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Hugh D.C. Smyth
Anthony J. Hickey *Editors*

Controlled Pulmonary Drug Delivery

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Controlled Pulmonary Drug Delivery

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Preface

The pace of new research and level of innovation repeatedly introduced into the field of drug delivery to the lung are surprising given its state of maturity since the introduction of the pressurized metered dose inhaler over a half a century ago. It is clear that our understanding of pulmonary drug delivery has now evolved to the point that inhalation aerosols can be controlled both spatially and temporally to optimize their biological effects. These abilities include controlling lung deposition, by adopting formulation strategies or device technologies, and controlling drug uptake and release through sophisticated particle technologies. The large number of contributions to the scientific literature and variety of excellent texts published in recent years are evidence for the continued interest in pulmonary drug delivery research. This reference text endeavors to bring together the fundamental theory and practice of controlled drug delivery to the airways that is unavailable elsewhere. Collating and synthesizing the material in this rapidly evolving field presented a challenge and ultimately a sense of achievement that is hopefully reflected in the content of the volume.

The spatial and temporal control of drug delivery to the airways as a general theme runs through the entire volume from discussions of micro and macro structure of the lung, particle engineering and polymer science, device design, to regulatory perspectives and science. The initial chapter topics were selected to provide a fundamental background to the problems and opportunities for controlled pulmonary drug delivery. In addition to providing an anatomical, physiological, and metabolic overview of the airways, the book provides unique guidance on specific microenvironments that exist in both health and disease within the airways – opening possible avenues to allow for targeted, triggered, or modulated delivery systems based on the physicochemical differences between target and bystander tissues and cells. The latter sections of the book explore technologies and tools available to facilitate controlled drug delivery to the airways, specifically covering topics such as, aerosol delivery technologies, materials and excipients, particle science, gene delivery, in vitro and in vivo tools including imaging. Finally, regulatory approval perspectives and the development of performance specifications complete the "tool box" that is provided by the text as a whole.

The authors who kindly agreed to contribute to *Controlled Pulmonary Drug Delivery* are acknowledged leaders in their respective fields, and many have initiated research programs in new and emerging research areas of relevance to the title of the volume. As a result, we hope that this text will provide a framework for interested researchers to find solutions to their drug delivery questions. The contents of the book should provide bridges between the multiple disciplines needed to successfully achieve controlled pulmonary drug delivery.

Austin, TX — Hugh D.C. Smyth
Chapel Hill, NC — Anthony J. Hickey

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Chapter 1
Macro- and Microstructure of the Airways for Drug Delivery

Kevin P. O'Donnell and Hugh D.C. Smyth

Abstract Both anatomy and physiology of the airways are critical for understanding and predicting the dynamics of drug delivery systems that are inhaled. This theme that intimately links the biology of the airways to the response of pulmonary drug delivery systems is present throughout other following chapters in this book. Therefore, it is ideal to introduce these concepts in this chapter by first addressing the lung architecture on the macroscale and how it influences drug delivery. Then, we discuss the microscale interactions between the airway environment and drug delivery system. By discussing the anatomy and physiology at these scales in the direct context of pulmonary drug delivery, we believe this chapter is unique and, hopefully, useful for those seeking the controlled release in the respiratory tract.

Keywords Anatomy • Cell biology • Deposition • Physiology

1.1 Macrostructure and Function

When considering drug delivery to the lung, one must first understand the general composite structure of the lung. The airway can be broken into two distinct zones: the conducting airway and the respiratory airway.

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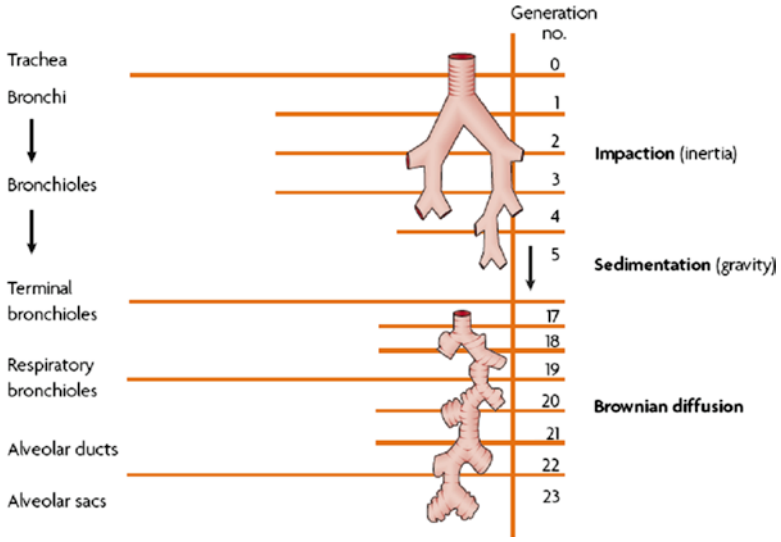

Fig. 1.1 Factors influencing lung deposition. Reproduced from Patton and Byron [53] with permissions

