Antibody-loaded polymeric nanoparticles as a promising strategy for inhalable lung cancer treatment

A new technological approach designed to combine targeted and controlled drug delivery while protecting antibody structure and bioactivity

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Abstract

Lung cancer has a high mortality rate among all common cancers. Conventional lung cancer therapy is usually administered intravenously, with low selectivity for tumor cells and severe side effects. Therapeutic proteins such as antibodies can be used as an alternative treatment or in combination with chemotherapy, demonstrating benefits due to higher specificity and bioactivity with cancer cells and markers, and lower toxicity compared to low molecular weight drugs. Inhalable drug delivery can facilitate localized delivery of drugs to the target tissue, directly to lung cancer cells, and may improve antitumor activity while reducing systemic adverse effects. For these reasons, antibody encapsulation into polymeric nanoparticles formulated into dry powders by spray drying is a promising strategy that combines targeted drug delivery with the ability to protect antibody structure and bioactivity. This article aims to show the potential of antibody-loaded polymeric nanoparticles as a promising option for inhalable lung cancer treatment, as it may address multiple drawbacks of conventional lung cancer therapy.

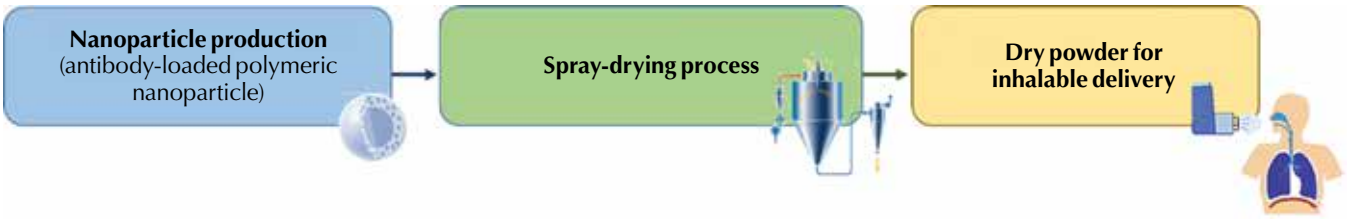
Introduction

Lung cancer is the second most diagnosed cancer worldwide and is the main cause of cancer death among all common cancers in people above age 50. Approximately 50% of cases are diagnosed at advanced stages, when treatments are no longer possible or are ineffective. Consequently, these patients have a low chance of survival, which results in high mortality levels of approximately 80%. Moreover, according to the American Cancer Society, 238,340 new lung cancer cases and 127,070 deaths are anticipated in 2023 [1]. Therefore, early diagnosis and treatment initiation are the main goals to improve the prognosis of the disease and increase the survival rate [2].

Conventional lung cancer treatment protocols depend on the cancer type and disease stage. They usually include local surgery in combination with other therapies, namely chemotherapy and radiation therapy [3-6]. Chemotherapy is generally the initial treatment, with platinum-based therapy used in most cases as the main support. It is administered in combination with other chemotherapeutic drugs such as paclitaxel, vinorelbine, topotecan and gemcitabine [7]. These conventional therapies are generally administered systemically. However, these drugs can cause serious side effects that compromise patients quality of life, including pain, nausea, fatigue, allergic reactions, mucositis, dysphagia, xerostomia, ototoxicity and nerve damage [5, 6, 8]. Furthermore, these drugs do not act selectively on tumor cells, which requires the administration of high doses to achieve therapeutically effective drug concentrations in the target zone and effective anti-tumor activity. These high doses result in damage to healthy cells and toxic effects, particularly in cells with high division rates such as hair, skin, spleen, liver and others. Therapeutic proteins, namely antibodies, are being used as alternatives or in combination with chemother-

Figure 1

Process phases to obtain an inhalable dry powder, which is the final product from the antibody loaded-nanoparticle formulation.



apy for lung cancer treatment, demonstrating higher specificity and bioactivity and lower toxicity compared to conventional therapies [9].

