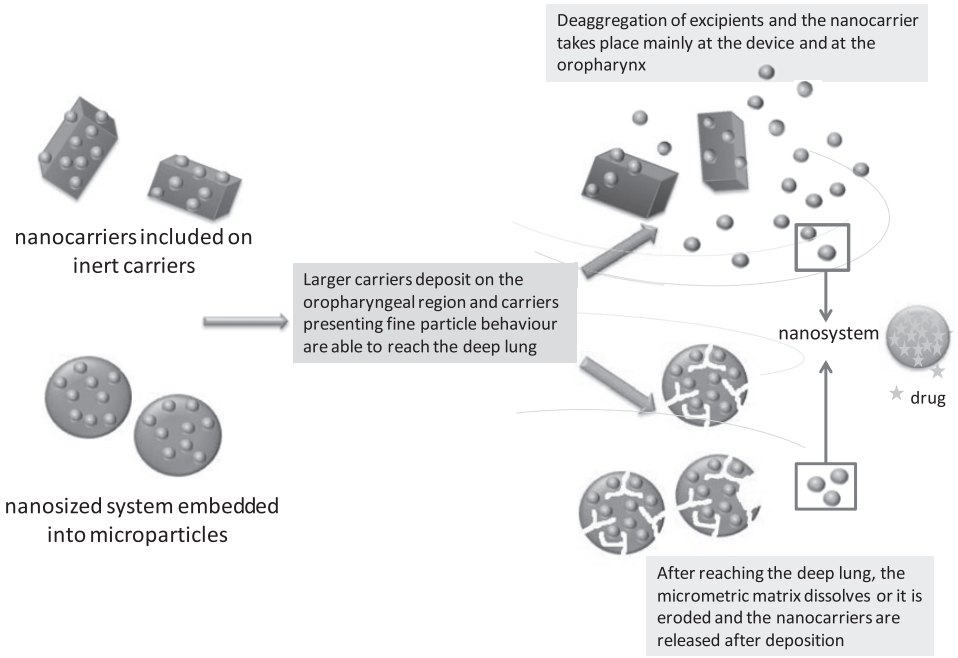

Figure 4. Improvement of the aerodynamic properties of the nanocarriers can be achieved following, e.g. the two different approaches that are represented in the scheme.

used as carriers or excipients for DPIs since they are approved by the FDA, non-toxic and degradable. The amino acid leucine is another candidate to be taken into consideration, since it prevents aggregation due to surfactant behaviour and antiadherent properties at low concentration. Dipalmitoylphosphatidylcholine (DPPC) is a phospholipid normally used to prepare nanosystems for pulmonary delivery because it is the major lipid component of lung surfactant and is relatively non-toxic.[50]

In order to prepare inhalable powders, the spray-drying technique is widely used. This method of producing dry powder is based on evaporating the solvent from a liquid or suspension, achieving solid-state particles presenting appropriate MMAD that ensures drug deposition in the tracheobronchial and deep alveolar regions. Lyophilization, or freeze-drying, has also been explored as an approach to produce stable dry powder that could be administered by DPIs or after rehydration in the appropriate buffer. Both methods produce a powder form that will enhance NP stability, avoiding polymer hydrolysis and drug loss.

As with other inhalable drugs, NPs should meet quality measures of isotonicity, sterility, neutral pH value between 3 and 8.5 (in European Pharmacopeia), biocompatibility, good aerosolization properties and production on an industrial scale.[51]

Current state of clinical therapy

Recently, the FDA approved an inhalation powder containing tobramycin (tobramycin inhalation powder, TOBI Podhaler™)[52,53] to treat *P. aeruginosa* lung infection in CF patients. This product is based on a tobramycin DDS prepared by means of PulmoSphere™ technology, which is an emulsion-based spray-drying process that enables the production of light porous particles. The success of this product has encouraged new developments in this field. In this regard, two more antibiotics, ciprofloxacin (Lipoquin® and Pulmaquin®)[54,55] and amikacin (Arikace™),[56] both as nebulized liposomal formulations, have reached Phase II and Phase III in clinical trials for CF and non-CF bronchiectasis.