1.1.1 Conducting Airways

The conducting zone constitutes the upper portion of the airways. It begins at the mouth/nose, and comprises the trachea, bronchi, bronchioles, and terminal bronchioles. This portion of the airway bifurcates approximately 17 times prior to reaching the respiratory zone of the deep lungs, which branches further and is discussed in the following sections. This branching allows for a progressive increase in surface area and a corresponding decrease in air velocity (Fig. 1.1) [43]. The presence of smooth muscle gradually increases from the trachea to the terminal bronchioles, connecting the incomplete cartilage rings of the trachea and eventually becoming a complete layer. Within the conducting region, no gas exchange takes place; its primary purpose, rather, is to transport the gas to the respiratory zone [1]. A secondary function of the conducting airways is to ensure that inspired gasses are humidified and heated so as to provide the alveoli with air identical to the preexisting environment. The dangers associated with the inspiration of dry and improperly heated gasses have been demonstrated [65]. The obvious problems associated with cold air inspiration are the loss of body heat (i.e., drop in core body temperature) due to heat transfer between the body and respired air, as well as water loss due to humid air expiration. Accurate humidification of respiratory gasses is crucial to ensure proper function of the airways. Improper humidity content of respired air can lead to extensive dehydration and loss of body weight [46]. Additionally, functional impairments may be rapidly observed including extensive impairment of the

mucociliary escalator. The following list describes the potential damages caused by dry gas inspiration [65]:

(a) Destruction of cilia and damage to mucous glands
(b) Disorganization and flattening of pseudostratified columnar epithelium and cuboidal epithelium
(c) Disorganization of basement membrane
(d) Cytoplasmic and nuclear degeneration
(e) Desquamation of cells
(f) Mucosal ulceration
(g) Reactive hyperemia following damage

Furthermore, overhumidified air poses dangers as well [71]. In fact, overhumid air may lead to water intoxication with the final effects being analogous to those listed for dehumidified air, in the opposing direction. Ultimately, improperly functioning conducting airways may lead to impaired respiration through increased surface tension and bronchoconstriction. This in turn will lead to inefficient drug delivery to the lung or, potentially, impaired pulmonary absorption ability. While damage to the ciliated epithelium may limit clearance of particles impacted in the upper airway, the body's natural defense system will also be harmed, resulting in a higher propensity for infection.

1.1.2 Respiratory Airways

Distal to the terminal bronchioles of the conducting zone lies the respiratory zone which consists of the respiratory bronchioles, alveolar ducts, and alveolar sacs. The alveolar ducts are typically 1 mm in length formed via connected groups of alveoli, polyhedral chambers with an average diameter of 250 μm characterized by a 0.1–0.4-μm epithelium and 70 nm liquid lining layer. Itoh et al. [38] provide excellent microscopy and 3D modeling images of the alveolar locality [38]. The primary function of this region is gas exchange, which may take place throughout the listed bifurcations. This region is ideally suited for gas exchange due to its inherent physical characteristics. The surface area of the distal airway is approximately 102 m^2, while the conducting airway is a mere 2–3 m^2, allowing for much greater contact with the inspired gas or therapeutic aerosol [52]. Second, the thickness of the cell layer, which makes up the respiratory region, is progressively reduced from approximately 60 μm in the upper airway to the aforementioned submicron thickness in the alveoli [53]. Similarly, the fluid layer at the cell surface decreases from 8 μm to approximately 70 nm in direct correlation with the decrease in cell thickness. Figure 1.2 depicts this change in breadth. The cell types allowing for this reduction in thickness are discussed in detail in later sections. Lastly, the partial pressure of oxygen within the alveoli is far less than that within the CO_2-rich blood present in pulmonary circulation. The pressure gradient coupled with a markedly thin diffusion pathway

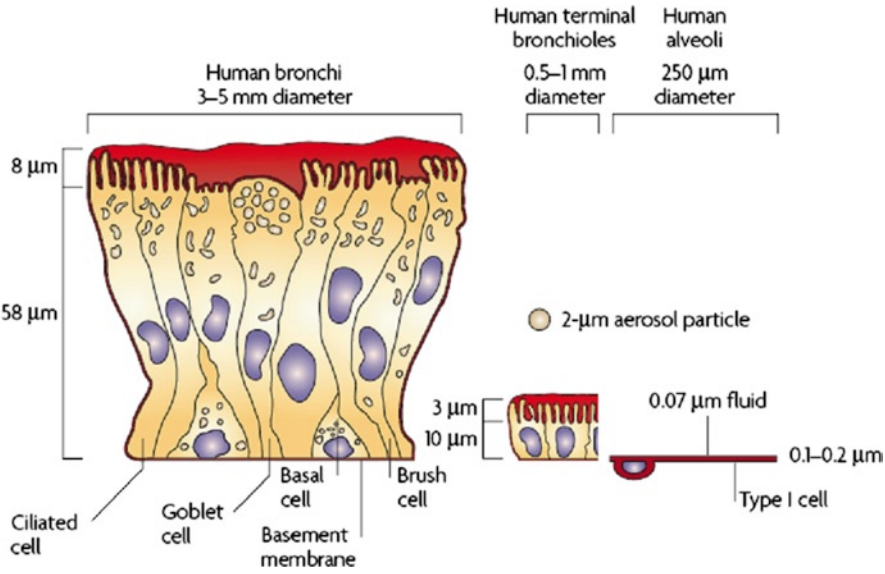

Fig. 1.2 Cell type and regional thickness across the regions of the airway. Reproduced from Patton and Byron [53] with permission

allows oxygen to diffuse from the alveoli into the blood, while CO_2 diffuses in the opposite direction. For a detailed explanation on the mechanisms of gas transport and development of the alveolar gas exchange, the reader is referred to Massoro (1996) and Hickey and Thompson (2003) [31, 47].