In recent years, several monoclonal antibodies have undergone clinical trials for lung cancer treatment and four are already approved by the United States Food and Drug Administration (FDA). These include Erbitux® (cetuximab; Eli Lilly and Company) and Portrazza® (necitumumab; Eli Lilly and Company), which target epidermal growth factor receptor (EGFR), as well as Avastin® (bevacizumab; Genentech, Inc.) and Cyramza® (ramucirumab; Eli Lilly and Company), which target vascular endothelial growth factor (VEGF) [10-12]. Programmed death-ligand 1 (PD-L1) inhibitors have also had a major impact on patients with non-small-cell lung cancer (NSCLC) in early-stage disease [13]. Tecentriq® (atezolizumab; Genentech, Inc.), Libtayo® (cemiplimab; Regeneron Pharmaceuticals, Inc.), Imfinzi® (durvalumab; Medimmune/AstraZeneca), Yervoy® (ipilimumab; Bristol Myers Squibb), Opdivo®(nivolumab; Bristol Myers Squibb) and Keytruda® (pembrolizumab; Merck & Company) are several checkpoint inhibitors already approved by the FDA to treat lung cancer [10, 11, 14].

Pulmonary drug delivery is localized to the target tissue, which can allow selective treatment with a lower dose than systemic delivery and consequently, fewer side effects [15]. However, antibody molecules are prone to instability during storage and processing, which can seriously influence their performance. Furthermore, they can become unstable upon administration, due to enzymatic degradation [16, 17]. In contrast, encapsulation of antibody molecules in polymeric nanoparticles for inhaled delivery may surpass these limitations, achieving a more effective and selective therapy while antibody structure and bioactivity could be maintained [18].

Spray drying is a well-established method used in formulation development to obtain dry powders from a liquid formulation. In addition, this process has been shown to be an appropriate strategy for the preparation of antibody formulations intended for pulmonary delivery [19-21]. One promising approach to surpass current lung cancer treatment limitations includes the use of nanotechnology to encapsulate the antibody into polymeric nanoparticles for pulmonary delivery upon formulation into dry powders (Figure 1). The nanoparticles are expected to maintain the antibody structure and bioactivity, and act as targeted and controlled drug delivery nanocarriers [22-24].

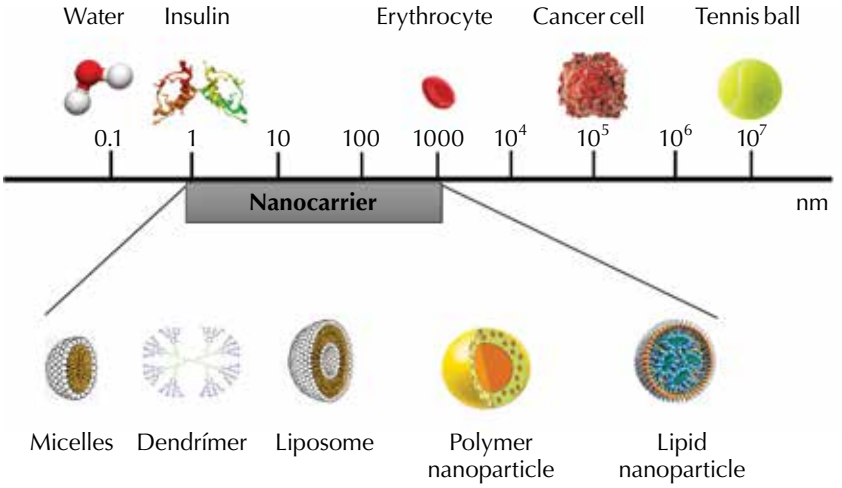
The main aim of this article is to provide an overview of the development of polymeric nanoparticles intended to obtain optimal features for loading therapeutic antibodies and subsequently be formulated into dry powders for inhalable lung cancer treatment. This strategy may reduce serious side effects and toxicity compared to conventional treatments and support therapeutic adherence, effectiveness and safety. The article will also discuss requirements, advantages and challenges for a dry powder formulation with respect to particle deposition profile in the respiratory tract and the spray-drying process.

