Chapter 2

Muco-ciliary clearance: A review of modelling techniques

Submitted to:

Journal of Biomechanics

Authors and affiliations:

Shayan M. Vanaki[a], David Holmes[a], Suvash Saha[b], Jinju Chen[c], Richard Brown[a], Pahala Gedara Jayathilake[c]

[a]School of Chemistry, Physics and Mechanical Engineering, Queensland University of Technology (QUT), Brisbane 4000 Queensland, Australia

[b]School of Mechanical and Mechatronic Engineering, University of Technology Sydney, Sydney, New South Wales 2000 Australia

[c]School of Engineering, Newcastle University, Newcastle upon Tyne, United Kingdom

Statement of contribution of co-authors for thesis by published paper

The authors listed above have certified that:

1. they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation of (at least) that part of the publication that lies

within their field of expertise;

2. they take public responsibility for their part of the publication, while the responsible author accepts overall responsibility for the publication;
3. there are no other authors of the publication;
4. potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
5. consistent with any limitations set by publisher requirements, they agree to the use of the publication in the students thesis, and its publication on the QUT ePrints database.

The authors specific contributions are detailed below:

Contributor	**Statement of contribution**
Shayan M. Vanaki	Conceived the study, conducted the literature research and drafted the manuscript
David Holmes	Supervised, critically reviewed and revised the manuscript
Suvash Saha	Reviewed the manuscript
Jinju Chen	Reviewed and revised the manuscript
Richard Brown	Supervised, reviewed and revised the manuscript
Pahala Gedara Jayathilake	Supervised, critically reviewed and revised the manuscript

Principal Supervisor Confirmation

I have sighted emails or other correspondence from all co-authors confirming their certifying authorship.

David Holmes

Name	**Signature**	**Date**

Abstract

The airways of the human respiratory system are covered by a protective layer, which is known as airway surface liquid (ASL). This layer consists of two relatively distinct sub-layers; a mucus layer (ML), and a periciliary liquid layer (PCL). In addition, the airways are lined with a dense mat of hair-like structures, called cilia, which beat back and forth in a co-ordinated manner and mainly propel the mucus layer. Such interaction between the cilia and mucus is called muco-ciliary clearance (MCC) which is essential to clear the respiratory airways from the inhaled toxic particles deposited on the mucus. The complex nature of lung clearance mechanisms limit the ability to conduct experiments to investigate micro-scale physiological phenomena. As such, modelling techniques are commonly implemented to investigate the effects of biological parameters on the lung muco-ciliary clearance.

In the present work, modelling techniques of cilia-ASL interactions – including continuum cilia modelling and discrete cilia modelling – are reviewed and the numerical procedures and level of complexity related to each technique are explained. This is followed by a detailed analysis of the airway surface liquid modelling approaches. In addition, findings of numerical investigations related to the effects of various parameters such as ciliary beat frequency (CBF), mucus rheology, metachronal waves of cilia, surface tension at the PCL-mucus interface, ciliary length, ciliary density, and airway surface liquid depth on the bronchial and tracheal ASL transport are reviewed. This review also explains how these biological parameters can alter the internal power required to perform ciliary beating. Lastly, the main limitations of current numerical works are discussed and significant research directions are brought forward that may be considered in future models to better understand this complex human biological system and its vital clearance mechanism.

2.1 Introduction

Mucus within the respiratory airways plays a vital protective role as inhaled aerosols (e.g. dust, particulate matter, bacteria, etc.) are trapped on this highly viscous layer and ultimately discharged from the airways. The mucus layer sits above a second

periciliary liquid layer (PCL) together referred to as the airway surface liquid (ASL) (Thiriet, 2012), as seen in Figure 2.1. Mucus is a complex biological fluid that consists of hydrogel-forming glycoproteins (mucins), proteins, DNA, lipids, ions, cells and cellular debris in a watery matrix which is about 98% (Carlstedt and Sheehan, 1989, Thornton and Sheehan, 2004). From the rheological point of view, mucus is referred to as a non-homogenous, non-Newtonian (shear-thinning), viscoelastic fluid (Puchelle et al., 1987). More detailed information on the biochemistry of mucus is available in Thornton and Sheehan (2004). On the other hand, the PCL is usually treated as a watery lubricating (nearly Newtonian) fluid layer with much less viscosity. The transport of the mucus blanket (with trapped foreign particles) from the airway is driven by cilia actions (Figure 2.1).During the effective (forward) stroke, cilia rise up and beat faster, while during the recovery (reverse) stroke they bend downward and move closer to the ciliated cell surface (Sanderson and Sleigh, 1981), leading to an effective forward mobility of the mucus layer (ML). Such cilia-driven mucus locomotion is called 'muco-ciliary clearance' (MCC), which is recognised as the principal clearance mechanism in the lung compared to other clearance mechanisms such as coughing, penetration into the epithelium, or phagocytosis (Albert and Arnett, 1955, Albert et al., 1969, 1973). The net transport of viscous fluid is created as a result of two key mechanisms: (1) asymmetric cilia beat cycle (Purcell, 1977); and (2) out-of-phase beating of cilia, known as 'metachronism' (Blake, 1972).

Developing a clear theoretical understanding of epithelial cilia-driven fluid flow is critical to individually evaluating the impacts of physiological and pathophysiological factors which have influence on muco-ciliary clearance and are commonly associated with respiratory diseases (Del Donno et al., 2000). Numerical modelling has been used widely as a key tool to achieve this. The previous review on theoretical modelling of MCC can be found in Smith et al. (2008b). To update significant developments that have occurred in the decade since, we review recent advances in modelling the cilia-driven airway surface fluid flow in the human lung. Also, a summary of findings on the effects of different biological parameters on the mucus transport during the MCC process is provided.

In the present review, we focus on 'cilia sublayer' models in which both the PCL and ML transport are considered, because the flow in both layers is of interest. As

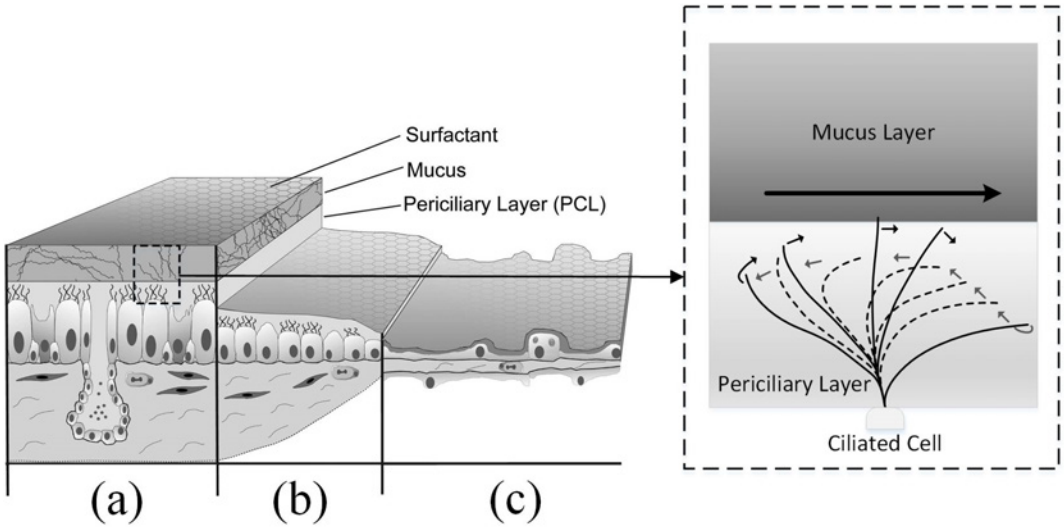

Figure 2.1: Left panel: morphology of the epithelial tissue in the (a) upper lungs (trachea and bronchus), (b) central lungs (bronchioles), and (c) deeper lungs (alveolus). Taken from Kirch et al. (2012). There are almost 200-300 cilia on each mature ciliated cell, which are from 0.2-0.3 μm in diameter and these decrease from 6-7 μm in the upper airways to 4 μm in the smaller airways (Chang et al., 2008). Right panel: diagram of ciliary beat cycle. Cilia are beating in the range of 815 Hz (Knowles and Boucher, 2002) in a periodic cycle including rest, recovery and effective (Sanderson and Sleigh, 1981).

discussed by Fulford and Blake (1986) and Smith et al. (2008b), there are two distinct types of sublayer model: (1) Continuum cilia also known as the 'traction layer' model, which is a phenomenological model representing the cilia with a spatially continuous volume force distribution, changing in time as the cilia beat; and (2) Discrete cilia modelling the cilia as discrete objects within the simulation. In addition, the muco-ciliary clearance modelling investigations under diseased ASL, as well as ciliary abnormalities, are reviewed.