1.2 Targeted Anatomical Sites for Aerosol Delivery

As can be derived from the above description of the airway structure, the premier target for drug delivery will be the alveolar region of the deep lung, which presents the largest surface area and thinnest diffusion pathway for dissolved material. Studies have demonstrated that the trachea presents >90% resistance to transport, limiting its potential in drug delivery [78]. While the nonrespiratory bronchioles are involved in many disease states, they too exhibit poor drug absorption; though due to the thinner cell layer, absorption may be marginally increased. This is most likely due to a reduction in intercellular and intracellular transport path lengths. Many disease states, such as asthma and chronic bronchitis, elicit their effects in the bronchioles or trachea; however, because of their poor drug reception ability, they are typically not a primary target. Drugs delivered via inhalation seldom achieve a dose of greater than 20% to the alveoli when administering via a pressurized metered dose inhaler (pMDI) [27]. While other devices, such as spacers, and patient parameters (synchronization of breath/actuation) can increase the dose well above 20%, a potentially large amount of drug may deposit within the conducting airways and

will subsequently be transported up the airway via the mucociliary escalator, ultimately resulting in the swallowing of a large portion of the dose by the patient. For a detailed description of the mucociliary system, the reader is referred to [54] in International Review of Physiology; Respiratory Physiology III. Following chapters within this publication discuss in great detail the clearance of particles from the lungs, as well as methods of targeting to various sites within the airway. Many targeting techniques often utilize receptor–ligand conjugation. Targeting of specific areas rich in the desired receptor can lead to greater therapeutic efficiency. A discussion of such receptors can be found in later sections.

1.2.1 Aerosol Particle Deposition Mechanisms

In order for a physiological effect to occur, inhaled particles must first deposit within the respiratory system. A large number of factors contribute to particle deposition within the airway including particle size, shape and density, airflow velocity and volume, interpatient physiological variations, and pause time between inspiration and expiration. While all of these factors and more contribute to total deposition, there are three generally accepted mechanisms by which particle deposition within the airway occurs: impaction, sedimentation, and diffusion. Two more mechanisms, interception and electrostatic precipitation, may also result in particle deposition; however, they are not discussed in detail here [43]. Impaction of particles upon airway surfaces is influenced by particle size, density, and velocity. As a result of inertial forces, it is most likely to occur in the upper conducting airways characterized by high particle velocities and drastic changes in airflow direction. This mechanism is most influential upon particles of sizes greater than 2 μm [64]. Sedimentation via gravitational force of particles within the airway is dependent upon particle mass. Particles will be subject to sedimentation if the product of their settling velocity and residence time is greater than the distance required for contact of the surface airway. Sedimentation typically influences particles between 0.5 and 2 μm. Particles less than 0.5 μm are subject to deposition via diffusion based on Brownian motion. A reduction in particle size and increase in residence time increase the probability of a particle to deposit through diffusion, and thus, breath holding may increase deposition via this mechanism [62].

1.3 Physiological Factors Influencing Deposition

1.3.1 Mode of Inhalation

While a large number of formulation factors will influence the mechanism and extent of particle deposition, patient dependent dynamics must also be considered. Regardless of the method of aerosol generation, the method of inhalation by the patient will strongly influence the degree and location of particle deposition.

The airflow pattern instigated by the patient will determine the extent of throat deposition, ultimately swallowed and considered a nontherapeutic dose, which will in turn determine the dose available to reach the lung [8]. Deposition within the throat and mouth can lead to a high degree of interpatient variability, and thus, adequate breath patterns can increase efficacy. In fact, it has been demonstrated that slow inhalation by the patient reduces throat deposition, increases lung deposition, and ultimately reduces patient variability [70]. While this slow inhalation may be applied during passive inhalation methods (i.e., nebulizers), it is not reasonable for devices such as pMDIs, which produce a high velocity aerosol with which the patient must time their breathing. Indeed, proper breath timing and inspiration pressure can increase the lung deposition of pMDI aerosols from 7 to 20% [51]. Similarly, slow air velocities may not be appropriate for dry-powder inhalers where sufficient airflow must be applied to generate the aerosol [83]. Therefore, an appropriate method of inhalation must be applied that is device specific to minimize conducting zone deposition and increase the therapeutic dose to the deep lung. The inspiration–breath hold–expiration cycle creates enormous variations in airflow patterns. These variations may be compounded by contractions or restrictions created by the aforementioned smooth muscle. These patterns will navigate inspired particles in conjunction with the physical properties of the particle. A detailed description of the influence and characteristics of airflow across the complex bifurcating human lung is given by [37], and the reader is referred there for further information.