More precisely, Phase II trial of ciprofloxacin formulations confirmed that a single administration of Lipoquin® was safe and capable of reducing the *P. aeruginosa* cfu account and improving lung function.[57] Another Phase IIb clinical trial, ORBIT-1 and ORBIT-2, focused on non-CF bronchiectasis patients suffering from *P. aeruginosa* infection. Both liposomal formulations were administered once daily with a 28 day treatment phase and a 28 day off stage with a follow-up period. In this case, patients presented a low incidence of adverse effects and the formulations were overall well tolerated.[55] Pulmaquin® is currently under evaluation in a Phase III clinical trial for the non-CF bronchiectasis population (ARD-3150-1201, ORBIT-3 and ORBIT-4).

Arikace™ is a formulation based on liposomes containing amikacin.[58] After nebulization, it is able to penetrate the characteristic sputum of CF patients. A Phase III trial is currently being conducted with CF patients colonized by *P. aeruginosa* in Europe, Australia and Canada (2011-000441-20, Insmed Incorporated). After analysis of some preliminary results, the authors postulated that liposomal amikacin was safe and effective. A single daily dose of liposomal amikacin showed better results than TOBI® twice daily, especially in terms of respiratory symptoms.[59,60]

New approaches for antibiotic DDSs

The efforts of the scientific community in the development of respirable DDSs have given rise to an extensive literature on antibiotic-loaded NPs that will be overviewed in the following section according to therapeutic group.

Macrolides

Moghaddam *et al.*[61] described an approach for the encapsulation of clarithromycin into PLGA NPs that were freeze- and spray-dried with different excipients, i.e. lactose, mannitol and leucine. Drug release studies showed a biphasic profile releasing 100% of the drug after 2 days (Figure 5). Finally, the aerodynamic study of the NPs was performed by means of a TSI using a Cyclohaler® device. It was observed that the addition of leucine to the formulations led to the best FPF (53.77%) and ED (75.85%). These results could be explained by the non-polar side chain of leucine, which improves flowability due to its antiadherent properties.

Quinolones

Cheow *et al.*[62] developed levofloxacin- and ciprofloxacin-loaded PLGA or poly-ε-caprolactone NPs that presented high activity against *Escherichia coli* in biofilm cells and biofilm-derived planktonic cells. In a subsequent study,[63] these nanoformulations were evaluated against *E. coli* in biofilms. NPs displayed biphasic release profiles over a 6 day period. This biphasic release permitted a high initial antibiotic concentration followed by an extended release profile presenting a drug concentration above the minimum biofilm inhibitory concentration value (i.e. >1.10 mg/L) that is able to inhibit biofilm growth of the surviving persisting *E. coli* cells for 4 days. This biphasic profile seems to be required for the successful eradication of the biofilm and to minimize the exacerbation due to the higher antibiotic susceptibility of the surviving cells.

Another work investigating quinolone encapsulation was reported by Duan *et al.*[64] The spray-drying technique was selected for moxifloxacin and ofloxacin encapsulation. *In vitro* aerosol dispersion of the spray-dried powders was performed using an NGI. When moxifloxacin was spray-dried along with DPPC, high values of ED (>90%) and FPF (>67%) were achieved, together with an appropriate MMAD (<5.24 μm) suitable for reaching the smaller airways without rendered crystallinity. However, ofloxacin powders retained partial crystallinity in certain compositions depending on the DPPC ratio. Hence, on this occasion, the use of DPPC improved the aerosol dispersion of moxifloxacin NPs after spray-drying, leading to powder-form carriers useful for the treatment of pulmonary infections.

Chono *et al.*[65] evaluated the aerosolization of ciprofloxacin incorporated into PEGylated liposomes. In the *in vivo* study, drug distribution in epithelial lining fluid (ELF) was analysed after the aerosolization of PEGylated liposomes and uncoated liposomes and it was observed that the elimination rate of ciprofloxacin from ELF was significantly slower for PEGylated liposomes compared with uncoated liposomes and also the AUC and mean residence time were higher. Moreover, the evaluation of their antibacterial effects against pathogenic microorganisms in ELF showed strong activity against bacteria such as *P. aeruginosa*, *Haemophilus influenzae* and *Streptococcus pneumoniae*. Finally, they also observed that the liposomes led to no lung tissue damage and that PEGylated liposomes did not show cytotoxic effects at the dose assessed. Altogether, the authors concluded that PEGylated liposomes may be a suitable pulmonary DDS allowing ciprofloxacin dose reduction against lung infections.