This paper is organised as follows. In Section2.2, muco-ciliary models of different levels of complexity are discussed in detail, by primarily focusing on the interaction between cilia and fluid flow. Section2.3 provides detailed analyses on the various airway surface liquid modelling techniques. In Section2.4, a thorough parametric investigation is carried out to describe the effects of various parameters on the mucus transport. Section2.5 explains how the internal power required to perform ciliary beat can be altered by different biological parameters. A summary of the present review and the future directions conclude this paper in Section2.6.

2.2 Muco-ciliary modelling techniques

2.2.1 Continuum cilia modelling

In the continuum cilia modelling technique, the effect of the ciliary carpet on the fluid is represented by a spatially continuous volume force. The earliest simplified continuum mathematical model of mucous flow caused by ciliary motion dates back to the work of Barton and Raynor (1967), considering the cilium as a rigid rod that is shorter during the reverse stroke than during the forward stroke. They used simplified 'resistance/drag coefficients' to approximate the effect of ciliary tips on the surrounding fluid which led to a an analytical continuum formulation to obtain the velocity field. Somewhat realistic flow rates were achieved while the PCL as well as the metachronal wave were not included in their model (Blake, 1973). Blake (1977) introduced a more appropriate model for ciliary propulsion which is known as 'active porous medium' model. Given that cilia are densely packed on the epithelial surfaces, the fluid motion can be considered as occurring through a porous medium, where the cilia are treated as active elements (solid phase) that exert propulsive forces on the fluid phase. By using the average velocity of the cilia over a beat cycle, a simplified formulation was obtained for the calculation of velocity profiles in a dense array of cilia.

Blake and Winet (1980a) and Smith et al. (2007b) were the first researchers who used the traction layer approach to model the resistive force of the cilia in both the PCL and ML. The impact of a dense mat of cilia on the periciliary flow was modelled as an active porous medium, similar to model of Blake (1977). They solved the governing equations of motion for the velocity and pressure fields due to a point force using the Stokeslet method, which is based on the solution of Stokes' flow.

Recently, Kurbatova et al. (2015) proposed a new physiologically based mathematical model to estimate the muco-ciliary transport in different generations of the lung. Their model was partially similar to that of Smith et al. (2007b) with identical resistance coefficient formulation, with the addition of taking into account mucus influx from previous generations, as well as the production of mucus by submucosal glands and goblet cells. To simulate the ASL flow, an analytical formulation

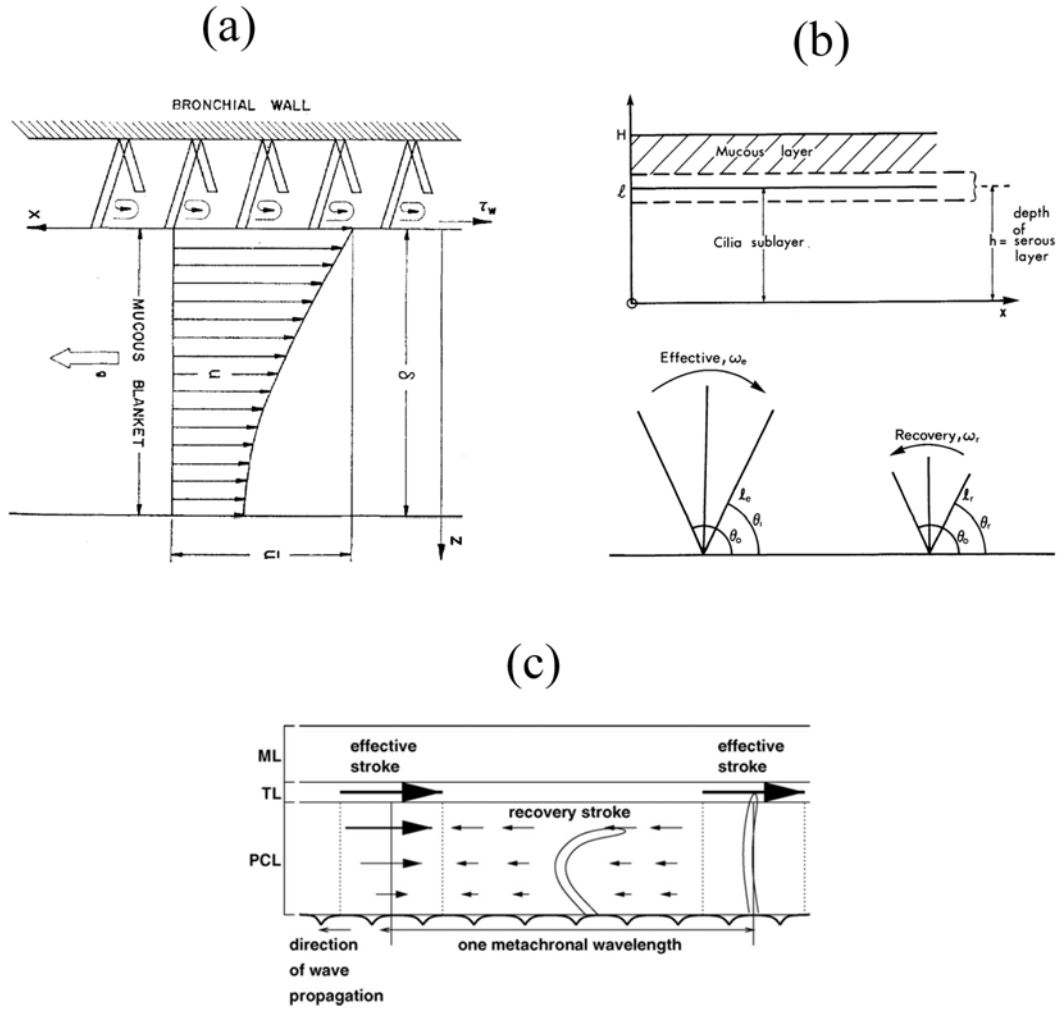

Figure 2.2: Continuum cilia modelling schematics of (a) Barton and Raynor (1967), (b) Blake and Winet (1980a), and (c) Smith et al. (2007b).

was obtained through a thin-layer approximation which was solved by using a finite-difference method. The aforementioned studies neglect variations of porosity along the metachronal wave, thus violating the conservation of mass in the cilia layer. To overcome this shortcoming, Hussong et al. (2011) accounted for spatial variations in the porosity along the metachronal wave in their continuum model, solving the volume-averaged Navier–Stokes (VANS) equations. They also presented their results using discrete cilia modelling (reviewed in the following section). Despite the robustness of their numerical continuum model for artificial cilia-driven fluid flows (see Figure 2.3), it is worthy of a detailed quantitative analyses to ensure the suitability of their continuum model for the muco-ciliary flow where the $Re << 1$ (Fulford and Blake, 1986).

