Biomaterials 34 (2013) 516–525

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Review

Airway delivery of peptides and proteins using nanoparticles

Christophe Y. Dombu [a], Didier Betbeder [a,b,c,*]

[a] PRES université Lille Nord de France, France
[b] EA4483, IMPRT, IFR 114, Faculté de médecine pôle recherche, Université de Lille 2, 59000 Lille, France
[c] Université d'Artois, 9 rue du temple, 62030 Arras, France

ARTICLE INFO

Article history:
Received 8 August 2012
Accepted 30 August 2012
Available online 6 October 2012

Keywords:
Nanoparticles
Airway
Peptide
Protein

ABSTRACT

Delivery of peptides and proteins via the airways is one of the most exciting potential applications of nanomedicine. These macromolecules could be used for many therapeutic applications, however due to their poor stability in physiological medium and difficulties in delivering them across biological barriers, they are very difficult to use in therapy. Nanoparticulate drug delivery systems have emerged as one of the most promising technologies to overcome these limitations, owing mainly to their proven capacity to cross biological barriers and to enter cells in high yields, thus improving delivery of macromolecules. In this review, we summarize the current advances in nanoparticle designed for transmucosal delivery of peptides and proteins. Challenges that must be overcome in order to derive clinical benefits are also discussed.

1. Introduction

Since the first attempts to administer insulin via inhalation in the early 1920s, airway delivery of proteins has gradually emerged as a serious non-invasive alternative for protein administration. The human respiratory tract is an easily accessible route with a large surface area, bypassing the first metabolic passage in the liver. Moreover, the low thickness of the epithelial barrier, the vast underlying vasculature, the low proteolytic activity, the low acidity and the thinner mucus layer compared to the gastrointestinal mucosa, make the airways suitable for both local and systemic applications [1]. However, the respiratory mucosa forms a very tight and complex barrier, hardly permeable to macromolecules such as proteins. Indeed, proteins alone are poorly bio-available in the airway, owing to their relative inability to cross the epithelial cell wall, proteolysis, rapid elimination by macrophages and muco-ciliary clearance. For example, insulin has an extremely low systemic bioavailability (only about 1%) following intranasal administration [2]. Among strategies developed to improve proteins' bioavailability and absorption through the respiratory mucosa, the use of colloidal carriers and especially nanoparticles is an emerging strategy under investigation. Nanoparticles in particular have been shown to provide several benefits, such as increasing proteins' stability, bioavailability, targeting, uptake and biological activity. In this review, we present the functioning of the respiratory tract and the challenges to be overcome in order to enable effective delivery of proteins. We will present a synthesis of studies from different fields of application, illustrating the potential of using nanoparticles for both local and systemic applications, including cancer therapy, central nervous system (CNS) targeting, allergy and vaccination applications.

2. Structure and function of the human respiratory tract

2.1. Description

The human respiratory tract is subdivided into three successive parts from the nasal and the mouth cavities to the lungs, as shown in Fig. 1 (ICRP, 1994): (i) the upper airways or extra-thoracic region are the gateway for incoming air, comprising successively, the nasal and mouth cavities, the pharynx and the larynx. They also participate in other functions such as phonation, olfaction and deglutition [3]; (ii) In the thorax, the upper airways give way to the tracheobronchial (TB) region, successively made of the *thoracic section of the larynx and the tracheobronchial tree containing the trachea and the two bronchi, which are divided in their deep parts into many successive branches with smaller sections named bronchioles. This region is responsible for air conduction and protection from inhaled particles.* (iii) The respiratory part of the airways made of the respiratory bronchioles, the alveolar ducts and the lungs. Lungs are massive spongy organs composed of respiratory bronchioles, pulmonary alveoli and capillaries. The lungs' alveoli are highly

* Corresponding author. EA4483, IMPRT, IFR 114, Faculté de médecine pôle recherche, Université de Lille 2, 59000 Lille, France. Tel.: +33 320 62 68 83.
E-mail addresses: didier.betbeder@univ-lille2.fr, dbetbeder@aol.com (D. Betbeder).

0142-9612/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2012.08.070

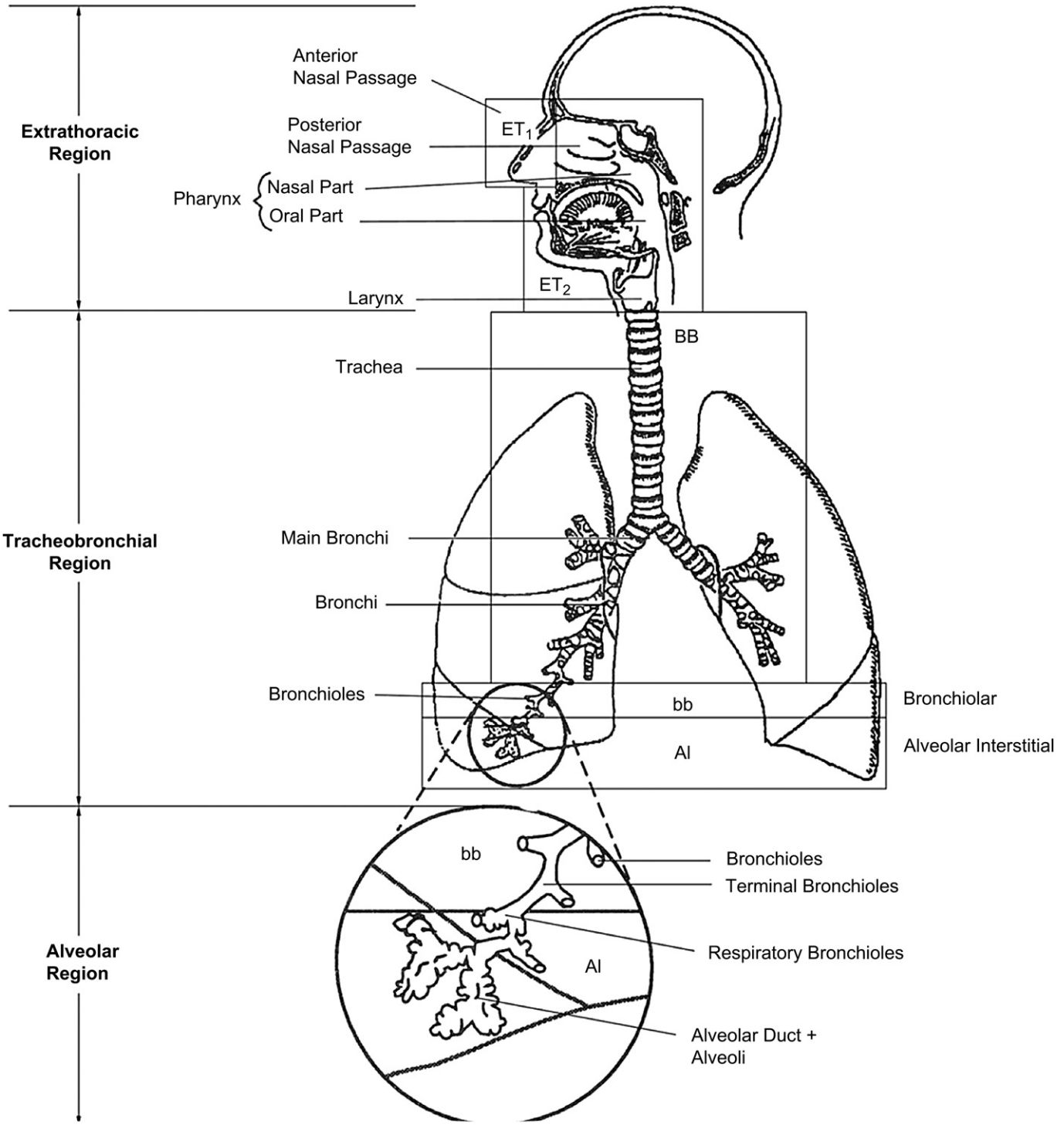

Fig. 1. Illustration of the major anatomical regions of the human respiratory tract (ICRP, 1994). Abbreviations: ET1: anterior nasal passages; ET2: posterior nasal passages, naso-oropharynx, and larynx; BB: bronchial region, including trachea and bronchi; bb: bronchiolar region consisting of bronchioles and terminal bronchioles; AI: alveolar-interstitial region, consisting of respiratory bronchioles, and alveolar ducts and sacs surrounded by alveoli [4].

vascularized small bags, made of thin wall pneumocytes, whose role is to ensure respiratory gas exchanges with the blood. From the upper to the deeper regions, the airways ducts' diameters gradually decrease while the number of branches and the surface area increase. Therefore, the surface area gradually increases from approximately $4m^2$ in the two bronchi, to 110–140 m^2 in the lungs. This unique organization ensures high volumes of gas exchange, to a total lung volume of about 5500 ml [4]. Moreover, this organization provides a large surface area for potential absorption of several drugs, including proteins.

2.2. The airway mucosa as the primary defense of the respiratory tract

The airway mucosa is responsible for airways homeostasis, humidification, mucous secretion, mucociliary clearance and

antibacterial defense [5]. The epithelium lining the airway is covered by a 10 μm film of mucus [6], a hypotonic fluid made of 95% water, 1% salts (1%), 1–2% proteins and glycoproteins (mucins), and other elements [7]. Mucins are secreted or membrane-associated high-molecular weight glycoproteins, produced by mucus secreting cells. They are highly glycosilated as 80% sugars are linked to a protein part rich in threonine and serine amino acids [8]. Several molecules secreted by airways cells are present in airways fluids and participate in protection against infections, cellular communication and cellular regulation. These molecules are mainly secreted by serous cells (proteases, antioxidants, lysozyme, lactoferrin, defensins, secretory leukocyte peptidase inhibitor, lactoperoxidase). Pneumocytes secret surfactant proteins and some peptides - proteins are also secreted by macrophages. Moreover, serum proteins are also present in the mucus as they diffuse through the epithelial cells by different mechanisms including transduction. The mucociliary clearance is the active transport of the mucus along the airways ducts to the digestive tract, due to the coordinated motion of cilia present on airways ciliated cells. Under normal situations, cilia motion is a constant mechanism at a frequency of 12–15 Hz, performed by airway epithelial ciliated cells under neuro-stimulation [9]. The mucociliary clearance, supported by the mucus production plays a major role in innate immunity, by entrapping and eliminating inhaled particles and pathogens present in the airways [10].

