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The role of mathematical models in designing mechanopharmacological therapies for asthma

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Healthy lung function depends on a complex system of interactions which regulate the mechanical and biochemical environment of individual cells to the whole organ. Perturbations from these regulated processes give rise to significant lung dysfunction such as chronic inflammation, airway hyperresponsiveness and airway remodelling characteristic of asthma. Importantly, there is ongoing mechanobiological feedback where mechanical factors including airway stiffness and oscillatory loading have considerable influence over cell behavior. The recently proposed area of mechanopharmacology recognises these interactions and aims to highlight the need to consider mechanobiology when identifying and assessing pharmacological targets. However, these multiscale interactions can be difficult to study experimentally due to the need for measurements across a wide range of spatial and temporal scales. On the other hand, integrative multiscale mathematical models have begun to show success in simulating the interactions between different mechanobiological mechanisms or cell/tissue-types across multiple scales. When appropriately informed by experimental data, these models have the potential to serve as extremely useful predictive tools, where physical mechanisms and emergent behaviours can be probed or hypothesised and, more importantly, exploited to propose new mechanopharmacological therapies for asthma and other respiratory diseases. In this review, we first demonstrate via an exemplar, how a multiscale mathematical model of acute bronchoconstriction in an airway could be exploited to propose new mechanopharmacological therapies. We then review current mathematical modelling approaches in respiratory disease and highlight hypotheses generated by such models that could have significant implications for therapies in asthma, but that have not yet been the subject of experimental attention or investigation. Finally we highlight modelling approaches that have shown promise in other biological systems that could be brought to bear in developing mathematical models for optimisation of mechanopharmacological therapies in asthma, with discussion of how they could complement and accelerate current experimental approaches.

KEYWORDS

multiscale models, mechanobiology, biomechanics, hyperresponsiveness, remodelling

1 Introduction

Asthma is a chronic lung disease affecting over 300 million people globally. Severe asthmatics suffer exacerbations requiring frequent hospitalisation and so contribute a significant burden to national health services. Although new therapies such as bronchial thermoplasty appear to provide a "cure" to severe asthmatics, in most cases it is only the symptoms that are treated. Asthma is characterised by inflammation, airway hyperresponsiveness (AHR) and airway remodelling. Inhalation of an allergen, such as pollen or dustmite, triggers an immune response causing infiltration of white blood cells (e.g., eosinophils) from neighbouring blood vessels into the airway. A normal response would see these cells return to the circulation but in asthma they remain elevated, causing inflammation of the airways. The eosinophils interact with resident cells (e.g., mast cells) in the airway and secrete a contractile agonist that causes airway smooth muscle cells in the wall to contract. Hyperresponsiveness refers to the rapid contraction of smooth muscle to lower doses of agonist in asthmatics than in non-asthmatics. Corresponding structural changes in the airway occur over longer timescales (airway remodelling), which include significantly increased smooth muscle mass along with increases in other cell types and extracellular matrix that support the smooth muscle, causing the airway to thicken with the capacity to worsen bronchoconstriction. There is increasing evidence that these three hallmarks are linked through both mechanical and biochemical factors. In parallel and in more general settings, the influence of mechanical factors on cell behavior and how this can affect pharmacological treatments is beginning to gain recognition, leading to the proposed area of mechanopharmacology (Liao et al., 2006; Krishnan et al., 2016); in this arena the role of mechanics and mechanobiology is acknowledged as a key factor to take into account when identifying and assessing the efficacy of pharmacological interventions. Additionally, mechanopharmacology includes consideration of combined or synergistic effects of mechanical and pharmacological interventions, as proposed in the study of Wang et al. (2019), in which tissue-level force oscillations are combined with a treatment that targets intracellular signalling and cytoskeletal stiffness via Rho kinase (ROCK). Given the multiple spatial and temporal scales associated with the underlying biochemical signals and tissue mechanics in asthma, along with interactions between multiple cell and tissue types, integrative multiscale mathematical models are increasingly useful tools for understanding links between inflammation, hyperresponsiveness and remodelling as well as synergies between mechanics and pharmacology. Such models together with novel biomechanics-based high throughput drug-screening technologies (e.g., (Park et al., 2015)) or organ-on-a-chip technologies (e.g., (Huh et al., 2010)) that can mimic physiological mechanical environments (including substrate stiffness, dynamic loading conditions), provide great potential for aiding the design or optimisation of new mechanopharmacological therapies.

In this review we will first consider an exemplar multiscale model (Figure 1) to illustrate how an understanding of cell–tissue interactions could be exploited to propose a potential mechanopharmacological therapy in asthma and acute bronchoconstriction. Noting that there is evidence that mechanotransduction and mechanobiology at the cell-level play important roles in emergent tissue and whole airway mechanics in longer time-scale processes, we then examine how mechanics play a role in linking inflammation to responsiveness and airway remodelling; integrative approaches are clearly necessary to understand these complex interactions at multiple timescales. We therefore also review the significant modelling efforts in respiratory cell and tissue mechanics in multiple respiratory disease settings, illustrating the role of mechanics in the multiscale processes described above. We emphasise that the focus is on recent work, rather than a complete background. Interested readers are referred to recent more extensive reviews for lung tissue mechanics and multiscale models of fibrosis (Burrowes et al., 2019; Leonard-Duke et al., 2020).

Given the importance of mechanical factors it is somewhat surprising that these have not yet been exploited in combination with pharmacological therapies. It therefore seems timely to consider mathematical models aimed at mechanopharmacological therapies in asthma in both: 1) acute situations, by understanding the role of deep inspirations (DIs) and breathing patterns on bronchodilation, and 2) chronic cases of airway remodelling where the combined role of mechanical stress and subcellular signalling govern adverse changes in tissue properties. Hence we will draw on exemplars to highlight how mechanopharmacology could be considered as a new focus for mathematical modelling in asthma to identify new targets e.g. how mechanical forces might influence the efficacy of a given drug or activate particular growth factors. We note however that:

(i) much more joint modelling and experimental work is needed. In this review we therefore highlight some hypotheses generated by models that remain to be tested experimentally and how these could advance this field, as well as key experimental questions and frameworks where existing modelling techniques could guide mechanistic understanding;

(ii) although this review focuses on subcellular, cellular, tissue, and airway-level models a significant amount of work has been done on networks of branching airways at the organ-level considering ventilation heterogeneity (Venegas et al., 2005; Tgavalekos et al., 2007; Winkler and Venegas, 2007; Bhatawadekar et al., 2015; Donovan, 2016; Leary et al., 2016; Donovan, 2017), multi-breath washout and forced oscillatory technique (Berger et al., 2016; Foy et al.,

2019), through to the effects of bronchial thermoplasty (Donovan et al., 2020). The challenge, which will be discussed later, is in upscaling from detailed tissue- and airway-level models, that include cell-level details, to these types of macroscale models;

(iii) the impact of mathematical modelling in respiratory research lags other biomedical areas and so we also highlight research areas where joint experimental–theoretical approaches have borne fruit (e.g., cardiovascular research) and discuss some associated methods from which we could learn.

Box 1 Key terms and definitions referred to in this review.

Airway hyperresponsiveness: greater reduction in airway calibre to low concentration of contractile agonist that occurs in asthmatics compared to non-asthmatics evidenced by leftward shift in dose-response curves (airway calibre as function of agonist concentration).

Airway remodelling: structural changes in the airway wall resulting from increased airway smooth muscle, extracellular matrix, epithelial and goblet cells, thus causing increased wall thickness or decreased luminal area.

Bronchoconstriction: a rapid contraction of airway smooth muscle resulting in airway narrowing.

Crossbridge cycling: repeated binding (and unbinding) of myosin crossbridges with actin binding sites within the airway smooth muscle cell resulting in a relative motion of actin and myosin filaments and thus shortening/contraction of the cell.

Deep inspiration (DI): a deep breath that induces a substantial mechanical perturbation compared to normal (tidal) breathing. DIs have a potent, long-lasting bronchodilatory effect in non-asthmatics but only a transient effect in asthmatics.