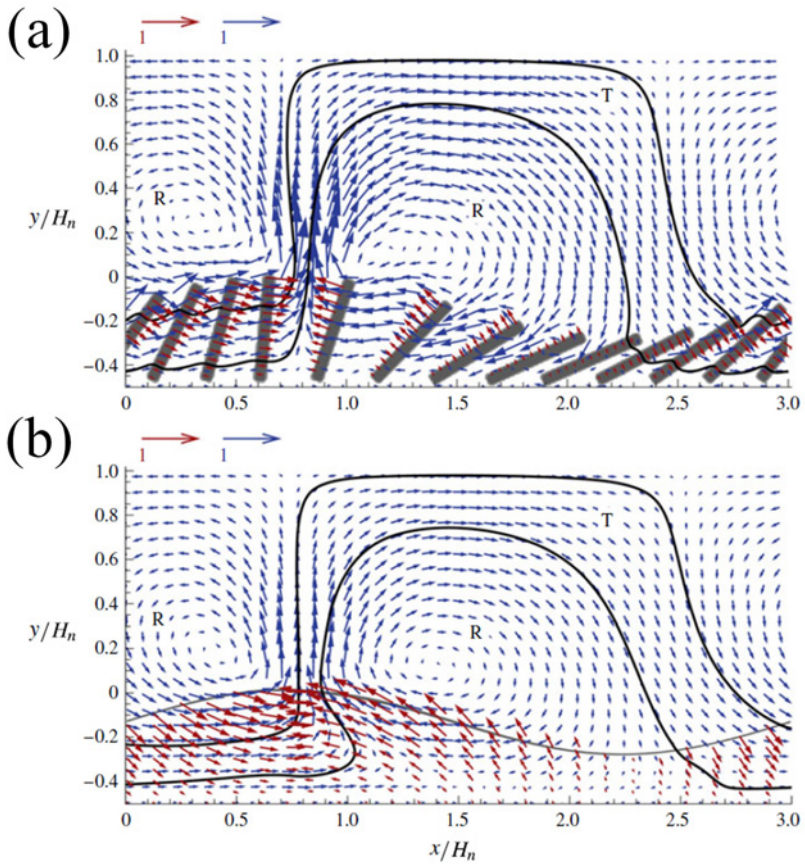

Figure 2.3: Qualitative comparison of (a) the discrete cilia modelling and (b) continuum cilia modelling in Hussong et al. (2011).

2.2.2 Discrete cilia modelling

Discrete cilia modelling approach models the actual beating cilia as the driving mechanism for fluid flow transport. The two major subdivisions of discrete cilia models are: (1) prescribed ciliary beating (PCB); and (2) fluid structure interaction (FSI) models. In the first, the predefined cilia motion is input to the model and this cilium motion drives the fluid. In the second, the ciliary motion emerges from the coupling of the internal cilium mechanics, the cilium elasticity and the external forces from the viscous fluid. In the present work, we also refer to these categories as 'one-way coupling' and 'two-way coupling' techniques, respectively.

The 'slender body theory' , developed by Hancock (1953) for Stokes flow, is known as the basis of discrete cilia modelling. In this theory, each cilium is divided into a number of elements (Figure 2.4a) and the force exerted by cilia is obtained along the cilias centreline. The immersed boundary methods (IBMs), first introduced by

Peskin (1972), have been one of the most popular techniques to couple the interaction between cilia and fluid. The IBMs have been developed over the years and successfully applied to many one- and two-way cilia models by researchers (Chateau et al., 2018, 2017, Dillon and Fauci, 2000, Dillon et al., 2007, Guo and Kanso, 2017, Jayathilake et al., 2015, 2012b, Lukens et al., 2010, Quek et al., 2018, Sedaghat et al., 2016). The popularity of these methods arises from to their ability to handle moving or deforming bodies (Mittal and Iaccarino, 2005). In contrast to body conformal mesh, the IBMs can significantly reduce the computational time of simulations as the fluid grids are not required to conform to the body geometry due to enforcing the no-slip boundary condition at the Lagrangian points by adding appropriate boundary forces. This feature makes the IBM an efficient method for the investigation of cilia-induced fluid flows.

2.2.2.1 Prescribed ciliary beating modelling

For two decades the model of Fulford and Blake (1986) has been the only PCB model in which discrete cilia operate within both layers of PCL and mucus. Exploiting the Stokeslet method, their model was limited, as it only considered the interaction of cilia with the time-averaged velocity field (Smith et al., 2008b). In addition to this, it is necessary to point out that although the Reynolds number of ASL is small enough to justify using Stokes flow equations, the Stokeslet method is suitable for constant viscosity fluids, not complex ones undergoing non-linearities as in ASL (Chatelin and Poncet, 2016).

Two-dimensional one-way coupled implicit-forcing IBMs were blended with the LBM (Sedaghat et al., 2016) and finite-difference method (FDM) (Guo and Kanso, 2017, Jayathilake et al., 2015, Lee et al., 2011) to simulate the two-layer liquid film lining in the pulmonary airways. In the case of IB-FDM, the the governing equations were solved using the fractional step/projection algorithm (Chorin, 1968, 1969) where the convection and the diffusion terms were treated by the Adams-Bashforth and the Crank-Nicholson methods.

Due to the creepy flow nature of muco-ciliary transport, the use of N–S equation together with the projection method may produce numerical errors as detailed in Guermond et al. (2006). This problem was overcome by the so-called ‘hybrid’ method

developed by Chatelin and Poncet (2013). In particular, it was a 3D hybrid numerical approach, mixing a novel efficient Eulerian approach for the elliptic Stokes equations and a particle method for the transport equation, and the interplay between fluid and ciliary motion was taken into account by means of a penalisation method (Angot et al., 1999). Contrary to the Stokeslet method, the mentioned hybrid method could be efficiently employed for creeping flows with variable viscosity. This method was later used in Chatelin and Poncet (2016) to simulate non-homogeneous mucus, and further extended in Chatelin et al. (2017) to study cystic fibrosis shear-thinning mucus.

Other detailed 3D modelling of cilium arrays immersed in the two-phase ASL was presented three-dimensionally by Chateau et al. (2017) and Chateau et al. (2018). They produced a 2D ciliary beat pattern (inspired from the work of Chatelin and Poncet (2013)) by using a transport equation and its associated boundary conditions, and used the explicit IB-LBM presented in Li et al. (2016) to model the fluid-cilia interaction. Unlike the previous PCB models in which only the immersed boundary forces are applied onto the fluid, the model of Chateau et al. (2017) took into account the feedback of the fluids onto the cilia as well. In particular, as seen in Figure 2.4b, the forces imposed on the mucus layer and the force imposed on the PCL are both projected on the cilium velocity vector at every Lagrangian point along each individual cilium. The obtained total projected force in the opposite direction is then treated as a torque with respect to the base point of the cilium. Based on this, the velocity of each cilium is tuned. Very recently, Quek et al. (2018) developed a 3D numerical model that was able to spontaneously create metachronal waves in an array of cilia beating by representing each cilium as a rod-and-spring network, as shown in Figure 2.4c. Linear elastic springs between every consecutive node, and torsion springs at these nodes provides resistance to stretching and bending, respectively. In the model, the active forces of cilia during the forward and reverse strokes were imposed as triangular loads on each cilium along with the switch mechanism to change the direction of the forces. This simplified ciliary dynamics model could study the effect of metachronal waves, avoiding non-linear, complex models to capture the internal mechanisms of cilia which are often computationally challenging and expensive to perform.