Aside from mucociliary transport, macrophage phagocytosis plays a major role in the protection of the respiratory tract. The two mechanisms are complementary and act in different manners along the airways. Indeed, the mucociliary clearance operates only in the airways ducts while macrophages operate in the lungs alveoli, and are responsible for particles clearance in the deep lungs, including fine and ultrafine particles [11,12]. Phagocytosis is facilitated by opsonization, which is the attachment of proteins on the particle surface making it more visible to surface receptors on phagocytic cells [13,14]. The airway is characterized by specific opsonins, such as the surfactant protein-A (SP-A) and surfactant protein-D (SP-D) which are principally secreted by type II alveolar cells and, to a lesser extent, by Clara cells and some submucosal glands cells [15–17].

2.3. The airway cells

The epithelium lining the airway is a continuous layer of cells bound together with multiple, tight junctions [18]. The integrity of these tight junctions is important to maintain impermeability toward xenobiotics and has also been demonstrated to play roles in cell signaling and proteins' expression [18,19]. This epithelium is made of several cell types with different but complementary functions, whose proportions vary all along the respiratory tract (Table 1). For example, the epithelium lining the larynx is a squamous stratified epithelium [3], while the trachea is covered by a pseudo-stratified layer of columnar ciliated epithelial cells. In humans, ciliated cells represent about 56% of epithelial cells in the trachea, with this proportion gradually decreasing to about 15% in the fifth generation bronchioles [20]. They persist throughout the major bronchi and in the respiratory bronchioles, and then give way to a cubical epithelium in the periphery. In the deep airways, the ratio of goblet cells to ciliated cells is estimated to be 1:5; thereafter, both ciliated and goblet cell numbers gradually decrease in smaller airways in favor of Clara and serous secreting cells [9,20]. Finally, in the lungs, the epithelium is mainly composed of alveolar epithelial cells, subdivided into two different cells types: type I and type II pneumocytes, the latter representing the majority.

Many secreting and non-secreting cells such as dendritic cells, macrophages, Clara cells, serous cells, goblet cells and mucous-secreting cells, also take part in the composition of the airways' mucosa [8]. There are responsible for mucus secretion, proteins secretion, xenobiotics metabolism, cell communication, and airway protection, maintenance and differentiation. Furthermore, some environmental stresses such as airways injury, reconstruction, inflammation, asthma and cystic fibrosis have been shown to influence the proportions and organization of these cells [21]. Finally, the ubiquitous exposure to airborne assaults make the airways epithelium a very dynamic tissue under constant renewal, with a total turnover estimated to be around 30–50 days, and supported by numerous progenitor cells [10,22].

3. Nanoparticles as airway delivery systems

Nanoparticles have several advantages as airway delivery systems. Their small hydrodynamic diameter allows them to partially avoid clearance and to deposit in every region of the respiratory tract, including the deep lung. Numerous studies, including in vivo experiments and computational modeling, have described the influence of particles' size on their local deposition along the airway [4,30,31]. It is widely accepted that, particles below 3 μm are more likely to reach the deep lung [32]. Moreover, smaller particles and especially nanoparticles (<100 nm) are

Table 1
Principal airway cells.

	Characteristics	Localization(s)	Function(s)	References
Squamous cells	Stratified non ciliated cells	Pharynx, larynx	Protection, secretion	[3]
Ciliated columnar cells	Pseudo-stratified ciliated cells	Trachea, bronchi, bronchioles	Mucociliary clearance, immunity	[20]
TypeII pneumocytes	Spherical cells with dense lamellar bodies	Lungs, respiratory bronchioles	Secretion, surfactant synthesis and regulation, gas exchange, xenobiotic metabolism, progenitor cells	[21,23]
TypeI pneumocytes	Thin cells	Lungs, respiratory bronchioles	Secretion, gas exchange	[21,23]
Clara cells	Ovoid non ciliated cells	Bronchioles	protection, immunity, progenitor cells	[24,25]
Goblets Cells	Electron dense large granules	Tracheobranchial tree, bronchioles (rare)	Mucous secretion	[9,20]
Serous cells	Electron-dense cytoplasm, much rough endoplasmic reticulum	Small bronchi and bronchioles	Mucous and proteins secretion	[26]
Macrophages	Large cells with dense granules	Alveoli, Connective tissue, pleural space, and pulmonary capillaries	Phagocytosis, antigen presentation, inflammation, neoplastic cells destruction, secretions	[11,27]
Dendritic cells	Large cells with dense granules and pseudopodia	NALT, Interepithelial space, below lamina propria, alveolar interstitium	Inflammation, innate/adaptative immunity, secretion	[28]
Endocrine, Kultchitsky and Feyrter cells	Dense-core granulated (DCG) cell	Surface epithelium in bronchi	Smooth muscle tone, mucous secretion and ciliary stimulation	[29]

preferentially expected to reach the deep lung, the tracheabronchial tree, and nasopharyngeal region [33]. In addition, nanoparticles are less susceptible to phagocytosis compared with larger particles. Indeed, several studies converge to a size-range of approximately 0.5–3 μm in which particles' phagocytosis is optimal [34,35]. For example, Lunov and co-workers showed that polystyrene particles with sizes between 0.25 and 3 μm were preferentially phagocytosed by macrophages [36]. Interestingly, this size range fits with the role of macrophages in pathogen clearance, as 1–4 μm represents the most common size of airborne and waterborne bacterial pathogens [37,38]. Several studies have shown that nanoparticle uptake by macrophages is highly dependent on the particles' physicochemical properties such as size, surface charge, shape, and the extent to which they undergo mucus interaction and endocytosis [12,39,40]. For example, a hydrophobic surface is known to facilitate nanoparticle phagocytosis, while the particles' shape influences attachment and internalization by macrophages [34,35].

In addition, nanoparticles can improve the bioavailability of molecules in the airway, due to their ability to be deposited in and adhere to the mucosa. Mucoadhesion is the particular interaction of a mucosal membrane with a synthetic surface [41,42]. Mucoadhesive nanoparticles were found to have longer residence time and improved bioavailability in the airway. The mucoadhesivity was shown to be influenced by nanoparticles physicochemical characteristics such as surface charge, size, porosity and the nature of the polymer [43]. Different mucoadhesive polymers have been reported in the literature, including chitosan (CS), alginate, polyvinyl pyrrolidone, polyacrylic acid copolymer, polyvinyl alcohol copolymers, and cellulose derivatives [44,45]. Among them, polysaccharides such as chitosan and its derivatives are of particular interest due to their biocompatibility, biodegradability and low toxicity [2,46–48]. Takeushi and co-workers showed that surface coating of liposomes with CS polymers highly improved their absorption to mucosa, compared to uncoated liposomes [49]. Moreover, PLGA nanospheres coated with CS exhibited improved uptake by A549 alveolar epithelial cells compared with non-coated nanospheres, without causing additional cytotoxicity [50]. Interestingly, it has been reported that coating nanoparticles with polysaccharide polymers may also limit their interaction with opsonins [51,52]. Recently, lectins have been developed as cytoadhesive polymers, due to their ability to preferentially bind to epithelial cells' surface receptors. Therefore, combining nanoparticles with lectins is an emerging strategy under investigation to improve their airway bioavailability by allowing direct attachment to the epithelial cells, and thus limiting interactions with the mucus [43,53].

Another advantage of nanoparticles is their ability to be highly endocytosed by airways' epithelial cells. The endocytosis pathway principally depends on the nanoparticles properties [54,55], and cell type under consideration [56]. In a recent study, 60 nm cationic maltodextrins nanoparticles (NP^+) were found to bind to anionic receptors on human bronchial epithelial cells, and this was followed by a fast internalization via the clathrin endocytosis pathway [57]. Indeed, nanoparticles with a cationic surface are known to bind anionic receptors, typically from the family of glycosaminoglycans (GAGs) such as heparan sulfate proteoglycans (HSPGs), syndecans, betaglycans and glypicans [58–61]. Similarly, the clathrin route has been described for the uptake of different nanoparticles by airways cells, including chitosan nanoparticles [62], PLGA nanoparticles [50,63], gold nanoparticles [64], and nanodiamonds [65]. On the other hand, other endocytosis mechanisms such as caveolae-mediated uptake, non-clathrin/caveolae uptake and macropinocytosis, have been described in the internalization of nanoparticles and viruses by airway cells [54,66]. For example, DNA nanoparticles were shown to penetrate BEAS-2B airways cells via a caveolae-mediated endocytosis [67]. In the same manner, macropinocytosis was found to be implicated in nanoparticles' uptake by airway macrophages, in addition to phagocytosis [68]. Interestingly, it has been reported that proteins' attachment on the nanoparticles' surface (opsonization) may influence their uptake. Indeed, opsonization of polystyrene particles with immunoglobulin was found to modify the mechanism of their endocytosis from clathrin and macropinocytosis pathways, to phagocytosis [36]. Other studies have shown that nanoparticles can cross alveoli cells by transcytosis and merge into the bloodstream [12,69,70]. This translocation of nanoparticles constitutes an interesting asset for systemic drug delivery following airways administration [70–72]. In summary, nanoparticles exhibit considerable advantages as airway delivery systems. They are able to be deposed efficiently in the airway tract, to avoid clearance mechanisms, and to take advantage of the endocytosis machinery of airway epithelial cells, making them highly potent vectors for macromolecules delivery systems.