Mechanobiology: the study of how cells sense and respond to mechanical cues.

Mechanopharmacology: the consideration of mechanobiological factors when evaluating therapeutic strategies, such as potential interactions or synergies between mechanical and pharmacological perturbations.

2 Deep inspiration and tidal breathing in asthma: An exemplar

During an asthma attack, release of contractile agonist at the cell level causes crossbridges from myosin filaments within the cell to attach and detach from binding sites on actin filaments. This crossbridge cycling causes filaments to slide past each other which in turn causes the cell to shorten along the smooth muscle fibre. This results in contraction of the airways and thus in shortness of breath and wheezing. Tidal breathing and deep inspirations (DIs) at the organ level transmits strain to the airways, the muscle fibres and thus to the cell and the contractile machinery. This perturbs the cycling dynamics, resulting in an interplay between subcellular and organ level processes. In particular, DIs have a potent bronchodilatory effect in normal subjects but only a transient one in asthmatics.

In order to understand why bronchodilatory effects of DIs are attenuated in asthmatics, a number of experimental investigations focus on the effect of cyclic strain on the cell, airway and organ. In particular it is well established that increasing the amplitude of oscillation applied to tissue strips causes a proportionate decrease in mean contractile force compared with application of isometric force (Fredberg et al., 1997; Wang et al., 2000; Bates et al., 2009). Dilation of constricted airways in precision-cut lung-slices (PCLS) on application of oscillations to surrounding parenchymal tissue also appear to indicate that such "breathing-like" oscillations are capable of reducing contractile force (Lavoie et al., 2012). The drop in force is attributed to perturbation of acto-myosin crossbridge binding. While this explained the effect of mechanical stretching on contractile force in *isolated* smooth muscle, results from other experimental investigations showed that when pressure oscillations are applied to whole airways the airways could not be dilated to the extent predicted by tissue and cell experiments unless the amplitude of the oscillations was extremely high (and not physiologically realistic) (LaPrad et al., 2010; LaPrad and Lutchen, 2011). A key question is: why are not the results from the cell and tissue levels recapitulated at the level of the intact airway (Figure 1A)? Furthermore, how does airway smooth muscle (ASM) force generation manifest at the tissue/airway-level, and in particular how do the dynamic effects of breathing differ between observations in *ex vivo* experimental assays and *in vivo*?

To answer these questions we previously developed and validated a multiscale mathematical model that couples models of dynamic force generation via crossbridge mechanics of airway smooth muscle at the subcellular level to an airway modelled as an axisymmetric hyperelastic cylinder under plane strain accounting for strain-stiffening properties of collagen-dominated extracellular matrix (Hiorns et al., 2014) (Figures 1B–D). Application of a time-dependent transmural pressure mimics the effect of breathing (Figure 1E). Most notably, this work shows that although length oscillations may cause a rapid drop in contractile force generated by ASM cells in tissue strips (the most common *ex vivo* experimental preparation), the additional variable of an operating transmural pressure from which strain oscillations are applied plays an important role in determining the effective stiffness of the intact airway (the slope of the pressure-radius curve which is a non-monotonic function of transmural pressure) and thereby the effectiveness with which strain is transmitted to the ASM cells. Thus, whether or not significant bronchodilation occurs, depends on the transmural pressure at which "breathing-like" oscillations are applied (Hiorns et al., 2014). Essentially, lower effective airway stiffness means greater strain is transmitted to the smooth muscle cells. Harvey et al. (2013) confirmed our theoretical finding by showing experimentally that oscillations applied at lower transmural pressure (i.e., at a more compliant part of the pressure-radius curve) generates greater bronchodilation at

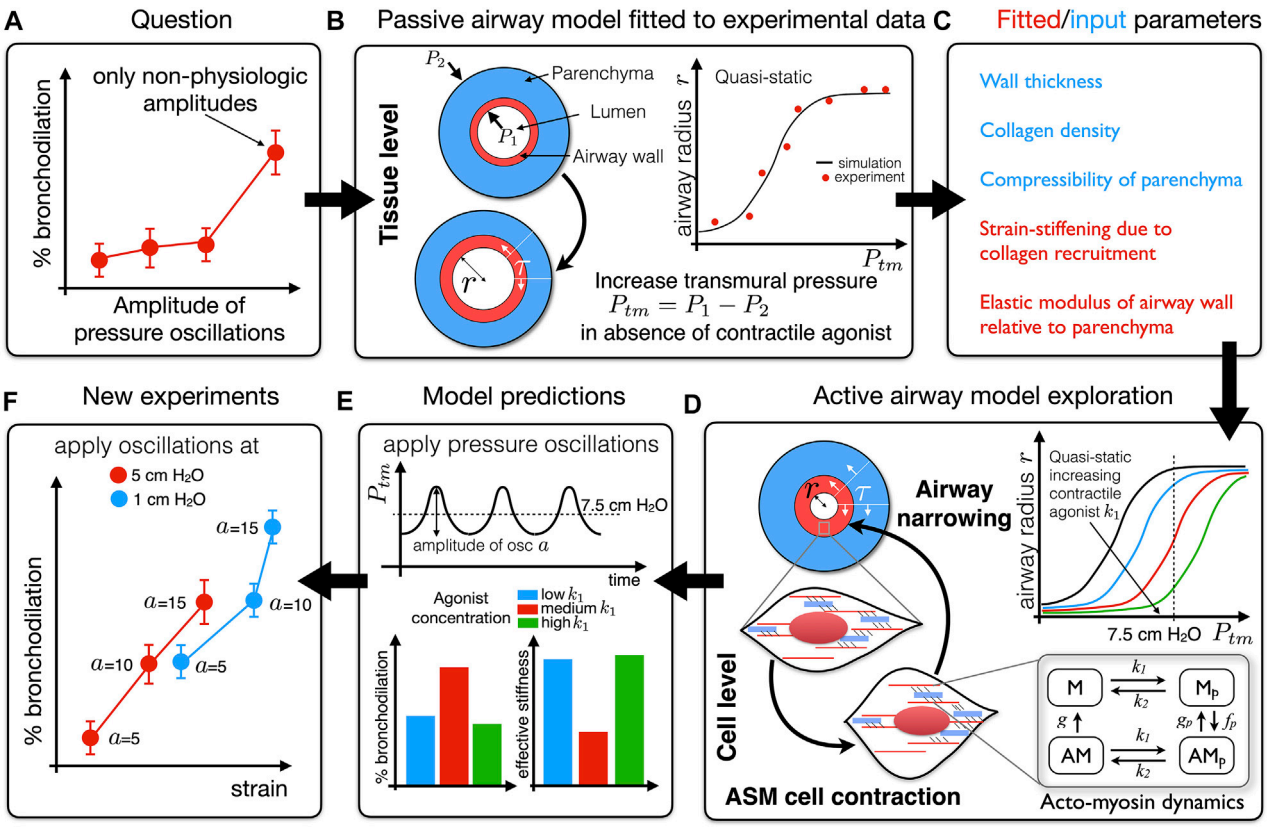

FIGURE 1
Schematic of an iterative process by which experimental data previously informed a mathematical model, exploration of which generated a hypothesis that could be tested experimentally. **(A)** The question: why is stretch-modulated reduction in contractile force at cell/tissue level not recapitulated in intact airways unless pressure oscillation amplitudes are unphysiologically high (LaPrad et al., 2010)? To address this question we developed a mathematical model of an airway that accounts for force generation via airway smooth muscle fibres and strain-stiffening via collagen fibres embedded in a non-linearly elastic axisymmetric airway wall. **(B)** In the absence of contractile agonist (passive airway) the quasi-static model is effectively of the form $P_{tm} = P_1 - P_2 = f(r; \mathbf{p})$; for each applied transmural pressure (P_{tm}), the elastic model allows us to predict the corresponding airway radius (r) for a set of parameters $\mathbf{p}$ in the form of a "pressure-radius" curve. We use quasi-static experimental data to infer mechanical properties as indicated in **(C)**. **(D)** The generation of contractile force at the cell-level is modelled via the Huxley-Hai-Murphy model and coupled to the elastic model in **(B)**. This allows us to investigate the effect of contractile agonist on the quasi-static pressure-radius curves. **(E)** Application of time-dependent pressure oscillations (amplitude a) about a particular transmural pressure allows dynamic changes in the contractile force to be predicted. This reveals that the amount of bronchodilation that can be achieved depends on the effective stiffness of the airway at that transmural pressure (reciprocal of the slope of the pressure-radius curves in panel **(D)**; $dr/dP_{tm}(7.5)$); the lower the effective stiffness the greater the ability of the oscillations to dilate the airway. We thus hypothesised that higher effective stiffness of an airway limits the strain that can be transmitted to the contractile machinery and so applying pressure oscillations at a more compliant part of the pressure-radius curve will generate greater bronchodilation **(B–E)** (Hiorns et al., 2014)). **(F)** In new experiments, pressure oscillations applied to intact airways at different P_{tm} and amplitudes a confirm this hypothesis (Harvey et al., 2013).