Ong *et al.*[66] worked on the development of ciprofloxacin-loaded liposomal NPs for the treatment of bacterial infections in CF and non-CF bronchiectasis. The characterization of nebulized aerosols by NGI studies revealed liposome diameters of 4.43 μm, similar to the free drug. The respirable fraction of the formulation was quantified at 70.5% ± 2.03%, i.e. the formulation was able to reach deep-lung regions. Moreover, when the nebulizer-adapted TSI was coupled to a Calu-3 cell culture, it was demonstrated that the formulation allowed slow and controlled release of the drug. In addition, >95% of the liposomal ciprofloxacin remained in the apical chamber of the inserts, meaning that the drug can be released where the bacterial infection takes place (Figure 6). Liposomal ciprofloxacin was found to be as active as the free drug against *P. aeruginosa* and *Staphylococcus aureus*. In addition, MBC testing showed that the liposomal formulation against *P. aeruginosa* presented a significantly lower value than the free

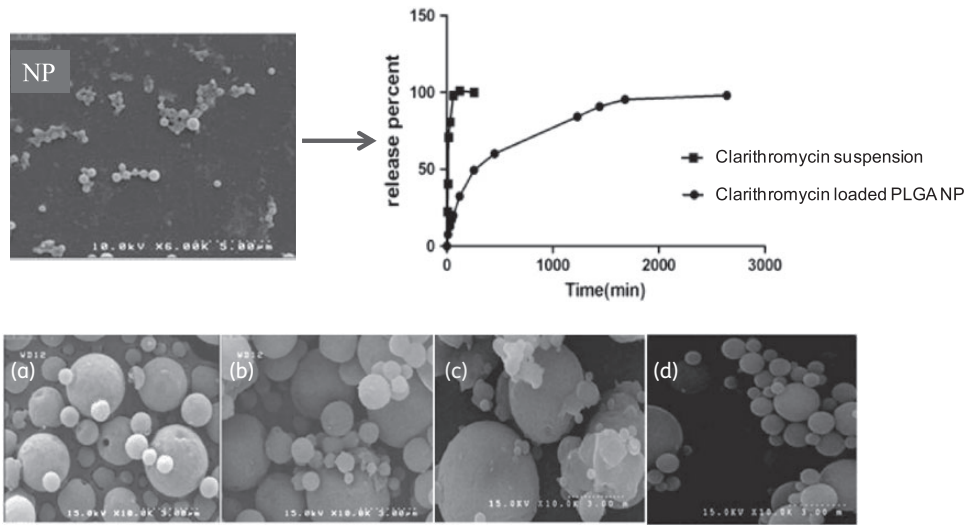

Figure 5. Top row, left: scanning electron microscopy image of clarithromycin-loaded NPs. Top row, right: release profile of clarithromycin-loaded polymeric NPs. Bottom row: four scanning electron microscopy micrographs of clarithromycin-loaded PLGA NPs after spray-drying with different excipients—(a) mannitol and L-leucine NPs; (b) lactose and L-leucine NPs; (c) mannitol; and (d) lactose. Reproduced with permission from Moghaddam *et al.*[61]