4. Nanoparticles for airway delivery of peptides and proteins

Associating nanoparticles with proteins is an interesting strategy to improve their bioavailability and biological activity following airway delivery. Indeed, using nanoparticle delivery systems is expected to provide benefits such as protein stability increase, sustained release, intracellular targeting, cell/tissue targeting and increased uptake by airway epithelial cells [73–75]. Numerous methods to load nanoparticles with proteins have been developed, including encapsulation, surface adsorption, and chemical linking [76]. For example, 50 nm self-assembled, cationic nanogels are known to strongly interact to form stable nanoparticles even in the presence of serum [77]. Particle coating with hydrophilic polymers such as PEG and CS was shown to improve their ability to deliver proteins across the nasal and intestinal mucosa [78]. Numerous nanoparticles have been developed to promote airway delivery of proteins, including mucoadhesive systems, tight junction disrupters, and macrophage targeting systems. For example, it has been shown that mucoadhesive nanoparticles can improve protein/peptide absorption and residence time in the mucosa [79,80]. Moreover, nanoparticles are expected to enable long-term bioavailability and controlled release of therapeutic proteins [75].

5. Applications

5.1. Airway delivery of therapeutic protein

So far, insulin is the most documented example of peptide delivery via the airway. Studies of inhaled insulin for the treatment of type 1 and 2 diabetes were reported in the 1920s, when Laqueur and Grevenstuk reported on insulin bioavailability after intratracheal administration (Laqueur and Grevenstuk, 1924). Intranasally administered insulin has more recently been shown to elicit long-term control of plasma glucose compared with the conventional subcutaneous administration [81–83]. However, many studies have also reported a low systemic bioavailability of nasally administered insulin (less than 1%) [2], thus requiring very high doses of insulin be taken (up to 10 times more than traditional doses) [84]. Although therapeutic peptides and proteins such as insulin can cross the air-blood barrier [85], their absorption across airway epithelial cells is strongly limited by the airways' clearance mechanisms, low penetration through cell membranes and the difficulty of determining effective doses depending on patients [84]. Numerous studies have shown that associating insulin to

nanoparticles can improve insulin's bioavailability in the respiratory mucosa. For example, PEGylated chitosan nanoparticles were shown to enhance insulin absorption to a greater extent compared with non-nanoparticulates forms of chitosan and insulin alone [86,87]. Chitosan nanoparticles were also found to enhance nasal absorption of insulin in rabbit, regardless of chitosan molecular weight [88]. Recently, Al-Qadi and co-workers reported that intratracheal administration of dry insulin powder microencapsulated in chitosan nanoparticles increased its distribution to the deep lungs, and facilitated release of a biologically active form of insulin to rats' blood. Moreover, they observed a more pronounced and prolonged effect compared to non-formulated insulin [89]. In addition, the use of mucoadhesive liposomes loaded with insulin has been shown to significantly reduce plasma glucose compared with non-mucoadhesive liposomes [90], and a significant reduction of glucose levels in blood was observed for more than 12 h in rat, via intranasal administration after [49]. Similarly, Sintov and co-workers showed that intranasal administration of an insulin microemulsion was able to control glucose levels in plasma with good permeation, pharmacokinetics/pharmacodynamics comparable to subcutaneous administration, and with no need for co-administration of an absorption enhancer [91]. Similarly, the L-penetratin cell penetrating peptide (CPP) was found to increase insulin permeability across nasal epithelial cells after intranasal delivery in male Sprague Dawley rats [92]. These results show that intranasal administration of nanoparticle-bound insulin is a promising non-invasive strategy to treat diabetes.

Aside from insulin, a large number of proteins have been evaluated for airway delivery, including peptide hormones (calcitonin, oxytocin, growth hormone, somatostatin, thyroid-stimulating hormone, follicle-stimulating hormone), growth factors (colony stimulating factor, granulocyte monocyte-colony stimulating factor, Insulin-like growth factor) and cytokines (IL-2, IL-12) [84]. Chen and co-workers showed that formulating calcitonin (a 32 amino-acid polypeptide hormone used in the treatment of bone diseases such as Paget's disease, hypercalcemia, osteoporosis and bone metastasis) in liposomes produced an activity comparable to subcutaneous injection, with a significant improvement of calcitonin absorption and hypocalcemia activity compared to calcitonin alone [93]. The same results were also obtained using different liposomes [94]. Positively and negatively charged gelatin nanoparticles were also shown to enhance calcitonin intranasal administration [95]. Moreover, pulmonary delivery of calcitonin loaded in gelatin-chitosan nanoparticles was found to produce a sufficient pharmacological availability with a pronounced hypocalcemic effect for more than 24 h [96]. In the same manner, incorporation of elcatonin (a calcitonin derivative) in chitosan-PLGA nanospheres produced a prolonged reduction in blood calcium for over 24 h compared with the drug solution and the unmodified nanospheres which was rapidly removed from lung [79].

5.2. Protein delivery for vaccination

Most of the antigens used for intranasal vaccination are proteins immunogenic enough to generate effective protection against the pathogen under consideration. However, due to barriers encountered in the respiratory mucosa, antigens alone do not effectively generate sufficient immunity and adjuvants are necessary to increase their immunogenicity. The main challenge in intranasal vaccination is to target the Nasal Associated Lymphoid Tissue (NALT). The NALT is mainly located in the pharynx as a ring of lymphoid tissue (Waldeyer's ring) just below the epithelial surface, and it contains many lymphoid follicles (B-cell areas), macrophages and dendritic cells (Fig. 2). The epithelium forming the NALT comprises ciliated epithelial cells, mucous goblet cells and non-ciliated cells similar to the M-cells present in the Peyer's Patches in the intestinal tract. These particular M-cells are responsible for uptake and transcytosis of antigens from the lumen to the NALT [97]. Numerous studies have shown that nasal immunization can not only produce local secretion of IgA in the airway mucosa, but can also produce systemic IgG [98]. Moreover, intranasal vaccination was shown to induce immune responses, on distal mucosal lymphoid tissues, including the oral and vaginal mucosa [99,100], and stimulate a protective immunity toward different HIV pathogens [101,102], the human papilloma virus [103] and the influenza virus.

Nanoparticles are considered to have high potential as delivery systems for antigens to the respiratory mucosa, being able to protect antigens against degradation and to deliver them to antigen presenting cells [104]. They have been shown to improve antigen protection, recognition and uptake by the lymphoid tissues, and in some case to lead modulation of the immune response [105]. For example, some nanoparticles such as chitosan, PLGA, and polystyrene nanoparticles were found to greatly improve antigens' uptake by the NALT [106–109]. Polysaccharide derivative nanoparticles, such as trimethyl chitosan (TMC) nanoparticles, were shown to enhance antigens' residence time in the airway mucosa, and the production of IgG and IgA [110]. Recently, TMC was shown to induce the production of several maturation markers by the dendritic cells [98,111]. Furthermore, surface coating of nanoparticles with mannose moieties was shown to improve their uptake by immune cells by enhancing macrophage targeting [112]. This strategy is of interest, as macrophages rather than dendritic cells have been found to be responsible for immunomodulation in some specific patterns of airway infections [113].

The surface glycoproteins hemagglutinin and neuraminidase are the most commonly used antigen proteins for influenza virus vaccination and numerous attempts at formulating hemagglutinin with nanoparticles have been made to develop a nasally administered 'flu vaccination. For instance, the polysaccharide nanoparticles Supra Molecular Biovector (SMBV) underwent clinical trials as a protein delivery system for intranasal vaccination against flu [114]. They were also shown to produce local and systemic immunity after intranasal vaccination in mice, using Hepatitis B antigens [115] or MenC meningitis antigen [116]. Moreover, SMBV were able to stimulate both Th1 and Th2 immune response, without modification of the IgG profile compared to intramuscular administration [116]. In the same manner, liposomes and liposome-based structures formulated with short, highly-conserved influenza peptides produced protective immunity against influenza virus infection [113]. In a recent study, Gupta and co-workers also showed that a single intranasal vaccination of mice with hemaglutinin using chitosan coated polycaprolactone nanoparticles increased the production of Th1 and Th2 antibodies compared to administering the antigen alone, without modifying antibodies' isotype profiles. Interestingly, they also showed that longer retention in the airway using a chitosan coating resulted in higher production of IgG antibodies [117].