more physiologic amplitudes (Figure 1F). It seems that the key to improving lung function in asthmatics would be to make their airways more compliant so that the bronchodilatory effects of DI can be restored. Can inhalation therapies be improved by exploiting these mechanics-based results, e.g., does breathing out first (rather than a DI) get the airways to a more compliant part of the pressure-radius curve? Can this be combined with a bronchodilator to optimize dilation? Appropriately validated multiscale mathematical models of the type described above can play an important role in addressing such questions and thereby enable the design and optimisation of mechanopharmacological therapies.

Furthermore, we showed that the effective stiffness of the airway wall differs significantly in dynamic compared to quasi-static conditions. This then led to an exploration of how dynamic stresses in an intact asthmatic airway differ from those in excised tissue strips (Hiorns et al., 2016). We show that airway geometry, and the interplay between dynamic active and passive forces, give rise to large stress and compliance heterogeneities across the broncho-constricted intact airway wall, that are absent in tissue-strips with identical properties. The study thus demonstrates how findings from a computational model can improve experimental design, suggesting a strategy by which the

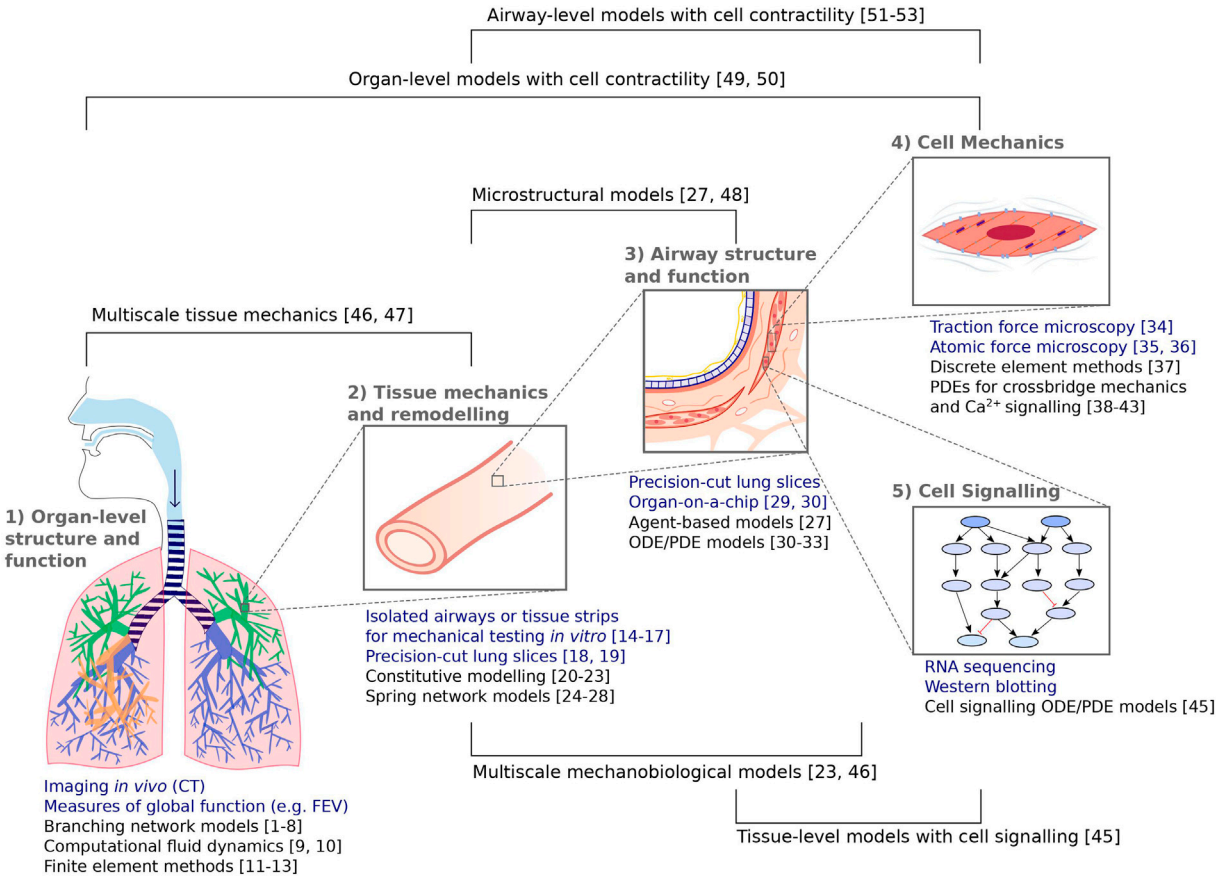

FIGURE 2
Summary of the experimental (blue) and complementary modelling (black) techniques discussed in these sections, spanning from organ-level structure and function to subcellular mechanics and signalling events. Numbers correspond to the following references: [1–8] Venegas et al., 2005; Tgavalekos et al., 2007; Winkler and Venegas, 2007; Bhatawadekar et al., 2015; Donovan, 2016; Leary et al., 2006; Donovan 2017; Foy et al., 2019. [9, 10] Choi et al., 2019; Yoon et al., 2020 [11–13] Berger et al., 2016; Tawhai et al., 2004; Werner et al., 2009 [14–17] Fredberg et al., 1997; Wang et al., 2000; Bates et al., 2009; LaPrad et al., 2010 [18, 19] Lavoie et al., 2012; Pybus et al., 2021b [20–23] Fung, 1974; Eskandari et al., 2019; Birzle et al., 2019; Hill et al., 2018 [24–28] Mead et al., 1970, Cavalcante et al., 2005; Iravani et al., 2020; Wellman et al., 2018; Oliveira et al., 2014 [29, 30] Huh et al., 2010; Kilic et al., 2019 [31–33] Chernyavsky et al., 2014; Chernyavsky et al., 2018; Pybus et al., 2021a [34] Lin et al., 2018 [35, 36] Sun et al., 2005; Smith et al., 2005 [37] Vargas et al., 2020 [38–43] Hai and Murphy, 1998; Mijailovich et al., 2000; Wang et al., 2008; Wang et al., 2010; Croisier et al., 2013; Croisier et al., 2015 [44] Irons et al., 2018 [45] Hao et al., 2015 [46, 47] Pascoe et al., 2020; Donovan et al., 2020 [27, 48] Wellman et al., 2018; Concha and Hurtado, 2020 [49, 50] Politi et al., 2010; Lauzon et al., 2012 [51–53] Hiorns et al., 2014; Hiorns et al., 2016; Brook 2014.

mechanical environment of smooth muscle cells can be replicated *ex vivo*; in particular we propose that if the tissue strip experiments are to represent the airway wall, then loading to the strip should utilise auxotonic loading characteristics at the outer wall as predicted by the finite thickness airway model not models based on the thin-walled Laplace approximation. The above work also suggests that integrative studies need to consider how feedback from resulting stress environments might affect ASM cell response over longer periods of time and how these stress environments might drive structural changes (such as activation of growth factors, ASM hypertrophy or hyperplasia and collagen deposition) and hence perturbations away from homoeostasis into pathology. This work has thus highlighted the need for combining multiple interactions, occurring at different spatio-temporal scales, governing bronchoconstriction in asthma, and illustrates the potential for exploiting mechanopharmacology as a potential route to developing new or more effective therapies.