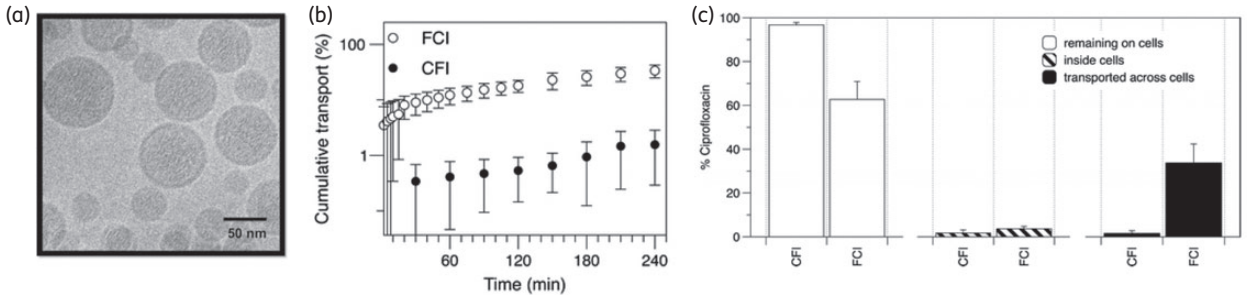

Figure 6. (a) Cryotransmission electron microscopy image of liposomal ciprofloxacin. (b) Apical–basal cumulative transport of nebulized free ciprofloxacin (FCI) and liposomal ciprofloxacin (CFI) on a Calu-3 air-interface cell line ($n \leq 5$, ±SD). (c) Intracellular distribution of ciprofloxacin, remaining on the Calu-3 epithelial cells and transported across the epithelial cells after 4 h, free ciprofloxacin (FCI) and liposomal ciprofloxacin (CFI). Reproduced with permission from Ong *et al.*[66]

Table 2. *In vitro* activity against *S. aureus* and *P. aeruginosa* of nebulized liposomal ciprofloxacin and free ciprofloxacin; reproduced with permission from Ong *et al.*[66]

	S. aureus		*P. aeruginosa*	
Formulation	MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)
Free ciprofloxacin	0.125	0.5–1	0.25–0.5	4
Liposomal ciprofloxacin	0.125	1	0.5–1	2*
Empty liposomes	>32	>32	>32	>32

*$P < 0.05$ compared with free ciprofloxacin.

drug. On the other hand, the ciprofloxacin-loaded liposomes did not provide an improvement in the bactericidal activity against *S. aureus*, very likely due to the presence of the dense peptidoglycan cell wall in Gram-positive bacteria (Table 2). Nonetheless, in order to provide an in-depth analysis of ciprofloxacin liposomes, the same group[67] used different *in vitro* and *ex vivo* methodologies to examine the release mechanisms from the inhalation delivery systems and their effect on drug disposition, comparing them with an *in vivo* assay performed by Yim *et al.*[68] As the results were qualitatively similar, they underlined the usefulness of *in vitro/ex vivo* models for the prediction of *in vivo* results.

Sweeney *et al.*[69] also developed a ciprofloxacin-loaded liposomal powder formulation using a spray- and freeze-drying process that showed adequate aerodynamic properties measured by ACI. By means of a numerical deposition model developed by Finlay *et al.*,[70] the drug concentration in the airway surface liquid was calculated to be 5 mg/L. This drug concentration would be above the MIC and thus could inhibit the growth of many pathogens, such as *P. aeruginosa*, *Streptococcus pyogenes*, *Neisseria gonorrhoeae*, *Bacillus anthracis* and many other aerobes. Nonetheless, more experimental outcomes should be provided in order to ensure the robustness of these estimations.

As another strategy, Liu *et al.*[71] encapsulated ciprofloxacin into liposomes presenting sustained *in vitro* release in simulated lung fluid over 36 h. Liposomes were administered to rats by intratracheal instillation. The drug concentration in the lung was higher for the liposomal antibiotic than for the free drug, e.g. liposomal ciprofloxacin presented 18.7 h $t_{1/2}$ in the lung and 151.2 mg/g C_{max}, representing 7.21- and 4.99-fold increases, respectively, over those of the free drug. Bioavailability results also confirmed that liposomal ciprofloxacin was able to reach the lung and provide high drug concentrations at the target site. In addition, an *in vivo* pulmonary irritation test showed ciprofloxacin liposomes were able to minimize modification and irritation of the lungs after intratracheal instillation in rats. From these results, it can be inferred that successful pulmonary delivery of a liposomal formulation was achieved with a high concentration of ciprofloxacin at the target site.