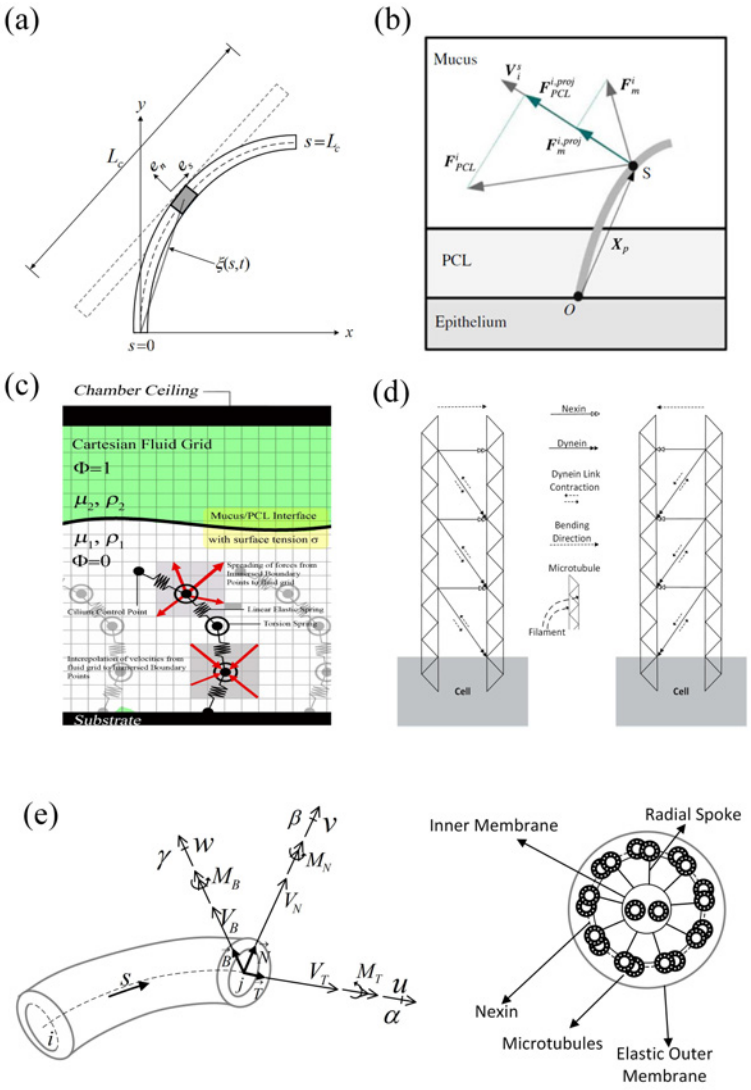

Figure 2.4: Discrete cilia schematic models. (a) Gray and Hancock resistive force approximation model. Redrawn from Smith et al. (2008b). (b) Fluid-cilia interaction model of Chateau et al. (2018, 2017). (c) The IB projection model of Quek et al. (2018). (d) Ciliary axoneme model implemented in FSI investigations. The bending occurs as a result of the contraction of the dynein links which leads to local sliding between the microtubules. Redrawn from Yang et al. (2008) (e) The model of Mitran (2007) (Redrawn). Left panel: curved beam element for microtubules. Right panel:transverse section of a cilium with the '9+2' microtubule structure (also known as axoneme): a ring of nine outer microtubule doublets and two central microtubule singlets. The adjacent microtubule doublets slide over one another due to the forces exerted by approximately 4000 active dynein molecules, leading to an overall bending of the cilia that drives the adjacent fluid. The connecting nexin as well as radial spoke links, were modelled by elastic springs, while the dynein molecular motors that are responsible for the bending of a cilium were assumed as forces which were tuned to obtain cilia metachronal wave patterns during the clearance simulations.

2.2.2.2 FSI modelling

Only a few studies (Dillon and Fauci, 2000, Dillon et al., 2007, Lukens et al., 2010, Mitran, 2007) have investigated the FSI simulation of the muco-ciliary system because it is numerically more complex than PCB modelling. The FSI coupling is made to evaluate the response of fluid to cilia motion and vice versa interaction. Section 2.5 will explain whether it is necessary to carry out a complex two-way coupling for muco-ciliary simulations.

Early attempts to model cilia internal mechanism can be found in the works of Gueron and Levit-Gurevich (1998), Gueron et al. (1997) that accounted for the viscous interactions between cilia and the fluid in which they are immersed and the boundary effects. Later, a more detailed numerical algorithm for the coupled fluid-cilia system was presented in the work of Dillon and Fauci (2000), incorporating discrete representations of dynein arms, the passive elastic structure of the axoneme including the doublets, and nexin links; see the schematic model of ciliary axoneme (Yang et al., 2008) in Figure 2.4d. A simple curvature control mechanism, suggested by Brokaw (1972) for flagella and cilia, was used. In this numerical algorithm, at each time step, the following detailed geometric information is obtained: the curvature of the microtubules, the stretching of nexin links, and the amount of shear or sliding between the microtubules. Having this information would lead to determining and controlling activation and connectivity properties of discrete dyneins. In the end, the force densities along microtubules of each cilium are spread to the fluid domain and the N–S equations are solved to obtain the velocity field

Implementing the same two-way coupled algorithm, Dillon et al. (2007) extended their previous work by including a mucus layer which was embedded by linear elastic elements in a Newtonian fluid. With the aid of the IBM, the flow around three elastic cilia was simulated using the finite-difference scheme. This study was further extended by Lukens et al. (2010) to investigate transport and mixing features near a cilium. To achieve this, the Eulerian velocity field obtained around a single cilium was used to compute finite-time Lyapunov exponent fields, whose maximal ridges identify Lagrangian coherent structures (LCSs) (Haller, 2001). Here, it is important to mention the ‘transcytosis’ , which is a type of transcellular transport, must be

implemented into MCC models if one is interested to study the mixing phenomena within the airway surface liquid layer. Instead of the IBM, Mitran (2007) employed an overlapping fixed-moving grid formulation to examine the ciliary movement and its effect on a Newtonian PCL and a viscoelastic ML. Each cilium was considered to have a '9+2 internal microtubule' structure, which was modelled three-dimensionally using large-deflection, curved, finite-element thin-walled beams, as seen in Figure 2.4e. This research would be the most complex model examined in the literature. Very recently, Stein and Shelley (2019) developed a more simplified FSI method using the Brinkman-Elastica model (derived from local-slender body theory), where the communication between Eulerian and Lagrangian frames was facilitated through a coarse-graining continuum model for elastic boundaries oscillating in a viscous Newtonian fluid. This allows for the efficient simulation of a dense matt of cilia, as run times increase slowly with ciliary density.

In the studies reviewed in this section, no model for how energy is used up by the dyneins has been included. Indeed, it is still unclear what energy source produces such beating or how dynein activation kinetics (the dynein motor) functions. This major unknown makes development of a full cilia-fluid coupled model challenging.

From the computational point of view, herein we provide a general insight into the computational expense of muco-ciliary clearance models. As described above, in the two-way coupling algorithm, detailed geometric information is obtained at every time step of the computations. This obviously requires huge computational resources in order to execute simulations compared to the PCB models. The volume force type model is computationally cheaper compared to the discrete models. This is due to the volume force formulation of the continuum method, while the discrete methods require the individual force assessment of each cilium. A more computationally efficient discrete force method was introduced in Chatelin and Poncet (2013), Chatelin et al. (2015), and Chatelin and Poncet (2016) – i.e. a combination of Stokes-transport coupling and penalisation method. It was found that the overall computational cost of this method is quasi-linear with respect to the number of discretisation nodes, without being dependent on number of cilia. These features make the introduced numerical algorithm a suitable choice for fast computations and for parametric investigations.

2.3 Airway surface liquid modelling

2.3.1 Viscoelasticity and shear-thinning modelling of mucus

Viscoelastic fluids are characterized based on the elastic (or storage) modulus G' and viscous (or loss) modulus G''. These parameters are obtained by performing small-amplitude oscillatory shear (SAOS) and large amplitude oscillatory shear (LAOS) tests for viscoelastic fluids within linear and non-linear viscoelastic regions, respectively. The obtained moduli can be used to find the relaxation time which is required as an input to viscoelastic models. Such experimental data can be found in Lutz et al. (1973), Gerber et al. (2000), Dawson et al. (2003), and Lai et al. (2009).

The viscoelastic properties of airway mucus have been taken into account in a limited number of numerical studies. In these works, the viscoelastic mucus has been described by: (1) linear models – including 'Maxwell' model (Ross, 1971, Smith et al., 2007b) and 'Jeffrey' model (Dillon et al., 2007, Lukens et al., 2010) ; and (2) non-linear models – including 'upper convected Maxwell (UCM)' model (Mitran, 2007) and 'Oldroyd-B' model (Guo and Kanso, 2017, Sedaghat et al., 2016).

In the linear models, the viscoelastic behaviour of fluid is simply modelled by a linear combination of mechanical elements like Hookean springs and Newtonian dashpots, representing elastic and viscous components (Deville and Gatski, 2012) respectively. Such models are applicable only for small deformations and linear materials (Cherizol et al., 2015). However, mucus is a complex heterogeneous biofluid in nature that demonstrates non-linear viscoelastic responses (Vasquez et al., 2014). For this reason, Guo and Kanso (2017) and Sedaghat et al. (2016) employed the Oldroyd-B model (Larson, 1999) which is suitable for polymeric solutions like mucus, where the viscoelastic stress tensor is decomposed into two parts; Newtonian solvent and polymeric elastic solute. The non-linear UCM model employed by Mitran (2007) is a differential generalization of the Maxwell model and more tractable from a numerical standpoint as it contains one parameter less (Deville and Gatski, 2012). The constitutive stress-strain relations of the introduced viscoelastic models are solved explicitly for the viscoelastic stress tensor which is then added as a source term into the momentum equation to update the velocity field. This potentially does not require

to modify the algorithms mentioned for PCB and FSI models. Generally, non-linear models are computationally more demanding compared to the linear and Newtonian fluids, as it contains an upper-convected time derivative.