3 Existing modelling approaches

Having considered the above example in some detail, we now review, more generally, modelling efforts in respiratory cell and tissue mechanics in multiple respiratory disease settings, illustrating the role of mechanics in the multiscale processes described above. Modelling efforts have targeted wide-ranging

questions across multiple scales, using discrete, continuum, or hybrid approaches. Some primary focus areas discussed in these sections are summarised schematically (Figure 2).

3.1 Organ-level structure and function

Clinical measurements for quantifying lung structure and function primarily occur at the organ scale, for example computed tomography (CT) scans, forced expiratory volume in one second (FEV_1), and forced vital capacity (FVC). The metrics for function are highly dependent on the mechanical properties of the lung, including airway stiffness, airway resistance, and the elastic recoil of the lung. Computational models at the level of the whole lung therefore focus on quantifying mechanics in addition to airflow, typically with finite element (FE) methods as well as computational fluid dynamics (CFD) approaches. In the context of asthma, 1D CFD models taking into account structural information from CT scans, airway resistance, and lung compliance, have been used to understand differences in flow and pressure distributions between asthmatic and non-asthmatic subjects (Choi et al., 2019; Yoon et al., 2020). These models capture hysteresis in pressure–volume curves, showing increased hysteresis (and thereby workload) in the asthmatic group. To understand organ-scale deformations, FE models are often used and built around patient-specific geometric data from CT scans (Tawhai et al., 2004; Werner et al., 2009), where the model-predicted deformations rely on a previously developed understanding of the mechanical properties of the tissue.

3.2 Tissue mechanics and remodelling

An early priority for developing computational models was to accurately characterise lung mechanics at the tissue level by finding constitutive relations to appropriately describe experimental stress–strain relationships from loaded tissue strips, isolated airways, or precision-cut lung-slices (PCLS) (Fung, 1974; Birzle et al., 2019; Eskandari et al., 2019). Towards more microstructurally motivated models, it can be desirable to consider the contributions and distinct material properties of different components of the tissue, for example, collagen, elastin, airway smooth muscle cells, and proteoglycans (PGs). In a recent example, a nonlinear continuum mechanics model considering distinct contributions of collagen and elastin was tuned to experimental data from uniaxially loaded PCLS with collagenase and elastase treatments, in which collagen and elastin were digested individually and in combination, allowing their contributions to be quantified (Birzle et al., 2019). Alternatively, discrete methods such as spring network models have also been used to similarly reproduce experimental stress–strain curves (Mead et al., 1970; Cavalcante et al., 2005). Collagen, elastin, PGs, and viscoelastic matrix were considered within a 2D spring network model for parenchymal tissue and, under uniaxial loading, the model was able to generate stress–strain behaviour and hysteresis loops characteristic of experimental curves (Iravani et al., 2020).

The inclusion of more explicit microstructural detail within continuum models is supported via multiscale methods such as two-scale asymptotic homogenisation. A recent model for a poroelastic material at the tissue-level, upscaled from a solid and fluid phase at the microscale, is based on realistic microstructural geometries from micro CT images of lung parenchyma and offers coarse-grained simulations with computational efficiency (Concha and E Hurtado, 2020). In addition to microscale structure, microscale mechanics have also been shown to be important in the modelling of airways. In the case of bronchoconstriction in asthma, airway-level mechanics and deformation are coupled bidirectionally to the force-generating actin–myosin crossbridge dynamics within airway smooth muscle (ASM) cells as considered in multiscale continuum models (Politi et al., 2010; Lauzon et al., 2012; Hiorns et al., 2014). In general the bidirectional coupling in such models cannot easily be modelled entirely via constitutive relationships; dynamic cell-level processes are currently explicitly accounted for which increases computational cost and new approaches for upscaling may be needed (see e.g. (Donovan, 2020)).

In the examples above the relevant constitutive relationships and tissue mechanics are effectively snapshots in time. Disease progression affects tissue structure and function over longer timescales, and hence we next consider modelling that accounts for the effect of such changes on lung mechanics.

3.3 Changes in lung mechanics with time and disease progression

In many cases, disease progression is characterised by adverse changes in tissue structure and function over time. Once reliable frameworks for simulating lung and airway mechanics are in place, it is necessary to be able to model these long-term changes, occuring via tissue remodelling. Although the manifestations of airway remodelling—changes in collagen and airway smooth muscle cell mass, changes in airway wall area and luminal area—are observed at the tissue-level, the underlying regulation occurs at cellular and subcellular levels via complex cell signalling and mechanobiological cues (Noble et al., 2014). To balance model complexity and efficiency, the decision to consider cellular processes explicitly will depend on the question of interest and the available data. For example, to model observed spatial patterning of airway remodelling in human asthmatic airway samples, it was sufficient to hypothesise a linear relationship between remodelling and the extent of bronchoconstriction (when present), representing mechanosensitive cellular regulation but not considering

pathways explicitly. This was simulated within a broader modelling framework for the whole lung, taking into account airway tree geometry, connectivity, ASM area and tone (Pascoe et al., 2020). Phenomenological relations like this can be hypothesised, tested, and verified in a specific context, making the models useful for further predictions. However, in order to understand the specific biological mechanisms behind bronchoconstriction-induced remodelling and possible pharmacological interventions, more detail would be needed about mechanobiological processes at the cellular and subcellular scales.

3.4 Airway structure and function

A cell-scale process of importance in asthma biology is that of phenotypic changes to ASM (Amrani and Panettieri, 2003; Wright et al., 2013); the current thinking is that most ASM *in vivo* likely exists in a contractile phenotype and that only small subpopulations transform to a synthetic or proliferative phenotype. On the other hand, in culture most of ASM quickly switches to a proliferative phenotype and small subpopulations can be driven back to contractile phenotype through serum deprivation (Halayko et al., 1999). An example of an early model that focuses on the role of phenotypic switching between proliferative and contractile ASM in airway remodelling is that of Chernyavsky et al. (2014) who use a relatively simple system of ordinary differential equations (ODEs) to investigate long term changes in ASM mass and phenotype in both deterministic and stochastic settings. A more detailed study is that of Hill et al. (2018), in which a multiphase mechanochemical, morphoelastic model is developed that combines the cardinal features of asthma (inflammation, hyperresponsiveness and remodelling) into a single integrative model. This model couples the mechanical state of asthmatic airways to (simplified) mechanobiological processes described by ODEs: inflammation due to prescribed allergen challenges mediates collagen deposition and phenotypic changes of smooth muscle cells from a contractile to proliferative phenotype. Tissue-level mechanics are considered via a multiphase model for a bilayered, cylindrical airway with constitutive relations prescribed separately for collagen and smooth muscle cells and the model captures feedback between contractility and inflammation. Parameter studies are carried out to simulate different asthmatic phenotypes (with parameters including the frequency of challenge events and clearance rate for inflammation). It provides a framework with which to test the hypothesis that while airway remodelling is initiated by inflammatory mediators, it is perpetuated by mechanical factors. The parameter study shows that persistent contractile tone observed in asthmatics could be a direct effect of the ASM cell's response to its mechanical environment, and identifies mechanisms that may contribute to the existence of different asthma phenotypes and predisposition to fatal bronchospasms. In parallel with this model development, Tatler et al. (2022) ran a set of model-informed experiments in the ovalbumin chronic mouse model of asthma to quantify accurately the temporal changes in airway constituents in response to inflammatory challenges. This resulted in the development of a computational tool for the extraction of large amounts of quantitative data of airway structure and composition from tissue histomorphological lung slice images, that bridges the experimental and theoretical approaches of both studies (Hill et al., 2018; Tatler et al., 2022). In addition to inflammation, mechanical stress regulates airway remodelling via other mechanosensitive cell signalling pathways and via the direct activation of growth factors such as TGFβ. The relevant stresses in the context of experimental precision-cut lung-slice assays have been simulated in a tissue-level biomechanical model using finite element methods and in a reduced model using asymptotic reductions (Pybus et al., 2021a). This framework forms an efficient and appropriate model for incorporating models of growth factor involvement (such as TGFβ activation), the associated remodelling, and feedback to the tissue-level mechanics. Further modelling studies informed by experimental measurements of protein levels and gene expression are under way (in other contexts; see later) and are much needed in understanding airway remodelling.