Aminoglycosides

Alhariri *et al.*[72] developed tobramycin-loaded liposomes incorporating bismuth-ethanedithiol (BiEDT) (LipoBiEDT-TOB). Previous work described that BiEDT in the presence of tobramycin has a synergistic effect against *P. aeruginosa* and *Burkholderia cepacia in vitro*.[73,74] The MIC of LipoBiEDT-TOB was 16-fold lower than that of free tobramycin and 4-fold lower than that of free tobramycin together with BiEDT. In a further *in vivo* assay, intratracheal administration of the liposomes was studied in rats chronically infected with *P. aeruginosa*. It could be observed that, after 24 h, LipoBiEDT-TOB decreased the bacterial counts in the lungs up to 10^3 cfu/lung, whereas untreated animals and the free antibiotic group displayed $10^{7.4}$ and $10^{4.7}$ cfu/lung, respectively. After the last dose of LipoBiEDT-TOB, no tobramycin was detected in the kidneys, whereas the free drug was found in the kidneys and lungs. Taken together, the authors concluded that pulmonary administration of LipoBiEDT-TOB could improve the treatment of chronic *P. aeruginosa* infection in CF patients.

Another tobramycin-loaded formulation was evaluated by Pilcer *et al.*[75] In this case, a mixture of microparticle and NP formulation was developed. The aerodynamic behaviour of the spray-dried tobramycin formulations was evaluated by an MSLI using an Aerolizer® as the inhalation device. It was confirmed that the NP-coated tobramycin increased the FPD during inhalation, which was explained by the fact that coating the drug with NPs could reduce powder agglomeration and cohesion with other particles. Similarly, it was found that an increase in the amount of sodium glycocholate in the spray-dried suspension led to an enhancement in FPF from 36% to ~61%. In conclusion, mixing tobramycin-loaded NPs and microparticle dry powders with low levels of sodium glycocholate resulted in a suitable DDS for treating lung diseases as it offered effective pulmonary delivery.

Tobramycin encapsulation was also described by Ungaro *et al.*,[76] although this group selected PLGA as the core polymer. Spray-drying was performed in order to obtain micrometre-sized dry powder particles using lactose as an inert carrier. The drug release was optimal, providing a burst release followed by maintained liberation of the drug for a month. Chitosan-coated PLGA NPs were able to penetrate through an artificial mucus layer. The MIC values of the PLGA formulations for *P. aeruginosa* planktonic cells were much higher than that of the free antibiotic. This could be due to the biphasic extended antibiotic release profiles of the NPs that in turn liberated small amounts of drug into the media that were very likely below the MIC. The aerosolization properties of the formulations were investigated *in vitro* using an MSLI coupled to a Turbospin®. The results confirmed that both powders presented suitable properties of MMAD and FPF with an ED of 100%. *In vivo* biodistribution studies in rats, after intratracheal delivery using the Penn-Century® device, showed that PVA-modified alginate PLGA NPs reached the deep lung, whereas chitosan-modified NPs were located to a greater extent in the upper airways. Hence, PVA preparations led to the development of respirable lactose–PLGA carriers suitable for lung delivery.

Rukholm *et al.*[77] proposed the encapsulation of gentamicin into liposomes. MIC and time–kill studies were performed with free and liposomal gentamicin against *P. aeruginosa*. The most remarkable difference among the two gentamicin preparations was observed for the MIC values, where liposomal gentamicin showed significantly lower values (32 mg/L) than those of the free form (512 mg/L) for a non-mucoid clinical strain of *P. aeruginosa* isolated from the lungs of a CF patient that was resistant to gentamicin. The authors explained these results by the possible fusion of the liposomes with the outer bacterial membrane, which may have led to increased penetration of the antibiotic. Finally, in *in vitro* time–kill studies, only the 4-fold MIC liposomal formulation demonstrated improved antimicrobial activity against the antibiotic-resistant strain by achieving complete bacterial eradication in 6 h, whereas the free drug needed 24 h to eradicate the bacteria. The authors concluded that the liposomal gentamicin formulation was effective, presenting an improved killing time and prolonged antimicrobial activity against *P. aeruginosa*.