Another interesting example of intranasal immunization is the vaccination against *Mycobacterium tuberculosis*. To date, the most common strategy against tuberculosis is vaccination with Bacillus Calmette-Guérin (BCG). However, BCG was shown to be mainly effective in the childhood, protection reducing with age [118], and BCG immunization of adults producing only reliable protection [119,120]. Therefore, nanoparticles have been increasingly investigated to provide vaccination and immunization boosts in adults, especially as recent studies showed that intranasal vaccination with antigens may boost immune protection in adults. The proteins ESAT-6, Ag85B, and ESAT-6/Ag85B (fusion protein) are immunogenic proteins commonly used for immunization against *M. tuberculosis* (MbT) [121]. PLGA nanoparticles formulated with

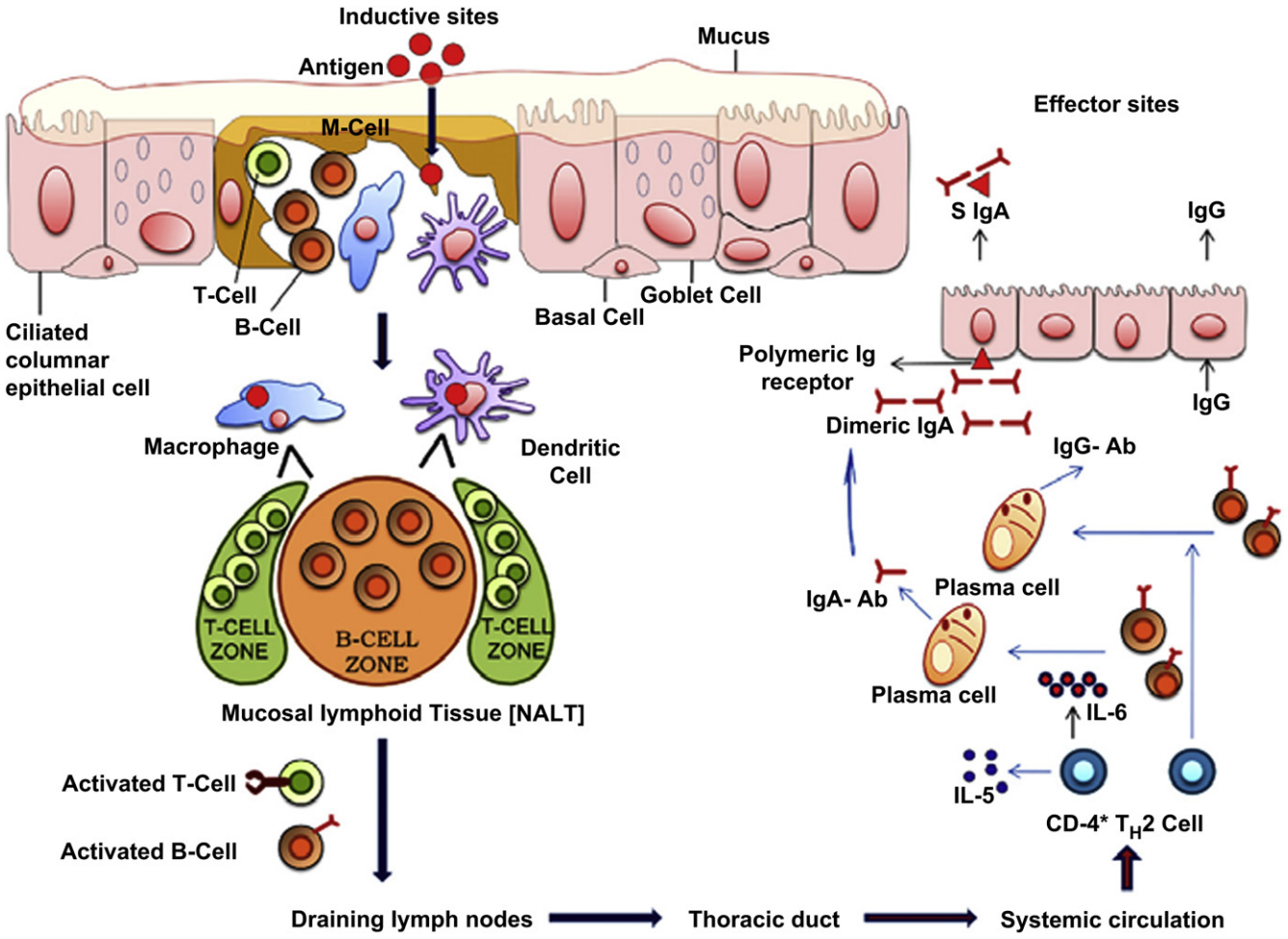

Fig. 2. The Nasal Associated Lymphoid tissue [117], showing the inter-connection between cell lining the airway and the underlying immune cells.

ESAT-6 or with Ag85B were shown to induce a strong immunity and memory T-cell response in the lungs and the thoracic lymph nodes after intranasal administration [122–124]. Similarly, adjuvanted Pluronic-stabilized polypropylene sulfide nanoparticles conjugated with Ag85B enhanced production of polyfunctional Th1 responses in the spleen, the lung and lung-draining lymph nodes, as well as Th17 responses, after intranasal administration [125]. These results offer encouraging perpectives for the development of new, non-invasive anti-tuberculosis vaccines, providing long-term immunity in both children and adults.

Finally, antigens loaded in nanoparticulate delivery systems have also been shown to generate immune protection against infections other than airway infections after intranasal immunization. For example, The HIV membrane glycoproteins gp120, gp124 and gp160 were shown to produce efficient systemic and mucosal immune response against HIV in animal, when formulated with various nanoparticles including liposomes [102], PLA nanoparticles [126], gamma-PGA nanoparticles [111] and polystyrene beads [127]. Although intranasal vaccines using nanoparticle/protein formulations are not yet available in the market, these research achievements are illustrative of the potential of nanoparticles for the delivery of protein antigens via the airway.

5.3. Cancer

Proteins play major roles in the processes of carcinogenesis and tumor persistence. They are involved in every cellular mechanism including cell signaling, proliferation, migration and apoptosis. Moreover, cancer cells are known to express/over-express several proteins markers. Therefore, numerous anti-cancer therapies take advantage of the protein-protein interactions to develop proteins and peptides based drugs, in order to specifically target membrane proteins or to impede intracellular machinery of cancer cells. Proteins drugs are developed for different anti-cancer strategies including diagnosis, antibody therapy, vaccination, and cancer targeting using proteins combined with anti-cancer drugs. However intranasal delivery of proteins for cancer therapy still presents serious difficulties, principally due to the airway barriers and the low uptake by tumors.

Different nanoparticles/proteins formulations are being developed to target specific protein receptors on tumors. For example, the epidermal growth factor receptor (EGFR) is a commonly targeted surface receptor, as EGFR is overexpressed in 80% of cancers, and this over expression has been associated with poor survival prognostics [128]. Biotinylated-EGF coupled with gelatin nanoparticles were shown to preferentially target lung adenocarcinoma cells (A549) *in vitro*, compared to normal lung cells. Moreover intranasal delivery to cancerous lungs mice showed accumulation in lung carcinoma cells. On the other hand, some approaches use specific antibodies to target EGFR. Cetuximab (or Erbitux, or C225) is a chimeric human-murine monoclonal antibody that specifically binds EGFR with high affinity, resulting in inhibition of cell proliferation, angiogenesis, invasiveness, metastasis, as well as enhanced apoptosis [129]. Recently, ultrasmall superparamagnetic iron oxide nanoparticles

(USPION) conjugated with cetuximab were shown to specifically bind EGFR and accumulate in nasopharyngeal carcinoma cells (CNE1 cells), showing their potential as non-invasive contrast agent for airway carcinoma [130]. In a same manner, various protein/nanoparticles have been studied, including cetuximab conjugated quantum-dot for tumor-specific imaging [131], Anti-ENO1-conjugated gold nanoparticles for non small cell lung cancer (NSCLC) [132] and bombesin coupled gold nanoparticles to target gastrin-releasing peptide (GRP) receptors in NSCLC cells [133].

Interestingly, proteins can be loaded in nanoparticles as airway anticancer drugs or associated with nanoparticles containing anti-cancer drugs, in order enhance their bioavailability and reduce undesirable effects [134]. El-mir and co-workers showed that, IL2 associated with maltodextrin nanoparticles was able to induce complete regression of a tumor and partial immune protection from tumor rechallenge, after intranasal administration in mice implanted with mammary adenocarcinoma TS/A tumor [135]. Recently, anti-EGFR antibody combined with hybrid plasmonic magnetic nanoparticles wasshown to impede EGFR signal transduction on NSCL cell culture, resulting in high cytotoxicity, autophagy and apoptosis [128]. Moreover, heparin-nanoparticles coupled with Cisplatin, was found to significantly increase their association on EGFR and the intracellular concentrations of cisplatin in non-small cell lung cancer H292, compared to the free drug alone [136]. Similarly, combining an anti H-ferritin monoclonal antibody to polylactide nanoparticles formulated with praclitaxel was shown to enhance their accumulation in A-549 cells lung carcinoma cells, leading to a much higher cytotoxicity on these cells.

Finally, therapeutic vaccines against cancer are under development using cancer proteins marquauers associated to nanoparticules. Indeed, nanoparticles are expected to enhance proteins immunogenicity, as these protein makers alone are generally unable to induce sufficient tumor immune responses due to their poor inherent immunogenicity. For example, Matsuo and co-workers recently showed that intranasal vaccination with poly γ-glutamic acid nanoparticles containing ovalbumin were able to induce antitumor immunity against cancer cells transfected with ovalbumin and injected in mice [137]. These achievements show encouraging perspectives for the use of proteins associated nanoparticles to treat airway's cancers.

5.4. Delivery to the central nervous system

Proteins and especially neurotrophic factors and recombinant antibodies have great therapeutic potential against neurological diseases. Recently, the brain targeting of proteins via the nasal route increasingly appears as a promising non-invasive route to deliver proteins to the brain, allowing bypassing the so selective blood brain barrier. Indeed, different pathways allowing direct passage of molecules from the nasal cavity to the brain have been characterized. (i) The olfactory nerve passage, operating by molecules transport along the axon to the brain through the manifold small holes of the cribriform plate of the ethmoid bone,after receptor mediated endocytosis in the olfactory epithelium locatedin the roof of the nasal cavity. (ii) The trigeminal nerves passage, which transports molecules to the posterior parts of the brain, such as cerebellum and brainstem. (iii) The vasculature route, in which molecules cross the blood-brain barrier after passing the epithelial barrier and melting into the circulation stream via the blood vessels draining the nasal mucosa. (Iv) the cerebrospinal fluid (CSF) and lymphatic system route, fitting into the nasal lymphatic's and the subarachnoid space containing CSF, accessible after nasal absorption [138,139].