1.3.2 Oropharyngeal Deposition

Aside from the method of inhalation, the individual's morphology will determine the success of drug delivery to the lung. Variations in diameter and length of individual generations create inherent volumetric and structural differences between patients [22, 34]. Figure 1.1 depicts this progressive change across the generations, while Table 1.1 demonstrates intersubject variation in oropharyngeal geometries.

Table 1.1 Oropharyngeal morphology in multiple subjects

Oropharyngeal designation[a]	Oropharyngeal volume/cm^3	Oropharyngeal opening[b]	Oropharyngeal centerline length/cm	Gender	Oral cavity inlet cross-section shape
1C	37.6	C	17.1	F	Rectangular
2C	53.4	C	18.7	F	Circular
3A	55.9	A	19.9	M	Rectangular
4A	61.8	A	17.8	F	Circular
5A	68.4	A	19.9	M	Circular
6B	75.1	B	21.6	M	Circular
7B	80.8	B	22.3	M	Rectangular

Reproduced from Ehtezazi et al. [22] with permission

[a] The designation sequence is rank of oropharyngeal volume/oropharyngeal configuration category

[b] *A* wide open space; *B* a moderate narrowing; *C* a marked constriction; *F* female; *M* male

Furthermore, these anatomical differences may be altered based on the disease state of the patient, a matter discussed in the following section. This physical difference in structure coupled with variations in respiration rate creates tidal volumes ranging from 460 to 900 ml in adults, capable of producing a twofold difference in particle deposition [33]. While larger particles are deposited by inertia at the 90° bend transition between the mouth and throat, small (i.e., submicron) particles may utilize the transition period between inspiration and expiration and sediment within the oropharyngeal space. It should be noted that residence times within any given portion of the respiratory tract may be subsecond, and the final dynamics influencing particle deposition within the selected region is a result of local aerodynamics, determined by local morphology and airflow changes throughout breathing [67].

1.3.3 Morphological and Deposition Changes Due to Disease

1.3.3.1 Obstructive Diseases

Obstruction of the airway due to disease may drastically reduce the ability to deliver drug to the lungs. Chronic pulmonary obstructive diseases (COPD) such as chronic bronchitis (CB), obstructive sleep apnea (OSA), and emphysema restrict airflow in an irreversible manner. CB is characterized by thickening and inflammation of the bronchial walls and increased mucus production, creating a restricted pathway for airflow and aerosols and an increased diffusion path for therapeutic entities. Emphysema, on the contrary, is the permanent enlargement of the gas exchange zone of the lungs, resulting in destruction of the alveolar walls [16, 24]. OSA is characterized by a reoccurring temporary stoppage of breathing during REM sleep and is prevalent in patients with a narrow oropharyngeal airway and low sleeping lung volume [79]. COPDs characteristically cause productive coughing in the patient, which may increase the clearance of delivered particles or further hinder inhalation ability. For a detailed description of the structural changes associated with COPD, the reader is referred to [17]. Asthma is a complex pulmonary disorder similar to CB in that bronchial inflammation and hyperactivity are observed [42]. Since particle deposition is highly dependent upon airflow patterns, any obstructive disease will alter the deliverability of drugs. For example, an obstructed throat causes recirculation in both directions, greatly altering flow characteristics [44]. It has also been demonstrated that patients suffering an asthmatic attack show a narrowing of the pharyngeal airway, which results in increased oropharyngeal deposition of an aerosolized dose and consequently, due to clearance and impaction, a reduction in total dose delivered to the lungs [69] While obstructions may lead to greater bronchial and central airway deposition due to impaction caused by altered flow patterns, it is possible to achieve a high degree of deep lung penetration in COPD patients. Maeyer et al. [45] demonstrated that low airflow rates could provide above 50% dose delivery

to the deep lung for particles between 2 and 4 μm (1–5 μm is considered the ideal particle size range). Furthermore, breath holding following reception of a dose may significantly increase deposition against COPD [45, 63]. Thus, while obstructions may greatly hinder the ability of the patient to breathe, it may still be possible to treat the condition via deep lung penetration. Owing to the chronic and reoccurring nature of these disease states, the ability to prolong release in a local manner would be of extreme benefit to the patient population by potentially reducing not only the number of doses required but also the occurrences of impaired breathing.