Nanomedicine therapeutic approach

Nanomedicine as a therapeutic approach, using nanotechnology systems, has appeared as a new paradigm for cancer treatment [6, 25, 26]. Nanomedicine concerns the use of nanoparticles ranging between 1 and 1,000 nm [27-29]. These nanostructures can load or bind therapeutics and act like drug carriers. They can be manipulated for controlled and sustained release, crossing biological barriers, prolonging blood concentration, and reducing or avoiding enzyme degradation, toxicity and immunogenicity of therapeutic agents [30-32]. They also can promote the accumulation of drugs in cancer tissues via an enhanced permeability and retention (EPR) effect [33]. Moreover, these systems can interact with therapeutic targets, among other markers present in cells, while delivering drugs to the target area, preserving their structure and bioactivity, and reducing the effects of treatment on healthy cells [30-32]. Antibody nanoencapsulation can allow the stabilization of antibody structure and bioactivity preservation while a controlled and targeted release occurs [23]. For these reasons, several nanocarrier structures are being developed as potential antibody delivery systems for lung cancer treatment [26] (Figure 2).

Figure 2

Organic nanocarrier systems aimed at pulmonary delivery of drugs. Adapted from [6, 34, 35].



In particular, polymeric nanoparticles have been studied for lung cancer therapy (Figure 2). These are optimal carriers that can offer several advantages for drug delivery, due to features for easy surface modification and nanosizing, as well as the ability to encapsulate various types of drugs. They also have better storage stability compared to lipid-based formulations [36]. Natural and synthetic polymers can be used to produce these carriers. The latter typically have higher purity, are prepared in precise and well-controlled processes, and are frequently associated with sustained release for days to several weeks compared to the relatively shorter duration of drug release provided by natural polymers [36, 37].

Polymers such as poly lactic-acid-co-glycolic acid (PLGA), polylactic acid (PLA), and polycaprolactone have demonstrated good performance in lung cancer therapy [36, 38-40]. PLGA is a biocompatible and biodegradable synthetic copolymer that undergoes complete biodegradation, in aqueous media, to glycolic and lactic acids. Here, the ratio between glycolic and lactic acid content drives the direct degradation kinetics of the delivery system in the body and, therefore, the release rates of loaded drugs. These monomers in the body can be metabolized and eliminated as carbon dioxide and water or excreted through the renal system. PLGA has been approved by the FDA for drug delivery applications [37, 41, 42].

However, PLGA nanoparticles are unstable in water and are highly recognized by the reticuloendothelial system (RES) of the liver and spleen. This can remove nanoparticles from blood circulation and/or reduce their residence time in the bloodstream, thereby considerably decreasing the delivery of nanoparticles to targeted tissues or cells. Additionally, the delivery of therapeutic proteins often results in interactions such as adhesion and aggregation between the hydrophobic surface of the polymeric system and protein molecules during storage, processing and release [41, 42].

Polyethylene glycol (PEG) is a hydrophilic molecule that can be used to stabilize nanoparticles in aqueous media, due to its properties that include electrical neutrality, significant spatial repulsion and molecular conformation [43]. In addition, PEG can expose uncharged hydrophilic groups, increasing its solubility and avoiding molecule aggregation by steric interference. These advantages may be useful during production, storage and application, for instance, by resisting protein and platelet adhesion [41, 44, 45]. PEG can also hinder fast recognition by the immune system and suppress the opsonization phenomenon, prolonging circulation time and accumulation in the tumor site. Moreover, it is classified as Generally Regarded as Safe (GRAS) by the FDA [45]. However, the insertion of too many ligands on the surface of nanoparticles, like excessive PEGylation, can alter the opsonization profile of serum protein patterns, leading to strong inhibition of cellular uptake and reduced binding to protein targets. Lastly, these effects can result in increased elimination of nanoparticles by the reticuloendothelial system [46, 47]. Consequently, the density of ligands around the nanoparticle must be balanced to achieve optimal distribution and penetration through the tumor mass.