Numerous attempts of proteins nose-to-brain targeting have been studied for the treatment of neurological deseases such as Alzheimer disease (IGF-1, NGF, BDNF and insulin), Parkinson disease (TGF-alpha, FGF) and traumatic brain injury (erythropoietin, CNTF). For instance, intranasal delivery of Insulin-like growth factor was shown to elicit IGF biodistribution in the brain and the spinal cord in rats [140]. Brain uptake via the nose can be improved using absorption enhancer such as surfactants, bile salts, lipids, cyclodextrins, chitosan and derivatives, poly-L-arginine, gelatin and poly(acrylic acid) or by using transporteurs such as nanoparticles [138,141]. For example, Vaka and co-workers showed a 14-fold increase in the bioavailability of intranasally administered NGF with chitosan compared to the formulation without chitosan, in rats [142]. Recently, nanoparticles are increasingly developed to improve CNS uptake of peptides and proteins via the nasal route. Though nanoparticles have already shown ability to overcome the blood-brain barrier via systemic delivery, recent studies are increasingly showing that brain targeting of proteins via the nasal route can be achieved by using nanoparticles[143]. One strategy to improve nanoparticles uptake by the nasal olfactory epithelium is to coat them with particular proteins such as the lectins Ulex Europeus Agglutinin I (UEA I) and wheat germ Agglutinin (WGA), or with homing peptides which furthermore have advantages of being more tolerated by mammalians [139]. For example, Liu and co-workers showed that association of nanoparticles with WGA enabled their targeting to rat brain using the olfactory and the trygemal nerves pathways [144]. Same results were observed using Solanum tuberosum lectin conjugated PEG-PLGA nanoparticles [145].

Interestingly, nanoparticles can be combined with therapeutic protein to improve nasal targeting of the CNS. For example, intranasal administration in mice of a vasoactive intestinal peptide (VIP) incorporated into PEG-PLA nanoparticles decorated with WGA was studied. The authors showed that WGA decoration induced a preferential affinity and localization of nanoparticles in the olfactory mucosa contrary to the respiratory mucosa. Futhermore, WGA decorated PEG-PLA nanoparticles were shown to efficiently transport VIP to the brain, following intranasal administration [146]. Recently, polysorbate-80 coated nanoparticles were shown to improve distribution of the neurotoxin-II (an analgesic peptide) in brain via intranasal administration to rat [147]. Moreover, Veronesi and co-workers observed a strong protection against glutamate toxicity in rat model's brain after intranasal delivery of thyrotropin-releasing hormone (TRH) incorporated in PLA nanoparticles, showing interesting potential for the treatment of epilepsy patients. Furthermore, this effect was greatly improved using nanoparticles compared with TRH formulation alone [148]. Although the mechanisms of protein delivery to the brain using nanoparticles are still poorly understood especially in terms of toxicity on the brain after repeated administration, these results enlighten the possibility of using nanoparticles as a new non-invasive carrier to deliver proteins to the brain using the nasal route and could bring considerable outcome for the treatment of neurological disease.

6. Conclusion and future perspectives

The ubiquitous role of proteins in a plethora of biological processes makes them molecules with a high therapeutic potential. However, their use in therapy remains a difficult challenge, principally due to the difficulty of targeting proteins in the body while simultaneously maintaining their stability and biological activity. Recently, the delivery of proteins via the airway has received increasingly significant attention as an alternative, non-invasive route of administration, able to bypass the first metabolic passage in the liver, while at the same time retaining proteins' biological activity. However, multiple barriers present in the respiratory tract combine to severely limit the systemic uptake of macromolecules

such as large peptides and proteins. In this review, we have presented numerous studies showing that, using nanoparticles as peptide and protein vehicles can significantly improve their therapeutic efficacy following airway delivery. Indeed, associating proteins with nanoparticles has been shown to improve they stability, residence time, cell targeting, and uptake by airways' epithelial cells. These properties were found to be dependent upon nanoparticles' physicochemical characteristics. For example, due to their small size, nanoparticles are able to carry proteins throughout every regions of the respiratory tract, including to the deep lungs. Moreover, using nanoparticles coated with specific polymers such as PEG was shown to protect proteins from proteolysis, mucus entrapment and clearance by macrophages, resulting in a higher bioavailability in the airways. In addition, proteins' residence time can be significantly improved in airways using mucoadhesive or cytoadhesive nanoparticles. This constitutes an interesting asset as different cell populations can be targeted by coating nanoparticles with specific cytoadhesive polymers such as lectins or mannose. These results show that developing nanoparticles as peptide and protein delivery systems is a promising strategy to improve the delivery of these potentially important therapeutic molecules to and through the airway. The application areas are numerous and include cellular imaging, anti-cancer therapy, vaccine development and CNS targeting.

Because of the current paucity of knowledge regarding the potential toxicity of airway-delivered nanoparticles, numerous studies have developed biodegradable nanoparticles, in particular because nanoparticles can pass directly into the brain after intranasal administration and consequences of repeated and/or long-term administration are poorly known. This lack of data is partly explained by the wide variety of nanoparticles, but also because there is currently no widely accepted and reliable methodology to study the toxicity of nanoparticles in the airways which mimics real exposure conditions. Studies have long been limited to the toxicity of airborne nanoparticles such as diesel [149], fullerene [150], titanium dioxide [151] or oil vapor nanoparticles [152]. A recent study suggested that nanoparticles' interaction with proteins can have a significantly influence on their genotoxicity [153]. In contrast, beneficial effect of nanoparticles were observed on both inflammation and allergies [154]. However, toxicity studies are usually performed with doses of nanoparticles and under experimental conditions that fail to realistically mimic exposure to nanoparticles.

Finally, the type of protein to be delivered via the airway is a crucial parameter to consider. Indeed, most success in delivery of proteins via the airways have generally been obtained with peptides or proteins of relatively low molecular weight, such as insulin (5.8 kDa), oxytocin (1 kDa), calcitonin (3.4 kDa), elcatonin (3.3 kDa), VIP (3.3 kDa) IgG fragments (approximately 25 kDa), rarely exceeding 50 kDa. This is principally due to the difficulty large proteins have in crossing the epithelial cell layer. Moreover, the blood bioavailability of airway-delivered proteins remains modest, even associated with nanoparticles: for example, the systemic bioavailability of airway-delivered insulin formulated in nanoparticles rarely exceeds 30%. Therefore, larger doses of protein are generally required in airway delivery. Regarding these parameters, the candidates for airway administration should be peptides or proteins of small molecular weight, with high biological activity, or preferentially developed for local applications.

References

[1] Agu RU, Ugwoke MI, Armand M, Kinget R, Verbeke N. The lung as a route for systemic delivery of therapeutic proteins and peptides. Respir Res 2001;2:198–209.

[2] Illum L, Farraj NF, Davis SS. Chitosan as a novel nasal delivery system for peptide drugs. Pharm Res 1994;11:1186–9.

[3] Pohunek P. Development, structure and function of the upper airways. Paediatr Respir Rev 2004;5:2–8.

[4] Hofmann W. Modelling inhaled particle deposition in the human lung - A review. J Aerosol Sci 2011;42:693–724.

[5] Vonk MJ, Hiemstra PS, Grote JJ. An antimicrobial peptide modulates epithelial responses to bacterial products. Laryngoscope 2008;118:816–20.

[6] Widdicombe JH. Regulation of the depth and composition of airway surface liquid. J Anat 2002;201:313–8.

[7] Houtmeyers E, Gosselink R, Gayan-Ramirez G, Decramer M. Regulation of mucociliary clearance in health and disease. Eur Respir J 1999;13:1177–88.

[8] Jeffery PK, Li D. Airway mucosa: secretory cells, mucus and mucin genes. Eur Respir J 1997;10:1655–62.

[9] Chilvers MA, O'Callaghan C. Local mucociliary defence mechanisms. Paediatr Respir Rev 2000;1:27–34.

[10] Crystal RG, Randell SH, Engelhardt JF, Voynow J, Sunday ME. Airway epithelial cells: current concepts and challenges. Proc Am Thorac Soc 2008;5:772–7.

[11] Brain JD. Mechanisms, measurement, and significance of lung macrophage function. Environ Health Perspect 1992;97:5–10.

[12] Geiser M, Casaulta M, Kupferschmid B, Schulz H, Semmler-Behnke M, Kreyling W. The role of macrophages in the clearance of inhaled ultrafine titanium dioxide particles. Am J Respir Cell Mol Biol 2008;38:371–6.

[13] Frank MM, Fries LF. The role of complement in inflammation and phagocytosis. Immunol Today 1991;12:322–6.

[14] Owens 3rd DE, Peppas NA. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. Int J Pharm 2006;307:93–102.

[15] van de Wetering JK, van Golde LM, Batenburg JJ. Collectins: players of the innate immune system. Eur J Biochem 2004;271:1229–49.

[16] Cortes G, Alvarez D, Saus C, Alberti S. Role of lung epithelial cells in defense against Klebsiella pneumoniae pneumonia. Infect Immun 2002;70:1075–80.

[17] Noah TL, Murphy PC, Alink JJ, Leigh MW, Hull WM, Stahlman MT, et al. Bronchoalveolar lavage fluid surfactant protein-A and surfactant protein-D are inversely related to inflammation in early cystic fibrosis. Am J Respir Crit Care Med 2003;168:685–91.

[18] Petecchia L, Sabatini F, Varesio L, Camoirano A, Usai C, Pezzolo A, et al. Bronchial airway epithelial cell damage following exposure to cigarette smoke includes disassembly of tight junction components mediated by the extracellular signal-regulated kinase 1/2 pathway. Chest 2009;135:1502–12.

[19] Polette M, Mestdagt M, Bindels S, Nawrocki-Raby B, Hunziker W, Foidart JM, et al. Beta-catenin and ZO-1: shuttle molecules involved in tumor invasion-associated epithelial-mesenchymal transition processes. Cells Tissues Organs 2007;185:61–5.

[20] Wanner A, Salathe M, O'Riordan TG. Mucociliary clearance in the airways. Am J Respir Crit Care Med 1996;154:1868–902.