While the examples above are associated with structural changes due to disease progression, mathematical models also have a role to play in understanding the effect of bronchial thermoplasty (a therapy involving ablation of ASM cells through radio-frequency heating) where the process is effectively considered to be a "reverse" of airway remodelling via targeted reduction of ASM mass. Theoretical models of bronchial thermoplasty have been informed by experimental *in vitro* data and validated by data from patients in a bronchial thermoplasty clinical trial (Chernyavsky et al., 2018). The significant finding from this study (that couples Joule heating due to the applied electrical current with bioheat transfer in the airway wall and surrounding parenchymal tissue) is that the acute post-heating phase of the procedure only results in a small fraction of the airway wall being heated to the threshold temperature required for ASM cell necrosis. Taken together with results from the corresponding *in vitro* study the authors propose that another mechanism must also contribute to the loss of ASM mass at the extent that has been reported by other investigators, over the longer term. This and other studies (Donovan et al., 2018) have generated some discussion in the literature (Brook et al., 2019). The new questions that have arisen from these studies have instigated the development of a new model that could explain longer term ASM loss and provide a possible explanation for why only some patients may benefit from the therapy (Pybus et al., 2021b).

In this section we considered models that account for structural changes at the tissue and airway level in asthma

and how these give rise to modified tissue mechanics and therefore modified function. Such models still require upscaling into network models of the branching airway tree to understand the effect of airway remodelling on the whole organ with the associated exacerbation of existing heterogeneities (e.g., (Donovan and Noble, 2021)). Additionally we note that current approaches assume particular phenomenological constitutive relationships but further work is needed to understand how cell-level changes can modify these constitutive relationships.

3.5 Cell mechanics and signalling: understanding the drivers of change

We now consider models that account for more detailed mechanobiological drivers of remodelling in the lungs. In the context of idiopathic pulmonary fibrosis (IPF), a discrete approach was taken to studying spatial patterns in remodelling by considering a 2D spring network for lung tissue mechanics coupled to an agent-based model for the effects of fibroblast cell signalling; namely, their stiffness-dependent activation and deposition of collagen which leads to further local stiffening (Wellman et al., 2018). This positive feedback loop generated spatial patterns (subpleural honeycombing) consistent with CT images of the lungs of IPF patients, supporting the idea that positive feedback is a key driver of this patterning. Here, the positive feedback was provided by tissue stiffening near the already stiffer pleura via the hypothesised fibroblast rules in the agent-based model, which are therefore demonstrated to be plausible mechanisms. However, this does not rule out other causes that do not feature in the model. Additional hypotheses could similarly be tested in agent-based models to see which are viable (i.e., which are able to produce the observed behaviour) or which should be ruled out, with the viable hypotheses then further explored experimentally. Also in the context of IPF, a continuum model using reaction–diffusion partial differential equations (PDEs) for different cell types (monocytes, M1 and M2 macrophages, alveolar epithelial cells, fibroblasts) and key extracellular proteins and cytokines (MCP-1, TGFβ, PDGF, TNFα, IL-13, MMP, TIMP) has been presented (Hao et al., 2015), although not yet coupled to the mechanical state or response of the tissue. These types of models provide steps towards a more detailed understanding of pro-fibrotic cell signalling but they require many kinetic parameters to be determined, especially in this case when diffusion and chemotaxis were considered. Although it is not explored in this paper, the spatial modelling framework has potential for further studying patterning in fibrosis, which has been shown to be important due to differing effects of heterogeneous vs. homogeneous fibrosis on the bulk modulus of the tissue (Oliveira et al., 2014). Models accounting for mechanotransductive signalling pathways in more detail will be essential for understanding the complexities of airway remodelling, which is stimulated by both mechanical and biochemical cues.

In addition to being incorporated into multiscale models, cell-level mechanics have been modelled in more detail separately. Actin–myosin crossbridge dynamics are critical to contractile force-generation by ASM and therefore observed airway-level contractile behaviour. These are described by a well-established Huxley-Hai-Murphy (HHM) PDE model accounting for crossbridge cycling via local attachment and detachment rates and myosin phosphorylation and dephosphorylation (Hai and Murphy, 1988; Mijailovich et al., 2000). An extension of this model includes explicit calcium dependence for myosin light chain kinase activation (Wang et al., 2008). Accounting for disconnectivity and reorganisation of the contractile units (Brook, 2014) provides closer agreement with the "banana-shaped" force-length loops measured in tissue strip experiments (Fredberg et al., 1997; Bates et al., 2009) but requires increased computational time.

There is increasing evidence that the ECM plays an important regulatory role in maintaining ASM and that hyperresponsiveness may result from changes to structural ECM in asthmatic airways (Parameswaran et al., 2006; An et al., 2009; Polio et al., 2019). Additionally cell-level mechanical processes, including cell–matrix and cell–cell adhesion via integrins and cadherins, are mechanosensitive and also coupled bidirectionally to tissue deformation and stiffness. These are beginning to be considered in modelling efforts (Irons et al., 2018; Vargas et al., 2020), where complementary experimental approaches include atomic force microscopy for studying cell adhesion strength, stiffness, and surface topography (Smith et al., 2005; Sun et al., 2005), and traction force microscopy for studying local displacements of extracellular matrix (ECM) during ASM cell contraction (Lin et al., 2018). In particular, Irons et al. (2018) show that bistability in adhesion strength emerges from mechanical cooperativity between the multiple scales involved in the cell-matrix interactions in airways. The model was used to investigate the effect of perturbations mimicking DIs (as described in the exemplar above) where, because of the bistability, a DI applied to the high adhesion state could either induce a permanent switch to a lower adhesion state or allow a return of the system to the high adhesion state. These findings provide a candidate mechanism for the hitherto unexplained transient bronchodilatory effect of a DI observed in asthmatics compared to a more sustained effect in normal subjects. To validate this bistability, a joint experimental–theoretical study was designed incorporating atomic force microscopy (AFM) experiments to study the integrin response to external oscillatory loading of varying amplitudes applied to live smooth muscle cells and to model the corresponding cell–ECM interactions (Irons et al., 2020). Experimentally a transition was observed between states of firm adhesion and of complete detachment as the amplitude of oscillatory loading increased, as well as switching behaviour (which can indicate bistability in stochastic systems) between the two adhesion states during single timecourses at intermediate amplitudes. The model

revealed two adhesion states qualitatively similar to the experimentally observed states, with a region of bistability where both the firm adhesion and detachment states can occur depending on the initial adhesion state. The model results provide a potential physical explanation for the experimental results and more importantly, taken together, suggest a means by which transient mechanical stimuli can induce long-term changes in adhesion dynamics, and thereby the ability of the cell to transmit force. While it still remains to be tested whether this region of bistability occurs in the physiologic range—the cell-level strain resulting from an organ-level deformation is yet to be determined—its occurrence is influenced by cell and ECM stiffness, thus structural changes in ECM could have multiple functional consequences for contractile force transmission in asthma.