PEG coating is a critical technique for improving biophysical and chemical properties of nanoparticles. By modifying the covalent bond of PLGA nanoparticles with PEG (PEGylation), hydrophilicity of the formulation can be increased and nanoparticles can be shielded from the immune system and uptake by the mononuclear phagocyte system, thereby increasing *in vivo* circulation time and probability the drug will reach its site of action [41]. The PEG-PLGA nanoparticle system has been shown to be non-toxic in various *in vitro* and *in vivo* studies, which makes it a promising polymeric system to be explored for the treatment of lung cancer [37, 45, 48].

Sousa, et al. encapsulated the antibody bevacizumab into PLGA nanoparticles using a modified solvent emulsification/evaporation method based on a w/o/w double emulsion technique. An association efficiency (AE) of 82.47 ± 0.56% and drug loading (DL) of 1.62 ± 0.01% were achieved, proving a successful method of encapsulation. An increase in mean particle size was also observed, from 168.4 ± 5.4 nm (unloaded nanoparticles) to 198.6 ± 5.4 nm (loaded nanoparticles) due to the large size of bevacizumab (149 kD). Attenuated total reflectance Fourier transform infrared (ATR-FTIR) analysis and circular dichroism analysis, as well as fluorescence spectroscopy and its spectrum, did not show relevant structural changes in comparison with the characteristic spectrum of native bevacizumab, even after release from nanoparticles. Moreover, *in vitro* studies showed bioactivity of bevacizumab was retained after encapsulation, even with its slow release from nanoparticles, which overall reveals the success of the bevacizumab encapsulation [18].

In this sense, antibody encapsulation into PEGylated PLGA nanoparticles for inhaled administration mediated by dry powders is a promising new strategy, which combines targeted and controlled drug delivery with the ability to protect antibody structure [18, 49].

Inhaled drug delivery

The delivery of drugs via inhalation has received attention due to lung characteristics. From a general point of view, the lung is a good target due to its large surface area with a thin epithelial/air barrier, high solute permeability and low proteolytic activity. Also, it comprises a non-invasive route for the systemic and local delivery of medicines [16, 50]. Moreover, if dry powders are selected, instability issues of aqueous formulations, such as aggregation and limited shelf life, could be overcome [15, 51].

Inhalable drug delivery also allows localized delivery of drugs to the targeted tissue: lung cancer cells. This allows selective treatment and use of high local concentrations, which may improve anti-tumor activity while reducing systemic adverse effects [15]. Therefore, delivery of antibodies for lung cancer treatment by inhalation can be a more selective, effective, efficacious and safe strategy than conventional approaches [16, 17]. However, antibody structure and bioactivity must be maintained during formulation development, storage and delivery to achieve safe and effective treatment. Spray drying of nanoparticles loaded with antibodies can allow their conversion into dry powders for appropriate inhalable therapy for lung cancer [18, 19, 23, 52, 53].

Nevertheless, inhalation of nanocarriers has an inherent limitation: a high probability of exhalation before particle deposition, due to the low inertia provided by the nanometric particle size [19, 54]. It is known that for whole lung (bronchioles to alveoli) drug delivery and deposition, it is essential to have particles with a mass median aerodynamic diameter (MMAD) of 1 - 5 µm or 1 - 100 nm. Particles larger than 5 µm will be deposited in the nasopharynx and swallowed. On the other hand, particles smaller than 1 µm and larger than 100 nm will remain suspended in air and exhaled before having the opportunity to deposit [55, 56].