1.3.3.2 Infectious Disease

Owing to direct tissue contact with the external environment via inspired air, the lung is susceptible to bacterial infections. While many pulmonary defense systems exist, infection is still possible, especially within immunocompromised patients. Owing to a mutated membrane embedded chloride channel (CFTR), patients suffering from cystic fibrosis exhibit a dehydrated airway liquid surface, resulting in an increase in mucus viscosity and poor mucociliary clearance. As a result of this altered physiological environment, this patient population has an increased incidence of pulmonary infection. Such infections include, but are not limited to, aspergillosis, zygomycosis, cryptococcosis, histoplasmosis, pneumocystis pneumonia, *Pseudomonas aeruginosa* infection, and tuberculosis (TB) [60, 66, 74]. In any case, pulmonary function may be impaired with common symptoms including acute dyspnea, pleuritic chest pain, chronic inflammation, and tachypnea. Tidal volume may be compromised, leading to insufficient inhalation ability. *Pseudomonas aeruginosa* rhamnolipid has been reported to cause cell membrane damage and inhibit epithelial ion transport, resulting in deteriorated mucociliary clearance mechanisms [2, 56]. TB can cause serious tissue damage, ultimately liquefying infected regions as a result of the host immune response creating an ideal growth medium. This liquid caseous material does not promote the growth or survival of alveolar macrophages, further reducing the innate immune ability [50]. It has been reported that targeted delivery directly to the site of infections results in high localized concentrations while maintaining relatively low systemic concentrations of therapeutic agents [75, 82]. This is desirable when delivering antibiotic or antifungal agents due to a high prevalence of adverse side effects associated with high systemic plasma concentrations. Furthermore, systemic delivery via the lung is still possible for infections that have disseminated to multiple locations within the host [68]. However, due to the aforementioned pulmonary impairments, sufficient delivery of inhaled therapeutics may be difficult. Reduced tidal volumes, altered mucosal physiology, altered epithelial function, and surface blockage due to colonization may all inhibit proper deposition. In cases of insufficient mucus clearance, the additional liquid yields a thicker diffusion pathway, presenting increased difficulty for diffusion as well as increased adhesive properties in undesired locations.

1.4 Airway Cells

1.4.1 Relevant Cells of the Conducting Airways

The columnar epithelium of the conducting airways is a gradually thinning barrier comprising a variety of cells. Below the luminal epithelium, and in no contact with inspired gasses, lie the basal cells. Basal cells are pyramidal progenitor cells that differentiate into the cell types found within the tracheobronchial epithelium [3]. Also, below the surface lie the neuroendocrine cells, such as K-cells, which contain and secrete peptide hormones. While their secretions may reach the surface, the cell walls themselves rarely do. Ciliated columnar cells make up a large portion of the conducting airway epithelium. These cells are a major component of the mucociliary escalator responsible for the clearing of foreign material from the airways, including deposited therapeutics. Interdispersed between the ciliated cells, and comprising approximately 25% of the tracheobronchial epithelium, are nonciliated goblet cells. Goblet cells are nonglandular cells involved in mucus secretion creating the viscoelastic layer lining the bronchial region. The most prevalent cell type in the epithelium is Clara cells. These nonciliated cells have a number of key roles including production of surfactant components, production of protease inhibitors, and metabolic detoxification [20, 21]. The cells of the epithelium are connected via tight junctions, preventing intercellular penetration of inhaled matter into the body. These junctions, however, may be broken or damaged by aerosolized pharmaceutical constituents such as chitosan. While such a component may serve to enhance the delivery platform, it may also be exposing the body to potential antigens while also inhibiting the body's natural clearance mechanisms [39, 73]. Other cells within the tracheobronchial epithelium include smooth muscle cells and mast cells, whose primary functions are contraction/relaxation of the airway and antigen recognition and response, respectively, and secretory gland cells (mucus and serous), which are discussed in a later section. Mast cell response must be considered during formulation due to the fact that upon recognition of an antigen or allergen, mast cells release inflammatory mediators creating a variety of biological responses including, but not limited to, airway constriction, swelling of tissue, blood vessel dilation, and permeation [40]. Particle size is of concern as well in avoidance of uptake by patrolling macrophages.

1.4.2 Relevant Cells of the Respiratory Airways

Entry into the respiratory zone is marked by a gradual transition as the bronchial wall is partially replaced by alveoli, with eventual complete replacement. The drastically thinner epithelium within this region is responsible for gas exchange and will be the primary target of most pharmaceutical scientists in drug delivery. As such, the two primary cell types of interest within this region are those responsible for gas and material exchange: alveolar type 1 (AT1) and alveolar type 2 cells (AT2).

Capillary endothelial cells and alveolar macrophages are also present and may be found close to the alveolar epithelium. AT1 cells cover over 95% of the alveolar surface area with AT2 cells accounting for the remainder, yet AT2 cells are far more numerous than AT1 cells, present at a ratio of approximately 2:1 [15, 20, 21, 58]. AT1 cells are approximately 50–100 μm in diameter while remaining extremely thin, 2 μm at the nucleus and 0.2 μm in cytoplasmic regions. These cells have shown the highest water permeability of any cell type. Furthermore, transport of macromolecules may be possible due to the presence of vesicles and caveolae invaginations, further reducing the membrane thickness. By contrast, AT2 cells are 10 μm in diameter, exhibiting a cuboidal shape. The primary role of AT2 cells is the production, secretion, and recycling of lung surfactant material. A secondary function of AT2 cells is the generation of AT1 cells. Upon division, AT2 cells may either proliferate into additional AT2 cells or may differentiate into AT1 cells to aid in repair and surface maintenance [14, 30, 47]. The alveolar cells are connected by both tight and gap junctions, creating a protective boundary between the environment and body while maintaining potential as a therapeutic target.