When considering inter- or intra-cellular signalling, mechanobiological models are often based on experimental observations from cell culture or co-culture experiments where single or multiple cell types are seeded onto a substrate (e.g., collagen gels) and their response to substrate stiffness, mechanical loading, or biochemical stimuli are studied *in vitro*. Changes in specific proteins or gene expression can be quantified by Western blotting and quantitative polymerase chain reaction (qPCR) or RNA sequencing, respectively, giving insight into mechanosensitive signalling pathways responsible for changes in cell behaviour such as proliferation or contractility. An example of a recent *in vitro* experimental framework that considers mechanical loading is the so-called 'bronchial chip' developed to simultaneously study airway epithelial and smooth muscle cells when epithelial cells are subject to a compressive stress mimicking bronchospasm (Kilic et al., 2019). The ASM cells were observed to rapidly contract before relaxing at later times, mediated by paracrine signalling from the epithelial cells in which there is both positive and negative feedback between the 2 cell types at different timescales. A simple ODE model for the production of spasmogens and relaxants was fit to the data, and potential mediators were identified by measuring secreted factors and changes in epithelial cell gene expression. This could inform more detailed mathematical models in future, for example one in which specific signalling pathways are considered. Further demonstrating the close coupling of mechanics and cell signalling, Wang et al. (2019) showed via an experimental study the synergistic effects of force oscillations, applied to isolated airway smooth muscle strips, and pharmacological inhibition of Rho kinase (ROCK), a key intracellular signaling molecule, on the reduction of contractile force generation. The proposed combination as a so-called mechanopharmacological treatment opens interesting questions and opportunities for future mechanobiological computational models that couple cell-and tissue-scale processes, in which such mechanisms can be considered and strategies for optimising bronchodilation can be determined *in silico*.

Acto-myosin dynamics are primarily driven by release of calcium from intracellular stores into the ASM cell cytosol. There is a long history of incorporating calcium dynamics into models of acute smooth muscle contractile response to agonists. In particular the combination of simultaneous calcium signals with tissue level deformation in PCLS have provided the means to develop multiscale models that directly link subcellular calcium signalling to airway narrowing in particular. Wang et al. (2008), Wang et al. (2010) demonstrate that calcium oscillations (rather than uniformly elevated cytosolic calcium concentration) play an important role in ASM force generation. Recent models include the study of Croisier et al. (2013) to account for store-operated Ca^{2+} entry into ASM cells and its effect on Ca^{2+} oscillations. Building on this model, and using data from PCLS, Croisier et al. (2015) then showed how sensitization of the Ryanodine receptor (RyR) could result in abnormal Ca^{2+} signaling. This work provides both theoretical and experimental evidence for a possible underlying mechanism that might drive normal airway smooth muscle into a hyperresponsive phenotype and generated new hypotheses that began to be tested in Sanderson's group (e.g. (Chen and Sanderson, 2017)).

4 Model-generated hypotheses

In Table 1 we highlight model-generated hypotheses that remain to be tested experimentally, focussing on areas with potential mechanopharmacological relevance.

5 Modelling approaches from other biological systems

5.1 Coupling cell signalling models to models of tissue remodelling

Detailed intracellular signalling in current airway models is mainly limited to cytosolic and store-operated calcium signalling associated with activation of acute ASM contractile responses. Activation of pro-remodelling growth factors are currently modelled using phenomenological relationships inferred from experimental studies which only account for downstream effects of the more complicated mechanosensitive signalling networks involved. While these are good starting points, depending on the questions being addressed (e.g., involvement of pharmacology), more detailed models may be required. Tissue remodelling regulated by mechanosensitive signalling at the cellular level has been modelled in a variety of other biological systems including the skin, heart, and vasculature. We briefly highlight some multiscale modelling approaches taken for coupling tissue mechanics to cell signalling, from which the methods could serve as alternatives or exemplars to build from when considering multiscale modelling approaches for airway remodelling.

Methods from arterial remodelling in particular contain many parallels; both airways and arteries are multilayered cylindrical structures with mechanical contributions from collagen and smooth muscle cells (SMCs). SMCs in both cases regulate passive and active contractile tone, and SMCs and fibroblasts regulate collagen turnover and tissue remodelling.

One multiscale tissue remodelling approach has been to couple finite element models for tissue mechanics to agent-based models for cell behavior. For example, in cardiac remodelling there is significant interest in scar formation via collagen deposition after myocardial infarction, and such approaches have been used to study the relation between local tissue mechanics and mechanosensitive fibroblast regulation of collagen (Rouillard and Holmes, 2014). In these models, disparate spatial scales require care to be taken over FEM mesh and ABM grid sizes (Lee et al., 2019). Finite element models can also be coupled to continuous systems of ODEs or PDEs at the cell level, as done in the context of wound healing in skin (Tepole, 2017). Here, PDEs capture transport and interactions between various growth factors and cell types, which influence cell proliferation, collagen deposition and fiber alignment, among other processes. Such reaction kinetics approaches at the cell level have also been used within multiscale models of arterial remodeling (Aparício et al., 2016; Marino et al., 2017), where here both models make use of an axisymmetric cylindrical geometry to simplify the tissue-level mechanics which do not require finite element methods. A major remaining challenge—especially as the number of modelled interactions increases—is the determination of kinetic parameters, which requires extensive timecourse data at the cell level.

In an alternative approach to reaction kinetics that more easily supports larger scale cell signalling networks, activation and inhibition interactions between network species can be written as a set of logic statements, with multivariable interactions implemented by using "AND" and "OR" logical operators. Traditionally, these logic statements would be implemented within a discrete Boolean update scheme where each network species either takes the value 0 or 1, representing an inactive or active state (Morris et al., 2010). Activation or inhibition reactions are then governed by Heaviside step functions and truth tables. Due to the limitations of a discrete approach, however, continuum logic-based methods have now been developed where network species can take any value in the [0, 1] range, where normalised Hill functions are used instead of step functions to model activation and inhibition, and where the logical "AND" and "OR" operators are interpolated between the values in truth tables to also be continuous functions (Wittmann et al., 2009; Kraeutler et al., 2010). One such method was initially proposed and validated against a reaction kinetics model for cardiac β-adrenergic signalling (Kraeutler et al., 2010), with a key advantage including a significantly reduced parameter set yet consistent predictive results. This method has since been used to study large scale intracellular signalling networks across different systems and cell types, with examples including cardiac fibroblasts (Zeigler et al., 2016), vascular smooth muscle cells and fibroblasts (Irons and Humphrey, 2020; Wang et al., 2020; Estrada et al., 2021), dermal fibroblasts (Cursons et al., 2015), brain endothelial cells (Gorick et al., 2022), and macrophages (Liu et al., 2021). Recently, using the cardiac fibroblast model, an *in silico* screen was carried out for 121 FDA-approved drugs to predict and understand the signaling mechanisms behind observed effects of pharmacological interventions (Zeigler et al., 2021). The flexibility to systematically introduce network perturbations that simulate external interventions make this an appealing framework for initial identification or shortlisting of pharmacological targets. In addition, these types of logic-based signalling networks can incorporate changes in mechanical stimuli and have recently been used within multiscale models, e.g., within a hybrid multiscale agent-based model for cardiac fibrosis (Rikard et al., 2019) and a continuum constrained mixture model for arterial growth and remodelling (Irons et al., 2021) in which pathways behind stress-mediated remodelling and feedback to and from tissue-level mechanics are considered. Similar mechanosensitive pathways are critical to airway remodelling, and multiscale models that consider coupling between airway remodelling, mechanics and detailed intracellular signalling pathways will provide important insight into inflammation- and bronchoconstriction-induced remodelling.