Pulmonary clearance of inhaled particles should also be considered. After lung deposition, particles immersed in the airway fluid (mucus or surfactant, depending on the region of the respiratory system), can permeate lung tissue and be absorbed or exposed to the natural clearance mechanisms present in the respiratory system [19, 56]. Particles smaller than 260 nm can experience reduced macrophage phagocytosis and particles less than 70 nm are not recognized by macrophages. Microparticles of 1 to 3 µm are easily phagocytosed by alveolar macrophages and cleared from the respiratory zone, and below these sizes, phagocytosis is easier the closer the size is to 1 µm [7, 55]. Particles that do not suffer macrophage phagocytosis may enter the lung epithelium if they are small enough in size [56, 57].

Large porous particles show better aerosolization properties. Therefore, a workable strategy to deliver nanoparticles to the lung is to convert them into microparticles, which can be performed by spray drying using matrix excipients. Microparticles may be tailored to exhibit desired aerodynamic properties for lung deposition. However, matrix excipients used for nanoparticle microencapsulation should be carefully selected to avoid toxicity concerns. These microparticles simply act as nanocarrier vehicles to the lung. Upon deposition, it is expected the matrix structure will rapidly dissolve within the lung lining fluid, releasing the nanoparticles, which are then ready to deliver the therapeutic protein to the target site [19, 58].

So, careful dry powder formulation and process optimization are needed for successful inhaled drug delivery. Various approaches have been developed to increase the size of antibody-loaded nanoparticles and result in deposition in the desired location upon inhalation. One of these approaches is the use of spray drying, which allows the conversion of nanoparticle formulations into dry powders suitable for pulmonary delivery [19].

Dry powder formulation

Spray drying involves a single-step procedure that converts a liquid formulation (solution, suspension or emulsion) into dry particles. The liquid dispersion passes through an atomizer nozzle to produce a spray inside the drying chamber, which is set to a temperature that enables solvent evaporation, leading to the formation of solid particles as a final product [19, 59, 60].

This process is known to offer several advantages and can be adapted to obtain the desired aerodynamic properties in the final dry powder. The morphology of that powder is usually hollow and wrinkled or dimpled, which provides its low density and, consequently, good aerodynamic properties for inhalation. However, the relationship between spray-drying parameters and *in vitro* aerodynamic performance of inhalable nanoparticle-based powders is also dependent on the formulation composition [61]. Spray drying is a simple, reproducible, scalable and cost-effective method that requires less time than other drying methods like lyophilization. In addition, it is well established as a rapid industrial drying process, raising interest in its use since there is the chance for continuous processing. All these factors may support a promising and quick solution for large-scale production [57, 59].

Spray-dried products are fine and free-flowing powders (compared to lyophilizates) which can facilitate precise particle engineering for particle size, density, morphology, aerodynamic properties and water content. Furthermore, it is a drying method appropriate for heat-sensitive compounds such as therapeutic proteins because of the short time materials are exposed to the high temperatures used in the process (usually on the order of a few seconds) [59, 62]. Moreover, if therapeutic proteins are loaded into nanoparticles before the spray-drying process, this exposure can be reduced even further [19]. Overall, the process can improve the speed and quality of biopharmaceutical production of products such as proteins and may provide both financial and health benefits [51, 63].

Still, for spray-drying of therapeutic antibodies, it is necessary to ensure their stability and maintain their bioactivity. This requires extensive investigation and study so complex and interrelated process parameters must be considered. The next section of this article will discuss various important aspects of the spray-drying process, which can influence physicochemical properties of the final product and the physical state of the formulation.

Spray-drying conditions and parameters

The critical parameters in the spray-drying process can be separated into two categories: feed solution characteristics and process factors.

The composition of the feed solution will impact the spray-drying process and the resulting solid particle properties, such as particle size, aerodynamic characteristics, morphology, surface-to-bulk structure and residual solvent content. Therefore, it is important to modulate the liquid feed properties such as concentration, viscosity, etc., using particle engineering to obtain the desired properties. Matrix excipients are also added to the liquid dispersion before spray drying. When nanoparticles are part of the dispersion, these excipients act as bulking agents, stabilizing the nanoparticles during processing and preventing exposure to stressful atmospheric conditions that may induce degradation. In this way, it is possible to obtain a dry powder composed of microparticles carrying therapeutic, antibody-loaded nanoparticles and achieve suitable properties for pulmonary delivery [51].