[21] Beers MF, Morrisey EE. The three R's of lung health and disease: repair, remodeling, and regeneration. J Clin Invest 2011;121:2065–73.

[22] Reynolds SD, Malkinson AM. Clara cell: progenitor for the bronchiolar epithelium. Int J Biochem Cell Biol 2010;42:1–4.

[23] Castranova V, Rabovsky J, Tucker JH, Miles PR. The alveolar type II epithelial cell: a multifunctional pneumocyte. Toxicol Appl Pharmacol 1988;93:472–83.

[24] Ayers MM, Jeffery PK. Proliferation and differentiation in mammalian airway epithelium. Eur Respir J 1988;1:58–80.

[25] Wong AP, Keating A, Waddell TK. Airway regeneration: the role of the Clara cell secretory protein and the cells that express it. Cytotherapy 2009;11:676–87.

[26] Rogers AV, Dewar A, Corrin B, Jeffery PK. Identification of serous-like cells in the surface epithelium of human bronchioles. Eur Respir J 1993;6:498–504.

[27] Fels AO, Cohn ZA. The alveolar macrophage. J Appl Physiol 1986;60:353–69.

[28] Lambrecht BN, Hammad H. Biology of lung dendritic cells at the origin of asthma. Immunity 2009;31:412–24.

[29] Sorokin SP, Hoyt Jr RF, Pearsall AD. Comparative biology of small granule cells and neuroepithelial bodies in the respiratory system. Short review. Am Rev Respir Dis 1983;128:S26–31.

[30] Longest PW, Holbrook LT. In silico models of aerosol delivery to the respiratory tract – development and applications. Adv Drug Deliv Rev 2012;64:296–311.

[31] Bartley DL, Vincent JH. Sampling conventions for estimating ultrafine and fine aerosol particle deposition in the human respiratory tract. Ann Occup Hyg 2011;55:696–709.

[32] Carvalho TC, Peters JI, Williams 3rd RO. Influence of particle size on regional lung deposition – what evidence is there? Int J Pharm 2011;406:1–10.

[33] Madlova M, Jones SA, Zwerschke I, Ma Y, Hider RC, Forbes B. Poly(vinyl alcohol) nanoparticle stability in biological media and uptake in respiratory epithelial cell layers in vitro. Eur J Pharm Biopharm 2009;72:437–43.

[34] Kawaguchi H, Koiwai N, Ohtsuka Y, Miyamoto M, Sasakawa S. Phagocytosis of latex particles by leucocytes. I. Dependence of phagocytosis on the size and surface potential of particles. Biomaterials 1986;7:61–6.

[35] Sharma G, Valenta DT, Altman Y, Harvey S, Xie H, Mitragotri S, et al. Polymer particle shape independently influences binding and internalization by macrophages. J Control Release 2010;147:408–12.

[36] Lunov O, Syrovets T, Loos C, Beil J, Delacher M, Tron K, et al. Differential uptake of functionalized polystyrene nanoparticles by human macrophages and a monocytic cell line. ACS Nano 2011;5:1657–69.

[37] Champion JA, Mitragotri S. Role of target geometry in phagocytosis. Proc Natl Acad Sci USA 2006;103:4930–4.

[38] Champion JA, Walker A, Mitragotri S. Role of particle size in phagocytosis of polymeric microspheres. Pharm Res 2008;25:1815–21.

[39] Kreyling WG, Semmler M, Erbe F, Mayer P, Takenaka S, Schulz H, et al. Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. J Toxicol Environ Health A 2002;65:1513–30.
[40] Oberdorster G, Oberdorster E, Oberdorster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 2005;113:823–39.
[41] Gurny R, Meyer JM, Peppas NA. Bioadhesive intraoral release systems: design, testing and analysis. Biomaterials 1984;5:336–40.
[42] Patil SB, Sawant KK. Mucoadhesive microspheres: a promising tool in drug delivery. Curr Drug Deliv 2008;5:312–8.
[43] Serra L, Domenech J, Peppas NA. Engineering design and molecular dynamics of mucoadhesive drug delivery systems as targeting agents. Eur J Pharm Biopharm 2009;71:519–28.
[44] Salamat-Miller N, Chittchang M, Johnston TP. The use of mucoadhesive polymers in buccal drug delivery. Adv Drug Deliv Rev 2005;57:1666–91.
[45] Morales JO, McConville JT. Manufacture and characterization of mucoadhesive buccal films. Eur J Pharm Biopharm 2011;77:187–99.
[46] Felt O, Buri P, Gurny R. Chitosan: a unique polysaccharide for drug delivery. Drug Dev Ind Pharm 1998;24:979–93.
[47] Illum L. Chitosan and its use as a pharmaceutical excipient. Pharm Res 1998; 15:1326–31.
[48] Cui F, Qian F, Yin C. Preparation and characterization of mucoadhesive polymer-coated nanoparticles. Int J Pharm 2006;316:154–61.
[49] Takeuchi H, Yamamoto H, Niwa T, Hino T, Kawashima Y. Enteral absorption of insulin in rats from mucoadhesive chitosan-coated liposomes. Pharm Res 1996;13:896–901.
[50] Tahara K, Sakai T, Yamamoto H, Takeuchi H, Hirashima N, Kawashima Y. Improved cellular uptake of chitosan-modified PLGA nanospheres by A549 cells. Int J Pharm 2009;382:198–204.
[51] Lemarchand C, Gref R, Couvreur P. Polysaccharide-decorated nanoparticles. Eur J Pharm Biopharm 2004;58:327–41.
[52] Rouzes C, Gref R, Leonard M, De Sousa Delgado A, Dellacherie E. Surface modification of poly(lactic acid) nanospheres using hydrophobically modified dextrans as stabilizers in an o/w emulsion/evaporation technique. J Biomed Mater Res 2000;50:557–65.
[53] Lehr CM. Lectin-mediated drug delivery: the second generation of bioadhesives. J Control Release 2000;65:19–29.
[54] Perumal OP, Inapagolla R, Kannan S, Kannan RM. The effect of surface functionality on cellular trafficking of dendrimers. Biomaterials 2008;29: 3469–76.
[55] Vacha R, Martinez-Veracoechea FJ, Frenkel D. Receptor-mediated endocytosis of nanoparticles of various shapes. Nano Lett 2011;11:5391–5.
[56] Dos Santos T, Varela J, Lynch I, Salvati A, Dawson KA. Effects of transport inhibitors on the cellular uptake of carboxylated polystyrene nanoparticles in different cell lines. PLoS One 2011;6:e24438.
[57] Dombu CY, Kroubi M, Zibouche R, Matran R, Betbeder D. Characterization of endocytosis and exocytosis of cationic nanoparticles in airway epithelium cells. Nanotechnology 2010;21:355102.
[58] Hartig SM, Greene RR, Carlesso G, Higginbotham JN, Khan WN, Prokop A, et al. Kinetic analysis of nanoparticulate polyelectrolyte complex interactions with endothelial cells. Biomaterials 2007;28:3843–55.
[59] Orr G, Panther DJ, Cassens KJ, Phillips JL, Tarasevich BJ, Pounds JG. Syndecan-1 mediates the coupling of positively charged submicrometer amorphous silica particles with actin filaments across the alveolar epithelial cell membrane. Toxicol Appl Pharmacol 2009;236:210–20.
[60] Zhang H, Xia T, Meng H, Xue M, George S, Ji Z, et al. Differential expression of syndecan-1 mediates cationic nanoparticle toxicity in undifferentiated versus differentiated normal human bronchial epithelial cells. ACS Nano 2011;5:2756–69.
[61] Mislick KA, Baldeschwieler JD. Evidence for the role of proteoglycans in cation-mediated gene transfer. Proc Natl Acad Sci USA 1996;93:12349–54.
[62] Huang M, Ma Z, Khor E, Lim LY. Uptake of FITC-chitosan nanoparticles by A549 cells. Pharm Res 2002;19:1488–94.