5.2 Data-driven modelling and machine learning

While an important aspect of the models developed above is the validation of model predictions via experimentally-obtained data, another equally important aspect of generating real impact in the development of therapies, is for the models to be *informed* by experimental data. Many models require assumptions to be made about the underlying biological mechanisms or constitutive mechanical laws for biological tissue; traditionally these assumptions are incorporated through phenomenological relationships based on experimental findings. More recently, however, data-driven modelling approaches are beginning to play an important role in addressing some of the challenges associated with accurately informing models such as sparsity of biological data, multiple candidate mathematical models and computationally expensive numerical simulations. Such approaches use deep learning neural networks to both solve systems of equations (data-driven solution) and identify or discover specific forms for the terms in systems of ODEs or PDEs that may not be easily postulated *a priori*, but can be "learned" through appropriate use of experimental data (data-driven discovery of PDEs) (Rudy et al., 2017; Raissi et al., 2019). From the point of view of developing mathematical models for biological applications, the most powerful aspect of these

approaches is that the governing system of equations are mechanistic so that each term can still be related to specific biological or physical mechanisms. A specific exemplar is the work of Lagergren et al. (2020) who, in an extension of the physics-informed neural networks described by Raissi et al. (2019), provide a data-driven methodology which is demonstrated using a case study of scratch assay experiments. The authors assume a set of governing reaction–diffusion equations in which the exact nonlinear forms of diffusivity and growth are not known. Using spatio-temporal data and cell density from the scratch-assay experiments (with space and time as inputs and cell density as an output to/from a deep neural network) they first approximate the solution of the governing dynamical system. The nonlinear forms of the diffusivity and growth terms in the dynamical system are then learned. Automatic differentiation is used on compositions of the different neural network models (i.e., cell density, diffusivity, and growth) to construct the PDE that describes the governing dynamical system. The governing system is used in an objective function associated with the full neural network to jointly learn and satisfy the governing PDE while minimizing the error between the network outputs and noisy observations. Such approaches could play an important role in identifying mathematical forms for hitherto unknown biological mechanisms, or where it is well known that *in vitro* data (and corresponding phenomenological relationships) may not accurately reflect *in vitro* behaviour. A specific example from our work that will benefit from the approach described above is in linking extensive spatio-temporal data from an *in vivo* mouse model of remodelling (Tatler et al., 2022) to mathematical models of remodelling (Hill et al., 2018) in which specific mathematical forms for the candidate mechanisms could be learned. The reader is directed to (Alber et al., 2019) for further reviews of machine learning in multiscale models.

5.3 Methods for transferring information across scales

Although detailed multiscale models of single airways linking multiple cell types to tissue level behaviour provides important insight into airway mechanics, whole-lung behaviour cannot be easily inferred from isolated airways. Inter-airway interactions as a result of being part of a branching network, as well as interdependence between airways and lung parenchyma need to be considered. For a comprehensive review of such models the reader is directed to Rampadarath and Donovan (Rampadarath and Donovan, 2021). Here we briefly focus on branching network models which need to account for the nonlinear, viscoelastic airway mechanics of bronchoconstriction, to understand, for instance, ventilation heterogeneities observed in hyperpolarised gas imaging studies. These models typically assume Poiseuille flow in individual airways with some correction (e.g., the earliest models of Pedley et al. (1971)) and the tissue mechanics require constitutive relationships of the non-linear pressure-radius or pressure-area relationships to characterise each airway within the network; most studies rely on the empirical observations of Lambert et al. (1982). These measurements and extrapolated relationships provide a reasonable description of passive purely elastic airway mechanics but the resulting branching network models are governed by a large system of differential algebraic equations that can be computationally costly to solve. Depending on the questions being addressed (e.g., heterogeneity of bronchoconstriction) the effect of contractile agonist on the ASM requires additional relationships as measured by LaPrad et al. (2010) and Harvey et al. (2013) but the relationships for each airway in the branching network may vary depending on airway size, agonist concentration, timescale of tidal oscillations or degree of airway remodelling as predicted by Hiorns et al. (2014), Hiorns et al. (2016) and Hill et al. (2018). To incorporate such changes we need appropriate methods for upscaling from individual airways, that exhibit potentially different mechanical characteristics, to branching network models in a computationally efficient way. Not every detail at the individual airway level is needed in the higher scale model and simplifications can be made at the outset; what those simplifications should be will depend on the specific question being addressed. Recently graph-theoretic perspectives have proven useful in characterizing transport in various examples of biological networks. Whitfield et al. (2020) use concepts from spectral graph theory to demonstrate that the resistive properties of realistic tree networks are characterized by a tree-specific operator called the Maury matrix, first introduced in (Maury, 2013) which provides a complete description of linear resistance relations on the network. They demonstrate how ventilation heterogeneity can be evaluated for four realistic airway network models (based on CT imaging) and how the Maury operator efficiently captures spatial patterns of ventilation heterogeneity, providing a new method for dimensionality reduction in these systems. The main limitations to this approach, however, is that it is restricted to fixed linear resistance relations. Future work should consider how the efficiency of such approaches can be exploited to effectively characterise other properties of the network such as dynamic airway compliance, resistance and viscoelasticity.

At the airway scale, we noted above that current phenomenological descriptions for constitutive relationships for the underlying biological tissue cannot easily account for ASM cells actively generating contractile force, or rapid remodelling of the cell cytoskeleton that is thought to occur on short timescales; again methods for determining constitutive relationships are needed. Recent examples for doing this in other biological applications come from bottom-up models of individual cells allowing for key cellular processes of

TABLE 1 Examples of hypotheses generated by mathematical modelling studies that are yet to be fully explored experimentally, where mechanics plays a fundamental role.

Area of Interest	Model-generated hypothesis	References
Pharmacological target for reduced ASM mass	In this combined experimental and theoretical study, azithromycin (an antibiotic with anti-proliferative effects) is shown to reduce ASM thickness by 29% in mice after 10 days, and to be similarly effective across proximal and distal regions of the airway tree. It is not yet known if this result translates to humans, but the authors simulate the organ-level consequences of a uniform reduction in ASM thickness in a computational model of a human tracheobronchial tree, and compare with simulation results from the more dramatic (75% reduction) yet localised effects of bronchial thermoplasty. The model predicts that pharmacological agents that provide lower but uniform reductions in ASM mass could restore function to an equal or better level than localised BT, and therefore warrant further consideration. These results show that there are direct mechanical consequences of uniform vs. heterogeneous reductions in ASM mass, and such models are promising for understanding and optimising possible mechanopharmacological treatments	Donovan et al. (2020)
Bronchial thermoplasty (BT)	Computational modeling of heat transfer and distribution suggests that the extent of observed ASM cell death during bronchial thermoplasty is only partially explained by the direct effects of the heat source, leading to the hypothesis of an 'active thermal bystander effect', in which local effects are propagated, perhaps due to a signalling cascade. *In vitro* studies of paracrine signalling in this context could provide further insight. Additional modelling studies suggest that other candidate mechanisms such as ASM–ECM interactions and their mechanoregulation of each other could play a role, and the interplay between ECM-dependent mechanical regulation of cell behavior and ASM–epithelial cell interactions should be further considered when seeking to understand the extent of cell death and designing optimal BT protocols	Chernyavsky et al. (2018), Donovan et al. (2018), Brook et al. (2019), Pybus et al. (2021b)
Mechanotransductive feedback loop in airway remodelling in asthma	In this modelling study, a mechanotransductive feedback loop is identified in which hyperresponsive airways exhibit increased remodelling via stress-induced release of pro-mitogenic/remodelling and procontractile cytokines after simulated exposure to contractile agonists. The possibility of a chronically increased contractile tone also emerges via either this same feedback loop, a failure to clear agonists from the tissue, or a combination of the two. The result remains to be tested experimentally, which could be done by comparing the model to spatio-temporal data of ASM and ECM area fractions within the airway, as well as cytokine levels, obtained (for example) in an *in vivo* mouse model of remodelling for which the exact timing of challenges are known. In the event of a confirmed pathological feedback loop, a priority would be to interrupt it by targeting any of the positive interactions including altered mechanical properties and mechanotransductive pathways	Hill et al. (2018), Tatler et al. (2022)
Spatial patterns in airway remodelling induced by bronchoconstriction	This study shows spatial patterns in airway remodelling in experimental data collected from human lung samples. Similar patterns are obtained in a computational model in which remodelling occurs only in the presence of, and linearly proportional to, bronchoconstriction. Currently, this relationship is hypothesised and the underlying mechanisms remain to be fully explored. This would require experimental measurements at the protein level of stress-mediated intracellular signaling pathways that regulate remodelling. An interesting factor noted in a small subset of experimental samples were local increases in inflammation which correlate with remodelling, leading to several open questions about the relationship between bronchoconstriction, local mechanics, recruitment or activation of inflammatory cells, and remodelling. A better understanding of the role of mechanics in regulating inflammation could open up the potential to identify new targets, including those related to ECM stiffness and remodelling	Pascoe et al. (2020)

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TABLE 1 (*Continued*) Examples of hypotheses generated by mathematical modelling studies that are yet to be fully explored experimentally, where mechanics plays a fundamental role.