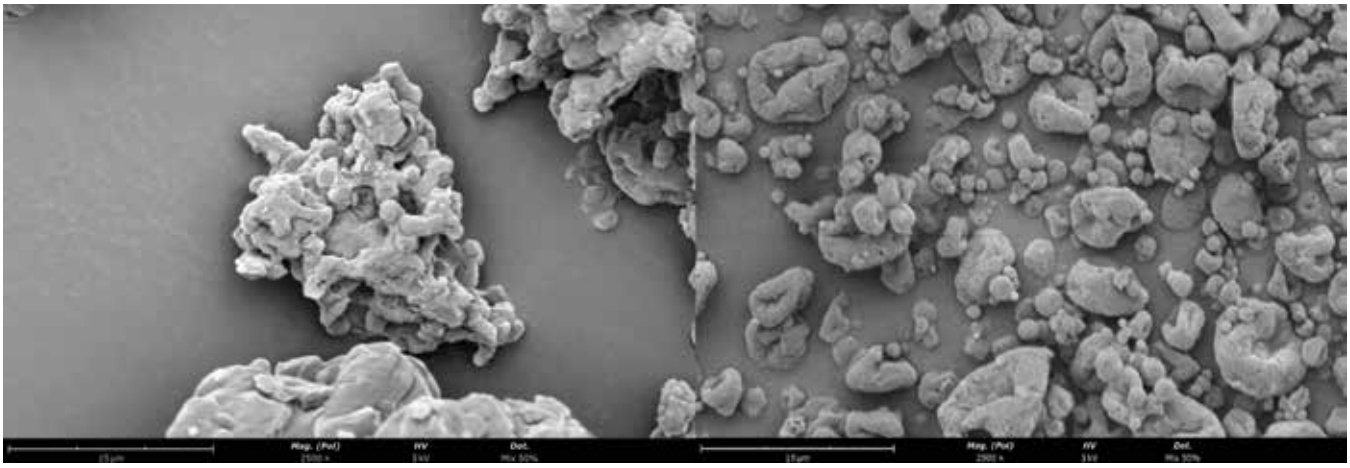
A limited number of excipients are approved for pulmonary delivery. Selected excipients should improve aerosol performance and powder flowability and foster a particle size that can target the deep lung. In addition, the ratio of nanoparticles to excipients has been shown to affect the surface morphology of the spray-dried microparticles [64].

Compounds from six chemical classes have been used as excipients for inhaled delivery: carbohydrates, amino acids, surfactants, salts, buffering agents, proteins and other molecules [51]. Mannitol has been the carbohydrate used most frequently with proteins (e.g., antibodies) in spray-dried formulations and commercialized products, due to low hygroscopicity and crystallization following drying [51, 65]. Moreover, as a non-reducing polyol, mannitol improves protein stability, whereas sugars like lactose have reducing behavior due to their free aldehyde groups and can react with protein amino acid groups through the Maillard reaction [65]. Finally, mannitol may improve lung function in patients with lung diseases due to hyperosmotic effects that can increase mucus clearance [51, 66]. L-leucine (or L-isoleucine) has also been used as a matrix excipient due to technological advantages it can provide during the production process, such as improved powder flowability and reduction of powder moisture content [67, 68]. Use of L-leucine can also result in reduction of powder adhesion to the walls of the spray-drying apparatus, reduction of impaction loss and increase in process yield [69-71]. Finally, L-leucine can acquire a crystalline structure after drying, which can lead to collapse of hollow droplets during water evaporation, resulting in corrugated surfaces and reduced moisture content in the powders. All these factors can improve powder flowability and aerosol performance [70-72].