Only a limited number of studies have modelled the shear-thinnnig rheology of the mucus. Craster and Matar (2000) used a bilayer system of immiscible Herschel–Bulkley materials (Herschel and Bulkley, 1923) and studied the effect of yield stress and shear thinning of mucus on surfactant spreading. Chatelin et al. (2017) carried out experiments on the samples of cystic fibrosis and bronchiectasis mucus to measure the shear-thinning rheological indices (i.e. flow consistency index k and power law index n). The rheological indices obtained were then used in the non-linear Carreau model (Carreau et al., 1979) to simulate the non-Newtonian behaviour of the mucus. Such rheological parameters can be found in Dawson et al. (2003), Serisier et al. (2009), and Tomaiuolo et al. (2014) for cystic fibrosis mucus and in Jeanneret-Grosjean et al. (1988), Zayas et al. (1990), and Serisier et al. (2009) for healthy mucus. Implementing the non-Newtonian (shear-thinning) models can result in an unstable solution of the Navier–Stokes equations. To avoid this, one would need to use smaller time steps, thus increasing the computational cost. To allow a different, smaller time step size, an explicit implementation of the constitutive equations would be preferred. Except for the stress tensor in the momentum equation, no other modification of the FSI algorithms is required to model a non-Newtonian mucus layer.

Although the mucus rheology has been modelled by various numerical formulations, an important question remains: to what extent a more complex fluid modelling can affect the accuracy of muco-ciliary results? This suggests the need of a comparative study on the effects of linear/non-linear viscoelasticity models as well as non-Newtonian models as compared with the simplest fluid rheology. This would help to select an optimum fluid modelling for the mucus layer, thus avoiding the unnecessary increase of the computational expenses of MCC models.

2.3.2 PCL modelling

Majority of numerical muco-ciliary studies in the literature have modelled the PCL as a Newtonian fluid with a constant dynamic viscosity similar to that of water, which

facilitates ciliary beating. However, it is argued in Boucher (2007) that the periciliary environment can be characterized as a 'grafted, polyanionic gel layer' (Randell and Boucher, 2006). To take into account the influence of such nonhomogeneity due to the existence of mucins in the PCL, Chatelin and Poncet (2016) defined the viscosity of airway lining fluid such that it varies (from PCL-epithelium interface to upper ML) as a function of mucins' ratio in the fluid.

2.3.3 PCL-mucus interface modelling

The electron micrographs from the experimental work of Matsui et al. (1998a) on the airway epithelial cell cultures show that cilia interact with the inner surface of the mucus blanket, revealing the existence of the PCL-mucus interface. Some authors (Blake and Winet, 1980a, Fulford and Blake, 1986, Guo and Kanso, 2017, Mitran, 2007, Smith et al., 2007b) have assumed a flat fluid-fluid interface between the PCL and mucus layer in their mathematical models. In these models, the boundary condition at the interface can be simply applied by imposing a continuous fluid velocity and stress across the interface. This assumption can be supported by the scanning electron-microscope (SEM) data of Sanderson and Sleigh (1981) obtained from cultured rabbit tracheal epithelium. To date, the structure of PCL-mucus interface is not well understood and measurements of the surface tension would be highly valuable.

Two-dimensional simulations such as Lee et al. (2011), Jayathilake et al. (2015) and Sedaghat et al. (2016) added the effect of the surface tension at the interface by computing the boundary force generated due to an 'imaginary elastic membrane' , where the elastic property of the flexible interface boundary was described by assuming a stiffness constant. In the 3D work of Quek et al. (2018), the level-set method (Osher and Fedkiw, 2006, Sethian, 1999) was used to solve the location of the fluid-fluid interface for two immiscible layers of Newtonian fluids.

On the other hand, the surface tension effects emerged intrinsically at the interface in the 3D models of Chateau et al. (2017) and Chateau et al. (2018) where the lattice Boltzmann model of Porter et al. (2012) was employed to model the two-phase ASL flow. Employing this model allowed: the minimisation of spurious currents near the PCL-mucus interface; the consideration of high viscosity ratios between the PCL and

mucus; a sharp interface at any time during the simulations; and keeping the two fluids separated during the penetration of cilia into the ML.

The experimental work of Button et al. (2012) shows that the ciliary beating facilitates the separation of PCL and ML, as it does not allow the mucin polymer chains to return into the PCL. The reason of having the mucus layer much more viscous compared to the PCL is essentially due to the presence of mucins (Button et al., 2012, Ehre et al., 2014). The transport of such macromolecules (i.e. transcytosis) in ASL flow has not been incorporated into MCC models so far, which may in future be developed. Indeed, it might be inferred that there is not a discrete interface between both PCL and mucus. Therefore, instead of a complex double-decker fluid with constant properties at each layer and the sharp PCL-mucus transition, Chatelin and Poncet (2013), Chatelin et al. (2015) and Chatelin and Poncet (2016) modelled the ASL as a continuum fluid, but varied fluid viscosity, as described in Section2.3.2. In these works, the order of magnitude obtained for the mean mucus velocity was the same as other published works in the literature. This suggests that MCC could be modelled using a less complex numerical approach when there is no experimental data available on the physical properties of the interface.

2.4 Parametric investigations of muco-ciliary clearance

2.4.1 Ciliary beat frequency

CBF has the largest effect on the mucus velocity. For example, Lee et al. (2011) and Sedaghat et al. (2016) reported an increase of nearly 700% and 400% by changing the ciliary frequency from $20\ rad/s$ to $140\ rad/s$, respectively. Also, Smith et al. (2007b) reported that decreasing the rate of CBF from $60 - 10\ rad/s$ can reduce the mucus transport by 83%. All these models found a linear relationship between the ML velocity and CBF. This is to be expected due to the negligible inertia forces (very low Reynolds number).

2.4.2 Mucus rheology

2.4.2.1 Viscosity

Sedaghat et al. (2016) did not observe any significant changes in the mean velocity of mucus when they changed the viscosity in the range of $0.002 - 0.2\ Pa.s$, whereas Lee et al. (2011) found that if the viscosity increases by two orders of magnitude ($10^{-1} - 10^{1}$), the mean velocity of mucus can reduce by approximately 40%. For a similar order of magnitude range of viscosity, Chatelin and Poncet (2013) and Chatelin and Poncet (2016) observed a reduction of $13 - 15\%$ in the surface mean velocity of the mucus layer when they used a linear profile for the viscosity variations across the ASL. Nonetheless, the results obtained from the mathematical model of Kurbatova et al. (2015) showed a very significant impact of highly viscous cystic fibrosis mucus; a five-fold increase in the viscosity resulted in the same amount of reduction in the tracheal mean velocity. While all of the above-mentioned studies noticed a decreasing trend with increased of viscosity, Smith et al. (2007b) predicted an increase in the mucus transport (i.e. 2%) for doubled mucus viscosity.

2.4.2.2 Viscoelasticity and non-Newtonian behaviour

The role of mucus viscoelasticity could not be elucidated by the model of Smith et al. (2007b) as both increasing and decreasing the relaxation time resulted in an increase in the ML transport. A quantitative investigation by Sedaghat et al. (2016) showed that the mean mucus velocity drops by 10 times the standard value for a viscoelastic mucus when compared with a fully Newtonian mucus. Nonetheless, when Guo and Kanso (2017) emulated diseased environments in their simulations by varying the viscoelastic properties and the thickness of the ML, it was revealed that the mucus elasticity depends largely on the ML thickness. That is to say, in diseased states (thinner PCL), the higher elasticity led to a decrease in the mucus transport, whereas in healthy states, the mean flow rate increased slightly with increasing the elastic part of mucus.

To asses the impact of shear-thinning mucus on the mucus transport, Chatelin et al.