[63] Tahara K, Furukawa S, Yamamoto H, Kawashima Y. Hybrid-modified poly(D, L-lactide-co-glycolide) nanospheres for a novel cellular drug delivery system. Int J Pharm 2010;392:311–3.
[64] Brandenberger C, Muhlfeld C, Ali Z, Lenz AG, Schmid O, Parak WJ, et al. Quantitative evaluation of cellular uptake and trafficking of plain and polyethylene glycol-coated gold nanoparticles. Small 2010;6:1669–78.
[65] Liu KK, Wang CC, Cheng CL, Chao JI. Endocytic carboxylated nanodiamond for the labeling and tracking of cell division and differentiation in cancer and stem cells. Biomaterials 2009;30:4249–59.
[66] Meier O, Boucke K, Hammer SV, Keller S, Stidwill RP, Hemmi S, et al. Adenovirus triggers macropinocytosis and endosomal leakage together with its clathrin-mediated uptake. J Cell Biol 2002;158:1119–31.
[67] Kim AJ, Boylan NJ, Suk JS, Lai SK, Hanes J. Non-degradative intracellular trafficking of highly compacted polymeric DNA nanoparticles. J Control Release 2012;158:102–7.
[68] Franca A, Aggarwal P, Barsov EV, Kozlov SV, Dobrovolskaia MA, Gonzalez-Fernandez A. Macrophage scavenger receptor A mediates the uptake of gold colloids by macrophages in vitro. Nanomedicine (Lond) 2011;6:1175–88.
[69] Nemmar A, Hoet PH, Vanquickenborne B, Dinsdale D, Thomeer M, Hoylaerts MF, et al. Passage of inhaled particles into the blood circulation in humans. Circulation 2002;105:411–4.
[70] Yacobi NR, Demaio L, Xie J, Hamm-Alvarez SF, Borok Z, Kim KJ, et al. Polystyrene nanoparticle trafficking across alveolar epithelium. Nanomedicine 2008;4:139–45.
[71] Choi HS, Ashitate Y, Lee JH, Kim SH, Matsui A, Insin N, et al. Rapid translocation of nanoparticles from the lung airspaces to the body. Nat Biotechnol 2010;28:1300–3.
[72] Cremaschi D, Porta C, Ghirardelli R. Different kinds of polypeptides and polypeptide-coated nanoparticles are accepted by the selective transcytosis shown in the rabbit nasal mucosa. Biochim Biophys Acta 1999; 1416:31–8.
[73] Almeida AJ, Souto E. Solid lipid nanoparticles as a drug delivery system for peptides and proteins. Adv Drug Deliv Rev 2007;59:478–90.
[74] Tan ML, Choong PF, Dass CR. Recent developments in liposomes, microparticles and nanoparticles for protein and peptide drug delivery. Peptides 2010;31:184–93.
[75] Illum L. Nanoparticulate systems for nasal delivery of drugs: a real improvement over simple systems? J Pharm Sci 2007;96:473–83.
[76] Husain Q, Ansari SA, Alam F, Azam A. Immobilization of Aspergillus oryzae beta galactosidase on zinc oxide nanoparticles via simple adsorption mechanism. Int J Biol Macromol 2011;49:37–43.
[77] Ayame H, Morimoto N, Akiyoshi K. Self-assembled cationic nanogels for intracellular protein delivery. Bioconjug Chem 2008;19:882–90.
[78] Vila A, Sanchez A, Tobio M, Calvo P, Alonso MJ. Design of biodegradable particles for protein delivery. J Control Release 2002;78:15–24.
[79] Takeuchi H, Yamamoto H, Kawashima Y. Mucoadhesive nanoparticulate systems for peptide drug delivery. Adv Drug Deliv Rev 2001;47:39–54.
[80] Kawashima Y, Yamamoto H, Takeuchi H, Kuno Y. Mucoadhesive DL-lactide/glycolide copolymer nanospheres coated with chitosan to improve oral delivery of elcatonin. Pharm Dev Technol 2000;5:77–85.
[81] Henkin RI. Inhaled insulin-intrapulmonary, intranasal, and other routes of administration: mechanisms of action. Nutrition 2010;26:33–9.
[82] Henkin RI. Intranasal insulin: from nose to brain. Nutrition 2010;26:624–33.
[83] Klingler C, Muller BW, Steckel H. Insulin-micro- and nanoparticles for pulmonary delivery. Int J Pharm 2009;377:173–9.
[84] Siekmeier R, Scheuch G. Inhaled insulin – does it become reality? J Physiol Pharmacol 2008;59:81–113.
[85] Brain JD. Inhalation, deposition, and fate of insulin and other therapeutic proteins. Diabetes Technol Ther 2007;9:4–15.
[86] Duan X, Mao S. New strategies to improve the intranasal absorption of insulin. Drug Discov Today 2010;15:416–27.
[87] Zhang X, Zhang H, Wu Z, Wang Z, Niu H, Li C. Nasal absorption enhancement of insulin using PEG-grafted chitosan nanoparticles. Eur J Pharm Biopharm 2008;68:526–34.
[88] Fernandez-Urrusuno R, Calvo P, Remunan-Lopez C, Vila-Jato JL, Alonso MJ. Enhancement of nasal absorption of insulin using chitosan nanoparticles. Pharm Res 1999;16:1576–81.
[89] Al-Qadi S, Grenha A, Carrion-Recio D, Seijo B, Remunan-Lopez C. Microencapsulated chitosan nanoparticles for pulmonary protein delivery: in vivo evaluation of insulin-loaded formulations. J Control Release 2012;157:383–90.
[90] Jain AK, Chalasani KB, Khar RK, Ahmed FJ, Diwan PV. Muco-adhesive multivesicular liposomes as an effective carrier for transmucosal insulin delivery. J Drug Target 2007;15:417–27.
[91] Sintov AC, Levy HV, Botner S. Systemic delivery of insulin via the nasal route using a new microemulsion system: in vitro and in vivo studies. J Control Release 2010;148:168–76.
[92] Khafagy el S, Morishita M, Isowa K, Imai J, Takayama K. Effect of cell-penetrating peptides on the nasal absorption of insulin. J Control Release 2009;133:103–8.
[93] Chen M, Li XR, Zhou YX, Yang KW, Chen XW, Deng Q, et al. Improved absorption of salmon calcitonin by ultraflexible liposomes through intranasal delivery. Peptides 2009;30:1288–95.
[94] Law SL, Huang KJ, Chou VH, Cherng JY. Enhancement of nasal absorption of calcitonin loaded in liposomes. J Liposome Res 2001;11:165–74.
[95] Morimoto K, Katsumata H, Yabuta T, Iwanaga K, Kakemi M, Tabata Y, et al. Evaluation of gelatin microspheres for nasal and intramuscular administrations of salmon calcitonin. Eur J Pharm Sci 2001;13:179–85.
[96] Makhlof A, Werle M, Tozuka Y, Takeuchi H. Nanoparticles of glycol chitosan and its thiolated derivative significantly improved the pulmonary delivery of calcitonin. Int J Pharm 2010;397:92–5.
[97] Illum L, Jabbal-Gill I, Hinchcliffe M, Fisher AN, Davis SS. Chitosan as a novel nasal delivery system for vaccines. Adv Drug Deliv Rev 2001;51:81–96.
[98] Slutter B, Bal S, Keijzer C, Mallants R, Hagenaars N, Que I, et al. Nasal vaccination with N-trimethyl chitosan and PLGA based nanoparticles: nanoparticle characteristics determine quality and strength of the antibody response in mice against the encapsulated antigen. Vaccine 2010;28: 6282–91.
[99] Neutra MR, Kozlowski PA. Mucosal vaccines: the promise and the challenge. Nat Rev Immunol 2006;6:148–58.
[100] Ninomiya A, Ogasawara K, Kajino K, Takada A, Kida H. Intranasal administration of a synthetic peptide vaccine encapsulated in liposome together with an anti-CD40 antibody induces protective immunity against influenza A virus in mice. Vaccine 2002;20:3123–9.
[101] Bradney CP, Sempowski GD, Liao HX, Haynes BF, Staats HF. Cytokines as adjuvants for the induction of anti-human immunodeficiency virus peptide