1.5 Airway Receptors

Therapeutic entities may elicit their cellular effect both pre and post internalization via cell receptor binding. As a combinatory example, many tumors overexpress receptors for hyaluronic acid, which upon coupling with a ligand results in rapid internalization of the bound molecule, which may further conjugate with an internal target. Thus, an understanding of cellular receptors within the pulmonary system creates potential for more effective inhalation therapy. Presented herein are only some of the most pertinent airway receptors in drug delivery. One of the most studied pulmonary receptor classes is β adrenergic receptors (βAR). β_2 adrenergic receptors may be found throughout the airway on the epithelium and smooth muscle; however, the greatest concentration may be found in the alveoli (on both AT1 and AT2 cells). More specifically, β_2 adrenergic receptors have been observed on the vascular endothelium, ciliated epithelium, mast cells, circulating inflammatory cells, Clara cells, and others. β_1 receptors lie primarily on alveolar walls [4, 9, 28]. Complexation with a β adrenergic agonist can lead to a number of physiological responses (Table 1.2), including bronchodilation via smooth muscle relaxation, which has lead to their targeting in the treatment of asthma and COPD. βAR activation also upregulates Na+ transport and accelerates clearance of fluid from the alveolar airspace, allowing for treatment of edema. Hanania and Moore [28] describe the influence on particular cell lineages and the reader is referred there if such information is desired. Desensitization of βAR can occur over prolonged or continuous exposure to agonists, for example via an uncoupling from the corresponding G protein responsible for stimulation [29, 49]. Activation of muscarinic receptors has proven to be more effective than βAR agonists in the treatment of COPD, but not asthma. Acetylcholine-lad bronchoconstriction is

Table 1.2 Documented physiological effects of β_2-adrenergic receptor stimulation in human lung

Airway smooth muscle relaxation
Prejunctional inhibition of acetylcholine release from parasympathetic neurons in airway smooth muscle
Stimulation of mucous and serous cell secretion
Stimulation of chloride ion secretion across the apical membrane of airway epithelial cells
Increase in ciliary beat frequency
Stimulation of surfactant secretion from alveolar type II cells
Inhibition of mediator release from lung mast cells and neutrophils

Reproduced from Bai [4] with permissions

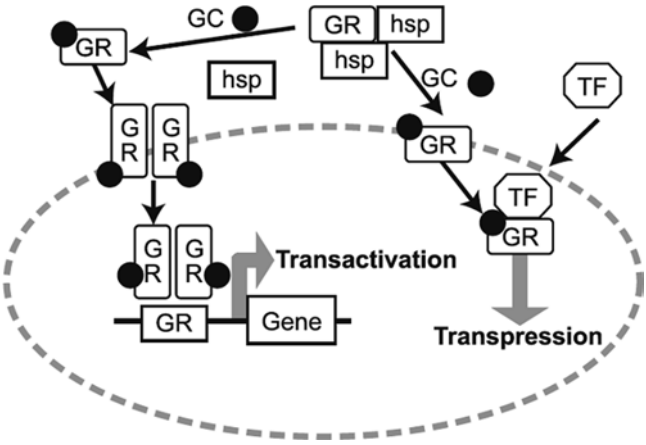

Fig. 1.3 Transactivation and transrepression pathways. *GC* glucocorticoid; *GR* glucocorticoid receptor; *hsp* heat shock protein; *TF* transcription factor. Reproduced from Hochhaus [32] with permissions

mediated by the M_3 receptor. Anticholinergics block M_1 and M_3 receptors, resulting in bronchodilation; however, selectivity away from the M_2 receptor (which mediates acetylcholine release) has proven to be challenging [6]. Endothelin receptors A and B (ET_A and ET_B) may be targeted with endothelin-1, a vasoconstrictor, for the treatment of pulmonary arterial hypertension [57]. Glucocorticoid receptors (GR) reside in the cytoplasm of pulmonary cells in an inactive complexed form. Upon binding to a corticosteroid ligand, its cytoplasmic complex disaggregates and is transported to the nucleus where dimerization occurs followed by interaction with response elements. Alternatively, bound monomers may interact with protein transcription factors potentially reducing proinflammatory cytokine production (Fig. 1.3) [32]. Many interstitial pulmonary diseases can result in an increase in lung GR, typically due to the increase in parenchyma cellular density associated with the disease state, leading to necessary alterations in treatment [61]. Figure 1.4 [23] presents binding affinities of various corticosteroids