(2017) computed the mucus velocity using a range of reported rheological indices obtained for both healthy and cystic fibrosis mucus. The lower indices, which correspond to the cystic fibrosis mucus, led to almost 28% loss of the mucus velocity compared to the healthy state, indicating the significant effect of the non-Newtonian rheology of mucus.

2.4.3 Metachronism

The experimental observations by Sanderson and Sleigh (1981) showed that there is a small phase lag between any successive cilia and they beat in such a way that the cilia tips form a moving surface waves which is known as 'metachronal' waves (MCWs). Depending on the direction in which the waves travel, there are two types of MCWs: (1) symplectic; the wave travels in the same direction as the flow with a phase lag of $-\pi < \Delta\phi < 0$ between two cilia; and (2) antiplectic; the wave travels in the opposite direction to the flow with a phase lag of $0 < \Delta\phi < \pi$. To understand which type of MCWs are more efficient for mucus transport, Chateau et al. (2017) and Chateau et al. (2018) investigated the effect of various wave types on MCC. The metachronal motion induced a stronger fluid displacement compared to the synchronized ciliary beat ($\Delta\phi = 0$). Particularly, the maximum displacement was found for the antiplectic MCW with a phase lag as small as $\Delta\phi \approx \pi/4$ (i.e. large wave length), showing nearly 700% and 480% increase in the displaced flow volume at $Re = 0.02$ and $Re = 0.01$, respectively. It is worth mentioning that, for smaller inter-ciliary distances (higher ciliary density), the synchronized motion could outperform the symplectic MCW with very small phase lags. Other numerical studies (Ding et al., 2014, Jayathilake et al., 2012b) predicted a better transport as well for the antipleptic MCW when they examined the fluid transport (in the absence of mucus) induced by ciliary carpets.

2.4.4 Surface tension at the PCL-mucus interface

Recent 3D modelling of Quek et al. (2018) suggests that a parameter like the surface tension σ at the PCL-mucus interface can affect mucus velocity and hence clearance. Assuming $0 \leq \sigma \leq 0.01\ Nm^{-1}$, their results indicated that the averaged fluid velocity is increased when surface tension is present at the interface, as shown in Figure

2.5a. This enhanced mucus transport was due to the suppression of the fluid velocity component perpendicular to the interface, which prevents vortices from forming in the overlying layer. Two-dimensional models of Lee et al. (2011) and Sedaghat et al. (2016), on the contrary, predicted insignificant changes in the mean mucus velocity when they varied the surface tension from $1\times10^{-7}-10\ Nm^{-1}$. As mentioned earlier, it seems that no experimental surface tension data have been reported yet for PCL-mucus interface. Thus all the selected values for σ have been assumed. Notwithstanding the contradictory results for the mucus mean velocity, all the studies presented in this section reported a flat interface in the presence of surface tension which has been observed experimentally (Sanderson and Sleigh, 1981).

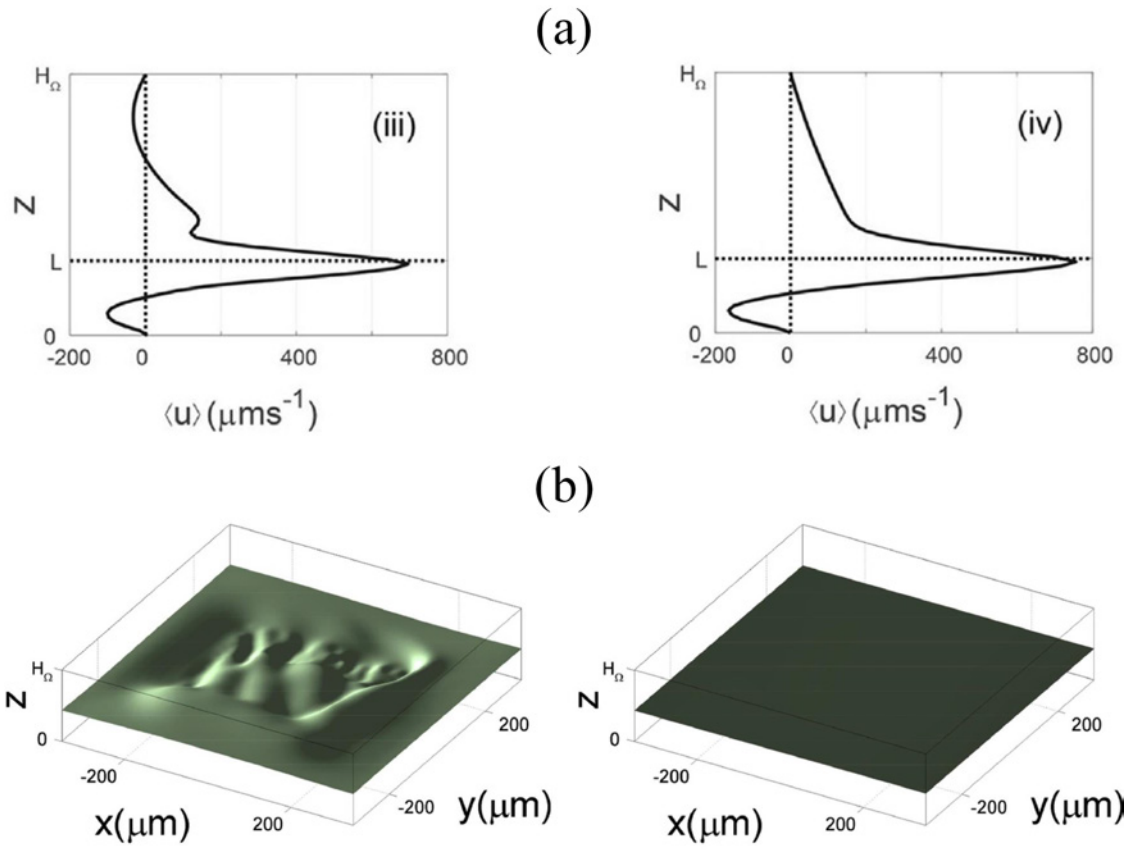

Figure 2.5: Example of the 3D MCC simulation results of Quek et al. (2018). (a) The fluid interface deformation and (b) velocity profiles in cases with (right) and without (left) surface tension.

2.4.5 Ciliary length, ciliary density, and ASL thickness

The effect of ciliary length on the MCC was first investigated in the work of Fulford and Blake (1986). Two cases were compared against each other: with and without cilia penetration in the ML. They observed that the case where cilia peneterated into the ML can enhance the mucus transport, but the extent of this enhancement was not reported. The 2D simulation of Jayathilake et al. (2015) and 3D simulation of Chatelin and Poncet (2016) shed light on the relation of muco-ciliary transport and the length of cilia. Both studies found that a slight increase in the length (i.e. slight penetration) can maximize the mean mucus velocity. Comparing the results within the same range of normalized ciliary length, Jayathilake et al. (2015) showed that the mucus transport rate can grow up to 700%, while Chatelin and Poncet (2016) reported a 135% increase for the mucus transport during the penetration as compared with the the smallest ciliary length. One of the reasons of such discrepancy can be attributed to 3D effects. That is to say, in the latter study, the transport of fluid is not only limited to two dimensions, given that the cilia beat three-dimensionally. However, in a 2D simulation, cilia only experience planar motions which might result in the overestimation of mucus transport. Using different cilia numbers, parameter sets as well as the adopted modelling approach could have been the other possible reasons for such deviation between the results.

A fairly limited number of studies have investigated the dependency of mucus velocity on ciliary density. Given that the reported value for the spacing between cilia is $0.35\ \mu m$ (Teff et al., 2007), further decreasing the spacing between two adjacent cilia can cause spurious numerical effects which needs an accurate implementation of cilia-cilia interaction. Although the model of Fulford and Blake (1986) predicted an independent relationship between the mucus velocity and the number of cilia, both Lee et al. (2011) and Chatelin and Poncet (2013) found a two-fold growth in the mucus layer transport, when they increased the number of cilia 3-fold and 36-fold, respectively. Once again, it should be stressed that a 2D MCC simulation should not be directly compared with a 3D study unless they both have exactly the same numerical settings as well as a similar numerical implementation.