In an attempt to decrease drug toxicity and improve dosing by drug targeting, Ghaffari *et al.*[78] encapsulated amikacin into SLNs for pulmonary delivery and a lyophilization step was carried out for the stabilization of the formulation. It could be determined that SLNs, whether as a freeze-dried powder or as a dispersion, were able to release >95% of the drug during 6 days of incubation. Both SLNs presented activity against *P. aeruginosa*. Nonetheless, testing of amikacin-loaded SLNs showed that SLNs increased the MIC and MBC values compared with the free drug. However, as the incubation period of this test was set at 48 h, it should be kept in mind that only the 25% of the drug was released from the SLNs; hence, the authors postulated that SLNs *in vivo* might present half the MIC and MBC compared with the free drug. The authors hypothesized that besides the sustained drug release profile, SLNs have the advantage of improving the antibacterial activity of amikacin due to diffusion enhancement across the bacterial membrane.

In a further study related to amikacin, Varshosaz *et al.*[79] analysed the biodistribution in the lungs and kidneys of ^{99m}Tc-labelled amikacin SLNs after pulmonary delivery to assess whether amikacin encapsulation could increase the drug concentration in the lungs and thus reduce side effects of CF treatment. The drug release profile displayed a continuous and sustained pattern for 144 h. In the subsequent *in vivo* experiment, ^{99m}Tc-labelled amikacin SLNs or free ^{99m}Tc-amikacin were administered by the inhalation route, detecting a similar signal in the lung for both formulations. It is worth mentioning that pulmonary-administered SLNs presented higher drug concentrations in the stomach than intravenous administration, which might be related to swallowing exhaled particles after administration. Finally, the authors concluded that SLNs seem to be a promising inhaled carrier for improving the efficacy of amikacin in CF as well as reducing the dose frequency due to sustained drug release and could, therefore, decrease drug toxicity, especially nephrotoxicity.

Polypeptides

Pastor *et al.*[80] recently reported the utility of lipid NPs for the encapsulation of sodium colistimethate. More precisely, SLNs and NLCs were elaborated. Both lipid NPs presented antimicrobial activity against clinically isolated *P. aeruginosa* strains at a concentration of 1–2 mg/L. Cell experiments using the A549 cell line showed that lipid NPs were able to significantly reduce antibiotic toxicity. Next, an *in vivo* biodistribution assay was conducted after nebulizing infrared (IR)-labelled NLCs into mice. It was observed that NLCs spread homogenously throughout the lungs, whereas no signal could be detected in other organs. The IR intensity was detectable 48 h after administration, suggesting that the dosing interval could be prolonged by the use of these NPs.

Conclusions

Pulmonary infections are often persistent and recurrent. A potential therapeutic approach is to target the delivery of antibiotics directly to the site of infection as a mechanism to increase and maintain the local drug concentration. In recent years, the encapsulation of antimicrobial drugs into nanocarriers has appeared as a powerful tool for enhancing therapeutic effectiveness against infectious diseases and minimizing side effects of the drugs. The inhalation route has gained much attention as a promising alternative administration route for the treatment of pulmonary infections. Tight control over the geometric size and morphology of particles resulted in aerosols with narrow aerodynamic size distributions that would be able to reach the deep-lung region and appropriately deliver the antibiotic to the site of infection.

Here, the current progress and challenges in synthesizing NP systems for delivering various antimicrobial drugs are reviewed. The published data stated that DDSs for inhalation therapy are able to decrease the antibiotic dose administered, thereby reducing toxicity as well as enhancing patient compliance and adherence to the treatment. Much has been studied in order to overcome the resistance of common antibiotics, yet additional efforts are needed. We need to gain insight into the complex context that surrounds the infection by better understanding the interaction of different fields, such as microbiology, physiopathology, immunology, pharmacokinetics/pharmacodynamics, pharmacology and microtechnology and nanotechnology.

Overall, the scientific community should pay attention to the formulation of DDSs to improve lung deposition and anti-infective therapy. Therefore, further tailoring of currently available DDSs is required in order to translate this technological advance into clinical benefits.

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