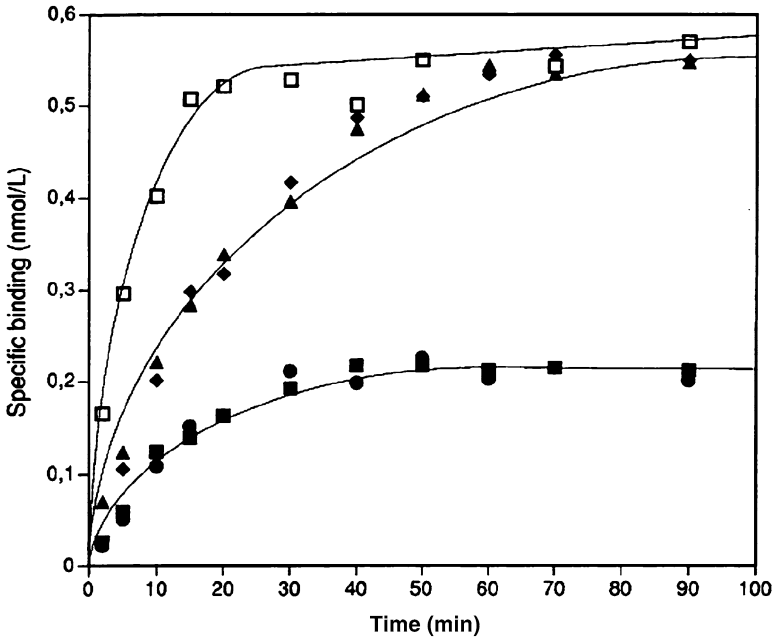

Fig. 1.4 Reproduced from Esmailpour et al. [23] with permission

used in inhalation therapy. Prostacyclin receptor (PR) agonists provide an anti-inflammatory response in asthma and COPD patients. Agonistic binding of bronchial epithelial PRs enhances the anti-inflammatory cellular response generated by glucocorticoids. Thus, combination therapies employing PR agonists may prove to be superior to steroid treatment alone [81]. A vast array of pulmonary receptors mediates clearance patterns, immunological responses, and particle uptake. Targeting such receptors or employing them synergistically can be highly beneficial in inhalation therapies.

1.6 Blood Flow

1.6.1 Bronchial and Pulmonary Circulation Systems

Airway circulation serves a number of roles in addition to providing nutrients to the region. These functions include heat–water exchange, regulation of airway caliber in the peripheral lung, clearance of biological substances, and recruitment of inflammatory mediators. Blood flow may be influenced by a number of factors including hyperventilation, airway pressure, inspired air temperature, and alterations in airway fluid content [13]. Blood is supplied to the pulmonary system via the right ventricle of the

Table 1.3 Blood flow and physiological parameters of various species

	Rats	Dogs	Humans
Body weight (kg)	0.25	10.0	70.0
Respiratory rate (per min)	97	21	16
Tidal volume (ml)	1.55	114	400
Dimensions of upper and lower airways, and of alveolar region			
Upper and lower airways			
Alveolar region		see Table 1.2	
Luminal volume (ml)	3.55	140	2,670
Surface area (cm^2)	45.7×10^4	754×10^4	$3{,}310 \times 10^4$
Blood flow (ml/min/whole tissue)			
Upper airway	1.05	11.0	44.2
Lower airway (1% of cardic output)	0.61	12.1	58.4
Alveolar region (95% of cardiac output)	60.8	1,150	5,550
Diffusion distance (cm)			
Upper airway (cm)	0.02	0.04	0.07
Lower airway (cm)	5% of lumminal diameter		
Alveolar region (μm)	0.4	0.48	0.62

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heart to the pulmonary arteries. In humans, the bronchial arterial system stems from the thoracic aorta with one bronchial artery supplying the right lung and two supplying the left lung. Intrapulmonary bronchial arteries provide at least two arteries for each bronchus. Branches enter the bronchial muscular layer to provide a submucosal vascular system. At the terminal bronchiole, arterioles branch to form a pulmonary capillary network to perfuse the alveoli. The diameter of the pulmonary capillaries is between 4.3 and 8.6 μm. Here, the capillary walls are in close proximity to the alveolar membrane and in many cases are fused directly to it to facilitate rapid diffusion. In such cases, the cumulative diffusion pathway from luminal air to the blood is less than 400 nm. For this reason, alveolar deposition has become a primary target for systemic drug delivery via inhalation [82]. Two pulmonary veins stem from each lung to transport oxygenated blood to the left heart via the left atrium and subsequently the left ventricle for systemic circulation. Table 1.3 defines the blood flow with corresponding cardiac output of the different airway regions in ml/min/whole tissue. For a more in-depth look at pulmonary circulation, the reader is referred to Chediak and Wanner [10, 11, 24, 55, 76].

1.6.2 Blood Flow Influence on Drug Delivery

The disease state of the patient may greatly influence perfusion of the airway. Asthma and COPD have shown to increase airway blood flow as a result of an increase in the number of blood vessels in the mucosa as well as the dilation of resistance arteries. Furthermore, increased blood vessel wall thickness, increased

microvascular permeability, and edema formation may all be observed. Lung hyperinflation potentially compresses vessels, resulting in increased arterial flow resistance, ultimately resulting in vascular remodeling. An increase in blood flow and permeability is beneficial for drugs meant for systemic treatment in that they will be transported throughout the body more rapidly. Indeed, transport processes are enhanced in the presence of flow. However, for drugs intended to act locally, this presents the problem of increased clearance and potential metabolism, making the treatment difficult. Furthermore, unintentional/undesired systemic exposure may occur for intended local treatments, especially in cases with elevated blood flow, creating potential adverse events. This is most prevalent for small-molecule drugs capable of quickly navigating the diffusion pathway into the blood stream. The extent of perfusion may inhibit long-acting drugs from eliciting a long-term effect if they are cleared rapidly, or conversely, may prolong the effect of intended short-action drugs [10, 17, 35, 36, 48].