To evaluate the influence of ASL thickness on the mucus transport, individual

assessment of both the mucus layer thickness as well as the PCL thickness should be performed. In the 2D works of Smith et al. (2007a) and Sedaghat et al. (2016), the transport slowed down by only 5% when the ML depth was assumed 50% higher than the standard depth. Almost the same percentage of the mucus transport reduction was obtained in Chatelin and Poncet (2016) even for a 600% deeper mucus than the standard case. Such findings could provide valuable insight into how the human body adapts to a thickening ML at the trachea. Thus, there is no need to have significantly faster ciliary beating in the upper airways to transport a greater volume of mucus. *In vivo* analyses (Tarran et al., 2001) revealed that the PCL depletes in cystic fibrosis. This motivated some authors to investigate the dependency of the mucus layer transport with decreased PCL thickness. Unlike the work of Sedaghat et al. (2016), other studies have observed a decreasing trend in the mucus transport when the PCL thickness becomes smaller. For instance, in the 2D numerical works of Lee et al. (2011) and Guo and Kanso (2017), the shallow PCL resulted in approximately 9 times smaller mucus mean velocity and 5 times smaller ASL mean flow rate, considering Newtonian and viscoelastic mucus, respectively. For a shear-thinning mucus, the three-dimensional MCC model of Chatelin et al. (2017) also reported a reduction in mucus velocity by 7% when the PCL layer was halved. It is important to note that, for beating cilia in non-Newtonian fluids at microscopic scales, the shear rate is radically different for a 2D vs 3D model (Montenegro-Johnson, 2017). Failing to account for this difference can misguide our understanding of non-Newtonian rheological effects on the muco-ciliary transport.

2.5 Energy expenditure by cilia

Powered by the action of dynein arms, the interaction of a set of microtubules within a cilium structure can produce the periodical beat patterns of cilia. Important questions to address are: how much energy is required by cilia to displace the fluid, and what is the effect of biological parameters on the power expanded by cilia? Answering these would help to better understand if a complex and computationally expensive two-way coupling is really required for muco-ciliary modellings.

Gueron and Levit-Gurevich (1999) theoretically calculated the energy expenditure from the observed cilium beat of *Paramecium* during a beat cycle (Sleigh, 1962) and reported the energy expenditure of $\approx 9\times10^{-16}$ Joules during the effective stroke and of $\approx 2 \times 10^{-16}$ Joules during the recovery stroke. However, Sleigh (1962) estimated that the amount of work required to overcome the viscous resistance during the effective action is approximately 10^{-16} Joules. The roughly 5-fold difference between the recovery and effective motions shows the gain in energy expenditure that is achieved through the slower recovery strokes.

Detailed parametric studies on the energy spent by cilia are found in Guo and Kanso (2016), Gueron and Levit-Gurevich (1999), and Dauptain et al. (2008). However, to address the questions raised at the beginning of this section, we shall be concerned with the muco-ciliary studies. Guo and Kanso (2017) studied the effects of viscoelasticity and mucus layer thickness on the mean internal power expenditure in such a way that the beating kinematics is not affected by any of these two parameters. They computed the average power expended by a cilium from the dot product of internal bending moments and the angular velocity vector generated by each cilium using the Kirchhoff model (Eloy and Lauga, 2012, Guo et al., 2014). For the healthy state with a normal PCL thickness, the internal moments required to perform the cilia beating kinematics are independent of the elastic properties of the mucus. Interestingly, stronger internal moments were found for the cilia when they beat in a fluid without the mucus layer. In addition to this, weaker internal powers were achieved for slightly thicker ML. This shows how the elastic property of the mucus can reinforce the reversal in the ciliary motion, thus requiring the cilia to spend less energy to perform their beating. On the other hand, in the diseased state with a very thin PCL layer, the required internal moments increased markedly as a function of the mucus elasticity which can potentially lead to a complete failure in the clearance process if the internal power is not larger than the energy budget of the dynein motors which is the case in lung diseases such as cystic fibrosis and chronic obstructive pulmonary. The phase lag between cilia $\Delta\phi$ was the other parameter whose effect was studied in the works of Chateau et al. (2017) and Chateau et al. (2018). The average power spent by a carpet of cilia was defined as the dot product of cilium velocity (which is modified/tuned as explained in Section2.2.2) and forces imposed on the ASL. The results showed that

$|\Delta\phi| < \pi/2$ encounters less viscous resistance, thus requiring less internal power by cilia. Within the same rang of $\Delta\phi$, smaller interciliary spacing resulted in less powers as well which is similar to what observed in the work of Gueron and Levit-Gurevich (1999). It is important to note that, due to the escape of fluid from the sides of cilia in 3D simulations, the calculated internal power would be more realistic compared to 2D simulations (Dauptain et al., 2008).

Analyses of the energy expenditure by cilia clearly show that it is indeed unnecessary to perform any FSI simulations for the healthy states because the internal energy required to perform the beating would not exceed the energy budget of cilia. Therefore, prescribing the ciliary beat in MCC models could be justified. However, in a mucosal diseased state such as cystic fibrosis, it is likely that the force exerted by the highly viscous mucus layer overcomes the internal driving force of cilia and consequently could deform them (Figure 2.6). Thus, prescribing the motion of cilia using the one-way coupling model would not be very accurate, which makes the two-way coupling technique a more suitable choice.

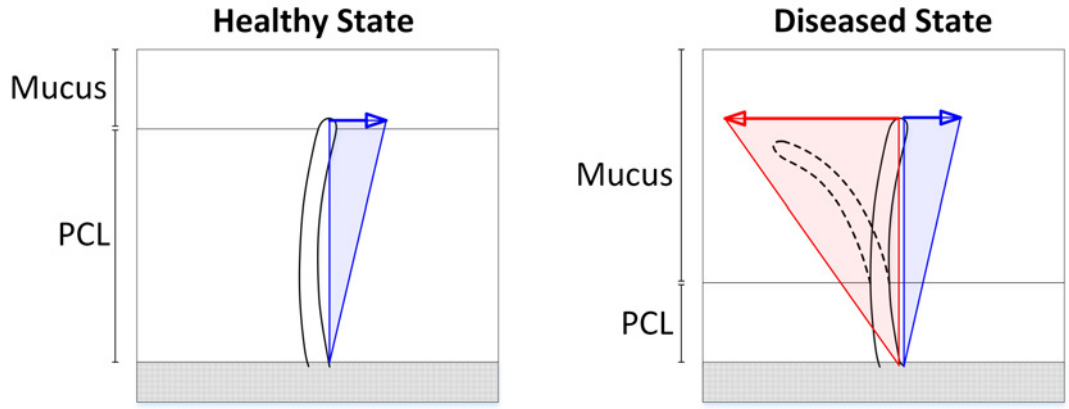

Figure 2.6: Schematic of the interaction forces between the viscous ASL and a cilium in healthy and diseased states. The force exerted by the highly viscous mucus layer (in red) overcomes the internal driving force of the cilium (in blue) and deforms it.

2.6 Summary and outlook

Table 2.1 provides a summary of numerically investigated muco-ciliary studies along with details such as type of model, numerical method, mucus rheology, and investigated biological parameters. Compared with discrete-cilia modelling, a limited number

of studies have used the continuum modelling. This is due to the potential drawbacks of such a model as it does not allow the simulation of actual ciliary beating and the associated boundary forces effect on the fluid flow. Implementing a more complex fluid rheology would also present a significant challenge to such a modelling approach (Smith et al., 2008b). It is evident that the PCB approach has been widely used in the literature by many researchers in recent years. This is obviously due to the lower level of complexity of implementation, as well as the higher speed of calculations of the PCB model when compared to the FSI model. For the FSI models, it is unclear how the dynein motor has been designed, or in other words, how the ciliay beating is produced. This remains an area of modelling that has no experimental benchmarks or accepted biological guidance. Another open problem which limits the use of two-way simulations is the way the dynein motor varies when the rheology of the airway surface liquid is altered. Thus, a deep understanding of the interplay between the immersed cilia and the ASL has remained elusive. Table 2.1 also shows that most researchers have exploited Newtonian fluid rheology in their investigations, as compared with more complex rheology such as viscoelastic and non-Newtonian models.