Our research group has been studying optimization of spray-drying excipients. After evaluating various excipient concentrations, we found the combination of D-mannitol 57% (w/w) and L-leucine 29% (w/w) was the better microparticle matrix for the microencapsulation of mPEG-PLGA nanoparticles (unloaded) 14% (w/w) in the dry powder (Figure 3, right). Dry powders with suitable characteristics for inhalation were obtained, with a reduction of particle agglomeration after spray drying, and satisfactory production yields up to approximately 60% were obtained. A strong difference was noted for microparticles composed of D-mannitol 80% and nanoparticles 20% (w/w) (Figure 3, left) which appear individualized,

Figure 3

Scanning electron microscopy (SEM) image of spray-dried microparticles of unloaded mPEG-PLGA nanoparticles using D-mannitol 80% (w/w) (left) and using the combination of D-mannitol 57% (w/w) and L-leucine 29% (w/w) (right) as matrix excipients (nanoparticle composition was 14% and 20% (w/w), respectively). Spray-drying process parameters: inlet temperature = 170 ºC; aspiration rate = 90%; atomizing air flow rate = 473 L/h; feed rate = 2 mL/min. The scale bar represents 15 µm. Adapted from [73].



with a spherical shape and in large agglomerations due to the D-mannitol bulking effect [73].

Jensen, et al., studied the effect of using mannitol, lactose and trehalose as matrix excipients on the spray drying of siRNA-containing PLGA nanoparticles intended for inhalation. They found by using mannitol, a higher yield (56.4%) and a lower moisture content (0.78% w/w) were obtained. An aerodynamic particle diameter of approximately 5 µm was also found. This work demonstrates the impact of various formulation conditions (namely, matrix excipient compositions) on spray-drying responses and shows some excipients may be suitable for inhalation while others are not [64].

Spray-drying process parameters are mainly instrument-related, such as inlet and outlet temperatures, feed rate, type of atomizing gas, nozzle type, atomizing airflow pattern and aspiration rate. Their effects on process outcomes, including spray-drying yield, powder moisture content, fine particle fraction (FPF) and aerodynamic particle size, can be complex so careful optimization is needed. For example, powder production yield and, consequently, process efficiency should be maximized. Yield can increase due to changes in atomizing air flow rate, inlet temperature, solids concentration, improved cyclone separation, reduction in air humidity and moisture content. Low powder moisture contents are usually required because increased moisture can negatively impact powder physical stability and aerosol performance. In contrast, final particle size can be increased through a decrease in atomizing air flow or an increase in feed rate and solids concentration [74]. So managing the interactions among these factors to optimize the spray-drying process is critical [59, 75].

Another key outcome in spray drying is particle size distribution, as it greatly impacts physical powder properties, including bulk density and aerodynamic behavior. An ideal process should yield particles of consistent size and shape, to promote predictable aerodynamic behavior. Control of droplet size and distribution can be influenced by adjusting the spray-drying parameters, feed solution composition and characteristics such as viscosity and solids content [59, 76].

Lastly, maintaining therapeutic antibody structure and bioactivity are also fundamental so spray-drying conditions, including inlet and outlet temperatures, humidity air flow and residence time, must be carefully selected. In addition, excipients such as cryoprotectants, added prior to antibody encapsulation, can assist in preserving structure and bioactivity [19].

Conclusion

Pulmonary delivery of dry powders can enable targeted cancer therapy to reach the lung. In turn, therapeutic antibodies used as lung anti-cancer therapy could be delivered by inhalation. As a first step, antibody nanoencapsulation may play a key role in antibody stability and maintenance of bioactivity. An additional process of microencapsulation through spray drying can provide nanoparticles with characteristics to support lung delivery. Overall, this article reviews the main concepts for development of an inhalable strategy for lung cancer treatment, as well as the administration of therapeutic antibodies in the form of dry powders, which can assist in developing a new paradigm for improved lung cancer therapy.

Further studies are necessary to evaluate antibody structure and bioactivity preservation after drying, as well as interactions with the microparticle matrix.

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