immunoglobulin G (IgG) and IgA antibodies in serum and mucosal secretions after nasal immunization. J Virol 2002;76:517–24.
[102] Sakaue G, Hiroi T, Nakagawa Y, Someya K, Iwatani K, Sawa Y, et al. HIV mucosal vaccine: nasal immunization with gp160-encapsulated hemagglutinating virus of Japan-liposome induces antigen-specific CTLs and neutralizing antibody responses. J Immunol 2003;170:495–502.
[103] Bermudez-Humaran LG, Cortes-Perez NG, Le Loir Y, Alcocer-Gonzalez JM, Tamez-Guerra RS, de Oca-Luna RM, et al. An inducible surface presentation system improves cellular immunity against human papillomavirus type 16 E7 antigen in mice after nasal administration with recombinant lactococci. J Med Microbiol 2004;53:427–33.
[104] Prieur E, Betbeder D, Niedergang F, Major M, Alcover A, Davignon JL, et al. Combination of human cytomegalovirus recombinant immediate-early protein (IE1) with 80 nm cationic biovectors: protection from proteolysis and potentiation of presentation to CD4+ T-cell clones in vitro. Vaccine 1996;14:511–20.
[105] Csaba N, Garcia-Fuentes M, Alonso MJ. Nanoparticles for nasal vaccination. Adv Drug Deliv Rev 2009;61:140–57.
[106] Amidi M, Romeijn SG, Verhoef JC, Junginger HE, Bungener L, Huckriede A, et al. N-trimethyl chitosan (TMC) nanoparticles loaded with influenza subunit antigen for intranasal vaccination: biological properties and immunogenicity in a mouse model. Vaccine 2007;25:144–53.
[107] Keijzer C, Slutter B, van der Zee R, Jiskoot W, van Eden W, Broere FPLGA. PLGA-TMC and TMC-TPP nanoparticles differentially modulate the outcome of nasal vaccination by inducing tolerance or enhancing humoral immunity. PLoS One 2011;6:e26684.
[108] Rajapaksa TE, Stover-Hamer M, Fernandez X, Eckelhoefer HA, Lo DD. Claudin 4-targeted protein incorporated into PLGA nanoparticles can mediate M cell targeted delivery. J Control Release 2010;142:196–205.
[109] Stano A, van der Vlies AJ, Martino MM, Swartz MA, Hubbell JA, Simeoni E. PPS nanoparticles as versatile delivery system to induce systemic and broad mucosal immunity after intranasal administration. Vaccine 2011;29:804–12.
[110] Slutter B, Bal SM, Que I, Kaijzel E, Lowik C, Bouwstra J, et al. Antigen-adjuvant nanoconjugates for nasal vaccination: an improvement over the use of nanoparticles? Mol Pharm 2010;7:2207–15.
[111] Akagi T, Wang X, Uto T, Baba M, Akashi M. Protein direct delivery to dendritic cells using nanoparticles based on amphiphilic poly(amino acid) derivatives. Biomaterials 2007;28:3427–36.
[112] Irache JM, Salman HH, Gamazo C, Espuelas S. Mannose-targeted systems for the delivery of therapeutics. Expert Opin Drug Deliv 2008;5:703–24.
[113] Tai W, Roberts L, Seryshev A, Gubatan JM, Bland CS, Zabriskie R, et al. Multistrain influenza protection induced by a nanoparticulate mucosal immunotherapeutic. Mucosal Immunol 2011;4:197–207.
[114] von Hoegen P. Synthetic biomimetic supra molecular biovector (SMBV) particles for nasal vaccine delivery. Adv Drug Deliv Rev 2001;51:113–25.
[115] Debin A, Kravtzoff R, Santiago JV, Cazales L, Sperandio S, Melber K, et al. Intranasal immunization with recombinant antigens associated with new cationic particles induces strong mucosal as well as systemic antibody and CTL responses. Vaccine 2002;20:2752–63.
[116] Baudner BC, Balland O, Giuliani MM, Von Hoegen P, Rappuoli R, Betbeder D, et al. Enhancement of protective efficacy following intranasal immunization with vaccine plus a nontoxic LTK63 mutant delivered with nanoparticles. Infect Immun 2002;70:4785–90.
[117] Gupta NK, Tomar P, Sharma V, Dixit VK. Development and characterization of chitosan coated poly-(varepsilon-caprolactone) nanoparticulate system for effective immunization against influenza. Vaccine 2011;29:9026–37.
[118] Andersen P, Doherty TM. The success and failure of BCG – implications for a novel tuberculosis vaccine. Nat Rev Microbiol 2005;3:656–62.
[119] Dantas OM, Ximenes RA, de Albuquerque Mde F, da Silva NL, Montarroyos UR, de Souza WV, et al. A case-control study of protection against tuberculosis by BCG revaccination in Recife, Brazil. Int J Tuberc Lung Dis 2006;10:536–41.
[120] Kaufmann SH. Is the development of a new tuberculosis vaccine possible? Nat Med 2000;6:955–60.
[121] Shegokar R, Al Shaal L, Mitri K. Present status of nanoparticle research for treatment of tuberculosis. J Pharm Pharm Sci 2011;14:100–16.
[122] Carpenter ZK, Williamson ED, Eyles JE. Mucosal delivery of microparticle encapsulated ESAT-6 induces robust cell-mediated responses in the lung milieu. J Control Release 2005;104:67–77.
[123] Griffiths G, Nystrom B, Sable SB, Khuller GK. Nanobead-based interventions for the treatment and prevention of tuberculosis. Nat Rev Microbiol 2010;8:827–34.
[124] Venkataprasad N, Coombes AG, Singh M, Rohde M, Wilkinson K, Hudecz F, et al. Induction of cellular immunity to a mycobacterial antigen adsorbed on lamellar particles of lactide polymers. Vaccine 1999;17:1814–9.
[125] Ballester M, Nembrini C, Dhar N, de Titta A, de Piano C, Pasquier M, et al. Nanoparticle conjugation and pulmonary delivery enhance the protective efficacy of Ag85B and CpG against tuberculosis. Vaccine 2011;29:6959–66.
[126] Lamalle-Bernard D, Munier S, Compagnon C, Charles MH, Kalyanaraman VS, Delair T, et al. Coadsorption of HIV-1 p24 and gp120 proteins to surfactant-free anionic PLA nanoparticles preserves antigenicity and immunogenicity. J Control Release 2006;115:57–67.
[127] Miyake A, Akagi T, Enose Y, Ueno M, Kawamura M, Horiuchi R, et al. Induction of HIV-specific antibody response and protection against vaginal SHIV transmission by intranasal immunization with inactivated SHIV-capturing nanospheres in macaques. J Med Virol 2004;73:368–77.
[128] Yokoyama T, Tam J, Kuroda S, Scott AW, Aaron J, Larson T, et al. EGFR-targeted hybrid plasmonic magnetic nanoparticles synergistically induce autophagy and apoptosis in non-small cell lung cancer cells. PLoS One 2011;6:e25507.
[129] Harding J, Burtness B. Cetuximab: an epidermal growth factor receptor chemeric human-murine monoclonal antibody. Drugs Today (Barc) 2005;41:107–27.
[130] Liu D, Chen C, Hu G, Mei Q, Qiu H, Long G. Specific targeting of nasopharyngeal carcinoma cell line CNE1 by C225-conjugated ultrasmall superparamagnetic iron oxide particles with magnetic resonance imaging. Acta Biochim Biophys Sin (Shanghai) 2011;43:301–6.
[131] Lee J, Choi Y, Kim K, Hong S, Park HY, Lee T, et al. Characterization and cancer cell specific binding properties of anti-EGFR antibody conjugated quantum dots. Bioconjug Chem 2010;21:940–6.
[132] Ho JA, Chang HC, Shih NY, Wu LC, Chang YF, Chen CC, et al. Diagnostic detection of human lung cancer-associated antigen using a gold nanoparticle-based electrochemical immunosensor. Anal Chem 2010;82:5944–50.
[133] Chanda N, Kattumuri V, Shukla R, Zambre A, Katti K, Upendran A, et al. Bombesin functionalized gold nanoparticles show in vitro and in vivo cancer receptor specificity. Proc Natl Acad Sci USA 2010;107:8760–5.
[134] Debotton N, Zer H, Parnes M, Harush-Frenkel O, Kadouche J, Benita S. A quantitative evaluation of the molecular binding affinity between a monoclonal antibody conjugated to a nanoparticle and an antigen by surface plasmon resonance. Eur J Pharm Biopharm 2010;74:148–56.
[135] El mir S, Casanova A, Betbeder D, Triebel F. A combination of interleukin-2 and 60 nm cationic supramolecular biovectors for the treatment of established tumours by subcutaneous or intranasal administration. Eur J Cancer 2001;37:1053–60.
[136] Peng XH, Wang Y, Huang D, Shin HJ, Chen Z, Spewak MB, et al. Targeted delivery of Cisplatin to lung cancer using ScFvEGFR-Heparin-Cisplatin nanoparticles. ACS Nano 2011;5:9480–93.
[137] Matsuo K, Koizumi H, Akashi M, Nakagawa S, Fujita T, Yamamoto A, et al. Intranasal immunization with poly(gamma-glutamic acid) nanoparticles entrapping antigenic proteins can induce potent tumor immunity. J Control Release 2011;152:310–6.
[138] Dhuria SV, Hanson LR, Frey 2nd WH. Intranasal delivery to the central nervous system: mechanisms and experimental considerations. J Pharm Sci 2010;99:1654–73.
[139] Malerba F, Paoletti F, Capsoni S, Cattaneo A. Intranasal delivery of therapeutic proteins for neurological diseases. Expert Opin Drug Deliv 2011;8:1277–96.
[140] Thorne RG, Pronk GJ, Padmanabhan V, Frey 2nd WH. Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. Neuroscience 2004;127:481–96.
[141] Di Colo G, Zambito Y, Zaino C. Polymeric enhancers of mucosal epithelia permeability: synthesis, transepithelial penetration-enhancing properties, mechanism of action, safety issues. J Pharm Sci 2008;97:1652–80.
[142] Vaka SR, Sammeta SM, Day LB, Murthy SN. Delivery of nerve growth factor to brain via intranasal administration and enhancement of brain uptake. J Pharm Sci 2009;98:3640–6.
[143] Wang X, He H, Leng W, Tang X. Evaluation of brain-targeting for the nasal delivery of estradiol by the microdialysis method. Int J Pharm 2006;317:40–6.
[144] Liu Q, Shen Y, Chen J, Gao X, Feng C, Wang L, et al. Nose-to-Brain transport pathways of wheat germ agglutinin conjugated PEG-PLA nanoparticles. Pharm Res 2012;29:546–58.
[145] Chen J, Zhang C, Liu Q, Shao X, Feng C, Shen Y, et al. Solanum tuberosum lectin-conjugated PLGA nanoparticles for nose-to-brain delivery: in vivo and in vitro evaluations. J Drug Target 2012;20:174–84.
[146] Gao X, Wu B, Zhang Q, Chen J, Zhu J, Zhang W, et al. Brain delivery of vasoactive intestinal peptide enhanced with the nanoparticles conjugated with wheat germ agglutinin following intranasal administration. J Control Release 2007;121:156–67.
[147] Ruan Y, Yao L, Zhang B, Zhang S, Guo J. Nanoparticle-mediated delivery of neurotoxin-II to the brain with intranasal administration: an effective strategy to improve antinociceptive activity of neurotoxin. Drug Dev Ind Pharm 2012;38:123–8.
[148] Veronesi MC, Aldouby Y, Domb AJ, Kubek MJ. Thyrotropin-releasing hormone d, l polylactide nanoparticles (TRH-NPs) protect against glutamate toxicity in vitro and kindling development in vivo. Brain Res 2009;1303:151–60.
[149] Hesterberg TW, Long CM, Lapin CA, Hamade AK, Valberg PA. Diesel exhaust particulate (DEP) and nanoparticle exposures: what do DEP human clinical studies tell us about potential human health hazards of nanoparticles? Inhal Toxicol 2010;22:679–94.
[150] Nielsen GD, Roursgaard M, Jensen KA, Poulsen SS, Larsen ST. In vivo biology and toxicology of fullerenes and their derivatives. Basic Clin Pharmacol Toxicol 2008;103:197–208.
[151] Park EJ, Yi J, Chung KH, Ryu DY, Choi J, Park K. Oxidative stress and apoptosis induced by titanium dioxide nanoparticles in cultured BEAS-2B cells. Toxicol Lett 2008;180:222–9.
[152] Wang YF, Tsai PJ, Chen CW, Chen DR, Dai YT. Size distributions and exposure concentrations of nanoparticles associated with the emissions of oil mists from fastener manufacturing processes. J Hazard Mater 2011;198:182–7.
[153] Merhi M, Dombu CY, Brient A, Chang J, Platel A, Le Curieux F, et al. Study of serum interaction with a cationic nanoparticle: implications for in vitro endocytosis, cytotoxicity and genotoxicity. Int J Pharm 2011;423:37–44.
[154] Hardy CL, Lemasurier JS, Belz GT, Scalzo-Inguanti K, Yao J, Xiang SD, et al. Inert 50-nm polystyrene nanoparticles that modify pulmonary dendritic cell function and Inhibit Allergic airway inflammation. J Immunol 2012;188:1431–41.