Despite the fact that modelling of MCC has taken place for several decades, there are a number of issues which remain unaddressed. Thus the following features may be considered into the existing MCC models: (i) incorporating a close-to-reality mucus rheology – considering the simultaneous effects of the shear-thinning behaviour of mucus viscosity and its viscoelasticity; (ii) inclusion of air layer in models – which would allow the simulation of a variety of external particles and bacteria entering the lungs, and examination of how they are trapped and subsequently expelled by mucus transport; (iii) inclusion of some key factors which influence the muco-ciliary transport such as temperature, humidity, ATP, epithelial differences, mechanosensing mechanisms, etc. (Pieterse and Hanekom, 2018). Implementing the above-mentioned features into future works would enhance model fidelity and robustness. It is important to note that adding any of these features into the existing models would increase the model complexity. Thus, it is vital to find a reasonable compromise between complexity and precision of the models.

To summarize the parametric investigations in the literature, it should be noted that parameters such as cilia beat frequency, metachronism, ciliary length, and ASL

thickness have had a large effect on the MCC. However, a moderate change in the transport has been observed by the variation of mucus viscosity and ciliary density. On the other hand, the impact of the surface tension variation at the PCL-mucus interface on the ML locomotion had been insignificant.

More importantly, little attention has been paid to the modelling of mucus transport and biofilm growth in mucosal diseases such as asthma, COPD, and cystic fibrosis. The existing MCC models are rarely validated against experimental data and hence it is needed to validate MCC models which can simulate some clinical settings. The proliferation of pathogens in the mucus as well as disease severity can alter biological parameters and thereby affect ASL transport (Chatelin and Poncet, 2016). Hence, relevant clinical data are required to estimate, for example, the mucus rheology (clinical data for mucus rheological properties can be found in Jeanneret-Grosjean et al. (1988), Zayas et al. (1990), Dawson et al. (2003), Serisier et al. (2009), Tomaiuolo et al. (2014), and Chatelin et al. (2017)). Lung diseases can also occur as a result of ciliary dysfunction. While some types of ciliary abnormalities have been modelled in a simplified manner, the pollutant-induced structural defect of cilia has not been included in any models, and may have significant influence on muco-ciliary clearance. To date, there is no investigation coupling a cell-scale muco-ciliary model with a lung-scale clearance model to develop a bottom-up model to simulate mucus clearance of the lungs. This requires conducting a multi-scale (i.e. millimetre to centimetre length scale) modelling of muco-ciliary clearance. That is to say, the emerging behaviour from the cell-scale model is fed to the lung-scale model to capture mucus clearance at large scale. This kind of multi-scale model would facilitate study of the effectiveness of treatment methods.

Finally, given the direct medical relevance of the mathematical modelling of epithelial cilia-driven fluid flow, the review of muco-ciliary models in the present survey is a useful basis to progress towards a quantitative understanding of clearance process in the common respiratory diseases such as cystic fibrosis and COPD. Such information is invaluable for an effective drug delivery for respiratory treatments, as the persistent of the inhaled drug carriers in the lung is significantly influenced by the muco-ciliary process, which consequently can minimize their bioavailability (Ruge et al., 2013).

Hence, with the aid of modelling techniques, the fate and transport of inhaled therapeutic particles via muco-ciliary clearance could be tested virtually before proceeding to clinical trials.

Table 2.1: Summary of numerical investigations of MCC concerned with 'cilia sublayer' models. Unless otherwise stated, the mucus viscosities for the given Refs. are assumed constant across the mucus layer. The investigated biological parameters include: ciliary beat frequency CBF, mucus rheology (i.e. the effects of viscosity μ, viscoelasticity, and non-Newtonian behaviour), PCL or mucus layer height $H_{PCL\ or\ ML}$, ciliary length L, surface tension at the PCL-mucus interface σ, flexibility of cilia, metachronal wave produced by ciliary motions (i.e the effect of phase lag between adjacent cilia $\Delta\phi$), and ciliary density (i.e. number of cilia / computational domain. For the investigated parameters given in the table , '✓' indicates that the authors have evaluated the effects of that biological parameter on the MCC (otherwise shown by '✗').

Studies	**Type of model**			**Numerical method**	**Mucus rheology**	**Investigated parameters**							
	Volume force	Discrete cilia				CBF	μ	$H_{PCL\ or\ ML}$	L	σ	Flexible cilium	$\Delta\phi$	No. cilia
		PCB	FSI										
Blake and Winet (1980a)	✓			Stokeslet	Newtonian	✗	✗	✗	✓	✗	✗	✗	✗
Fulford and Blake (1986)		✓		Stokeslet	Newtonian	✗	✗	✗	✗	✗	✗	✓	✓
Dillon et al. (2007)			✓	Finite-difference scheme + IBM	Newtonian Viscoelastic – Lagrangian Mesh network	✗	✗	✗	✗	✗	✓	✓	✗
Mitran (2007)			✓	Stokes Eqs. (finite-volume) + Finite-element thin-walled beams + overlapping fixed-moving grid	Newtonian Viscoelastic non-linear Maxwell model	✗	✗	✗	✗	✗	✓	✓	✗
Smith et al. (2007b)	✓			Stokeslet	Newtonian Viscoelastic linear Maxwell model	✓	✓	✓	✗	✗	✗	✓	✗

Table 2.1 (continued).

Lukens et al. (2010)			✓	Finite-difference scheme + IBM + LCSs	Newtonian Viscoelastic Lagrangian Mesh network	✗	✗	✗	✗	✗	✓	✗	✗
Lee et al. (2011)		✓		Navier–Stokes Eqs. (projection method) + IBM	Newtonian	✓	✓	✓	✗	✓	✗	✗	✓
Chatelin and Poncet (2013)		✓		Stokes Eqs. + transport Eqs. + penalisation method	Newtonian ASL – variable mucus viscosity	✗	✓	✗	✗	✗	✗	✗	✓
Jayathilake et al. (2015)		✓		Navier–Stokes Eqs. (projection method) + IBM	Newtonian	✗	✗	✗	✓	✗	✗	✓	✗
Kurbatova et al. (2015)	✓			Thin-layer Eq. (finite-difference)	Newtonian	✗	✓	✗	✗	✗	✗	✗	✗
Sedaghat et al. (2016)		✓		LBM + IBM	Newtonian Viscoelastic Oldroyd B model	✓	✓	✓	✗	✓	✗	✗	✗
Chatelin and Poncet (2016)		✓		Stokes Eqs. + transport Eqs. + penalisation method	Newtonian ASL variable mucus viscosity	✗	✓	✓	✓	✗	✗	✗	✗
Guo and Kanso (2017)		✓		Navier–Stokes Eqs. (finite-volume) + IBM	Newtonian Viscoelastic Oldroyd B model	✗	✗	✓	✗	✗	✗	✗	✗
Chatelin et al. (2017)		✓		Non-linear Stokes Eqs. + transport Eqs. + penalisation method	Non-Newtonian ASL – variable mucus viscosity	✗	✓	✓	✗	✗	✗	✗	✗

Table 2.1 (continued).

Chateau et al. (2017)	✓	LBM + IBM	Newtonian	✗	✗	✗	✗	✗	✗	✓	✗
Quek et al. (2018)	✓	Navier–Stokes Eqs. (projection method) + IBM	Newtonian	✗	✗	✗	✗	✓	✗	✗	✗
Chateau et al. (2018)	✓	LBM + IBM	Newtonian	✗	✗	✗	✗	✗	✗	✓	✗

Conflict of interest

The authors declare that they have no conflicts of interest associated with the presented work.

Acknowledgments

Shayan M. Vanaki gratefully acknowledges Queensland University of Technology (QUT) for support through the QUTPRA scholarship. Jinju Chen and Pahala Gedara Jayathilake acknowledge funding from the Engineering and Physical Sciences Research Council (EP/K039083/1). The authors would like to thank Dr. Hanliang Guo (University of Michigan, Ann Arbor), Dr. Raymond Quek (A*STARs Institute of High Performance Computing), and Dr. Mahmood Norouzi (Shahrood University of Technology) for their technical advice. The authors would also like to thank the reviewers for all of their careful, constructive and insightful comments in relation to this work.