1.7 Airway Secretions

As mentioned, while discussing respiratory cellular composition, two primary cell types are responsible for the majority of secretions found in the airways: serous cells and mucous cells. In the cartilaginous tracheobronchial region, serous and mucous cells form glands beneath the epithelial surface, connecting to the lumen via ducts [25]. Glands comprised primarily of serous cells have been shown to secrete both antimicrobial and immunological agents, including lysozyme, IgA, and antimicrobial peptide LL-37, making them crucial in certain disease states. Glands formed of mucous cells as described by their nomenclature are the primary producers of mucus and mucoproteins [5]. Produced mucins may be either secreted by these glands into the mucus layer or may be membrane-associated, in which case antigen recognition and cellular signaling may be of primary purpose. Secreted mucus, along with the perciliary fluid produced by the epithelial goblet cells, creates an adhesive, viscoelastic fluid layer covering the airway surface. The viscosity of the mucus layer decreases with increasing strain in a time-dependent manner, allowing for proper control of clearance rates. Mucus is composed of 95% water and approximately 5% solids, which contribute to its gel-like nature. These values vary regionally within the lung. The solids of the mucus include the mucin glycoproteins, lipids, DNA, actin, and minerals. The mucin glycoproteins, which vary greatly in length, are approximately 75% glycosylated and provide binding sites for large carbohydrate formations [7, 77, 80]. The variation in protein length creates changes in the swelling capability of the mucus network, and thus, directed cleaving of the S:S bond of apomucin polymers may drastically accelerate mucus distribution and clearance [19]. Primary functions of the mucus layer include binding (via adhesion) and clearance of particles (mucociliary clearance), hydration of the cell surface, humidification of respired air, and lubrication of the airways [18, 41]. A list of the primary glandular secretions may be found in

Table 1.4 Major secretory products of airway gland cells

Serous cells	Mucous cells
Mucin (MUC 2, MUC 7)	Mucin (MUC 2, MUC 5B)
Proteoglycans	Antimicrobial peptide LL-37/hCAP-18
Lysozyme	
Lactoferrin	
Secretory IgA	
Antimicrobial peptide LL-37/hCAP-18	
Antileukoprotease	
β-defensin 1	
β-defensin 1	
Proline-rich proteins	
Albumin	

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Table 1.4, and for a more detailed discussion on individual components of the mucus, the reader is referred to [7]. The extent of mucus production is dependent upon individual physiology of the patient, including disease state, and is primarily activated via cholinergic stimulation mediated by the aforementioned $M_{1\text{-}3}$ receptors, though innervations via other means may also be influential. An in-depth discussion of glandular and goblet cell motor control is provided by [59]. Cholinergic innervations of the glands may also influence the epithelium by instigating active ion transport with associated passive water flux, as well as secretion of large molecules such as albumin. For example, CB is characterized by hypersecretion of mucous with concurrent enlargement of the secretory glands due to hyperplasia. This may increase not only the duration but also the frequency of pulmonary infection and can result in airway plugging [16]. Cystic fibrosis exhibits improper secretory gland function as well owing to the fact that the glands are unresponsive to the synergistic effect of vasoactive intestinal peptide (VIP) acting with acetylcholine. This improper mucus production, in conjunction with the epithelial damage discussed previously, creates an environment with increased susceptibility to infection [12].

1.7.1 Importance in Drug Delivery

Particles delivered to the lung will, unless expired, inevitably contact the lung lining fluid. As such, its production, properties, and interaction with the inhaled material will all impact therapeutic delivery. If soluble within the mucosal matrix, dissolution kinetics will determine the extent of solubilization and absorption of the drug. However, poorly soluble materials with low dissolution velocities, i.e., hydrophobic formulations, as well as material with a high affinity

for mucus protein binding will be subjected to clearance, preventing absorption [53, 82]. The clearance mechanisms vary based on pulmonary location and are beyond the scope of this chapter but are discussed in Chaps. 2 and 6. Drugs that do enter solution can cross the epithelial membrane via passive diffusion with the rate controlled by physical properties of the therapeutic entity (i.e., hydrophobicity, molecular weight). Material that is not dissolved, nor cleared from the system, may be physically translocated to the epithelial surface allowing for particle–cell interactions and potential internalization. In either case, the mucus presents a physical barrier. Variations in surface tension created by various disease states as well as particle morphology will determine the extent of submersion into the liquid lining layer [26]. Thus, surface and formulation modifications that increase dissolution (i.e., cyclodextrin complexation) or translocation (i.e., particle size control, surface wetting agent) may be extremely beneficial in enhancing therapeutic efficiencies of inhaled particles [72].

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