Area of Interest	Model-generated hypothesis	References
Bronchoconstriction and responses to deep inspirations	In this study, computational models predict bistability of cell–matrix adhesion density, leading to different levels of contractile force transmission. In systems with bistability it is possible to observe two different outcomes after a perturbation, such as the transient vs. sustained bronchodilatory effects of deep inspirations seen in asthmatics vs. non-asthmatics. Experimentally, a suitable test to see if this bistability exists and can be found in a physiological range is to gradually increase and then decrease oscillatory loads (symmetrically), while looking for hysteresis in total contractile force. If present, responses to DIs could potentially be manipulated by altering binding rates or modifying mechanical properties such as cell or ECM stiffness, which are predicted to shift the bistable range	Irons et al. (2018), Irons et al. (2020)
Cell adhesion dynamics regulated by substrate stiffness	In this more fundamental study on mechanosensing, a discrete element method was used to simulate stress fibers, cell–cell and cell–matrix adhesion for 3D cell pairs on a 2D substrate with results compared to traction force microscopy experiments. The model recreates an experimentally observed phenomenon of cell decoupling as substrate stiffness increases and importantly provides a hypothesis for the underlying mechanism, which is a mechanosensitive competition between different fiber-adhesion configurations (focal adhesion (FA) to adherins junctions to FA, vs. FA to FA), which lead to the presence of a threshold stiffness at which cell–matrix adhesion is favored over cell–cell adhesion. Decoupling has been observed in ASM cells as ECM stiffness increases (Polio et al., 2019), and understanding the underlying mechanisms and potential relevance to asthma (where ECM stiffness is elevated) is key since changes in cell–cell and cell–matrix connections can alter contractile force transmission as well as intracellular signalling. This phenomena was modelled for cell pairs on a static substrate and it remains to be tested if similar thresholds can be found with more than 2 cells as well as in the presence of oscillatory loading, and if such a threshold remains within the physiologic range of stiffnesses relevant to healthy or asthmatic conditions	Vargas et al. (2020)
Positive feedback loop between stiffness and fibrosis	With an agent-based model coupled to a 2D spring network for tissue mechanics, the authors hypothesise a positive feedback loop where increased tissue stiffness leads to enhanced fibroblast activation and collagen deposition. Additionally, springs reach a high enough strain (induced by stiff neighbouring springs) to undergo rupture. When imposing these rules in their model, spatial patterning consistent with subpleural "honeycombing" patterns seen in CT images of IPF patients is obtained, provided fibroblasts begin in a low activation state. Such agent-based models help to identify plausible hypotheses for experimental observations, but the proposed mechanisms require experimental verification. As with other pathological positive feedback loops mentioned, verification (or therapeutic strategies) could involve targeting ECM stiffness or mechanosensitive fibroblast pathways in order to disrupt this feedback	Wellman et al. (2018)

proliferation, adhesion, and short- and long-range signalling to be included (Osborne et al., 2017). Popular approaches for modelling tightly-packed cells are the off-lattice vertex-based models in which each cell is typically modelled as a polygon (each side modelled as a spring) to represent the cell membrane with multicellular tissue effectively modelled as a network of elastic springs. Each cell vertex moves according to a balance of forces due to limited compressibility, cytoskeletal contractility and cell-cell adhesion with additional rules governing cell neighbour rearrangements, proliferation and apoptosis. These types of models have generally been applied to epithelial tissues (e.g., (Nestor-Bergmann et al., 2018)). Preliminary studies suggest that this approach appears to be the most promising avenue for modelling multicellular arrangements of ASM cells in which intracellular contractile apparatus can be introduced and changes in phenotype can be incorporated through modifications to their contractile machinery and therefore ability to respond to contractile stimuli and also through cell shape (to represent spindle-shaped contractile cells versus more rhomboid proliferative phenotype). Tissues built up from individual-

based cell models can then be used to represent confluent cell layers in traction force microscopy experiments providing validation of the underlying model, and from which stress–strain relationships emerge (on simulated application of load) that account for the cellular processes of contraction and phenotypic switching.

One of the greatest contributors to computational cost in simulating the cell- or tissue-level models discussed above is that of solving the HHM model to determine distributions of bound cross-bridges and thereby the force generated by ASM cells via acto-myosin crossbridge interactions. Isolated finite-thickness airway models that use the HHM model suffers from significant computational cost (e.g., (Hiorns et al., 2014; Hiorns et al., 2016)); furthermore the cost becomes prohibitive for whole lung branching networks. To provide computationally efficient ways of coupling muscle cell contraction to tissue behaviour, simplified models, first proposed by Zahalak (Zahalak, 1981) for the Huxley model for striated muscle, have been developed for ASM and corresponding HHM models by Rampadarath and Donovan (Rampadarath and Donovan, 2018). The idea behind this is that an ansatz is made regarding the form of probability distribution functions (PDFs) of the cross-bridge distributions, enabling the reduction of the governing PDEs to a system of ODEs in terms of the distribution-moments of the PDF. The idea is further extended to generalised distribution-moment approximations in (Donovan, 2020) and provides potentially viable alternatives to the full HHM model for incorporating ASM activity into branching network models. Other possibilities are the development of Gaussian process emulators used successfully in cardiovascular fluid dynamics (Melis et al., 2017), the pulmonary circulatory system (Noè et al., 2016) and ventricular mechanics (Di Achille et al., 2018). Essentially the computationally expensive mathematical model is replaced with a computationally cheap statistical surrogate model (the emulator) obtained via numerical simulations of the underlying partial differential equations covering as much of the parameter space as possible combined with multivariate smooth interpolation between solutions using methods from non-parametric Bayesian statistics based on Gaussian processes. As far as we are aware such approaches have not been used in the context of cell–tissue interactions in airways but could be a promising avenue allowing for more accurate representation of the underlying biophysical mechanisms.

6 Conclusion and outlook

The dependence of ASM cell responses on airway mechanical environment and loading conditions is now well-established in acute events such as agonist-induced bronchoconstriction and over longer timescales in airway remodelling. Mechanopharmacology and the importance of considering such mechanobiological effects when identifying and optimising pharmacological interventions has been acknowledged in clinical and experimental communities, and should be of high priority in asthma where the interplay between mechanics and cellular processes—including active contraction generated by crossbridge cycling within ASM cells as well as pro-remodelling and pro-inflammatory biochemical signalling cascades—are so closely coupled. One key example of a mechanopharmacological strategy noted above is the study by Wang et al. (2019), in which relaxation of ASM was observed in tissue strips with synergistic effects between the application of force oscillations and low dose β_2 agonists and/or a ROCK inhibitor, which reduce contractility and cytoskeletal stiffness respectively. It is thus proposed that either of these interventions would allow for the beneficial effects of pressure oscillations to be obtainable with lower amplitude oscillations. This observation also indicates that expected effects of these pharmacological agents when measured under static conditions may not translate well to the *in vivo* setting, due to the expected synergy when combined with oscillatory loading. So far, the proposed area of mechanopharmacology has focused on encouraging the use of experimental systems where physiological loads can be applied, and in developing high-throughput methods for such systems (Krishnan et al., 2016). In this review, we aim to have highlighted the potential for using mathematical models to complement this approach in asthma, where numerous modelling frameworks currently exist across scales that consider various aspects of lung and airway mechanics. Although model extensions and integration are still required, there is considerable opportunity to then design or optimise new mechanopharmacological therapies, where *in silico* exploration can yield improved mechanistic insights into these multiscale processes as well as reducing the experimental burden.

We highlighted some model generated hypotheses that remain to be experimentally validated (Table 1), but it is also worth highlighting some specific experimental approaches that could benefit from complementary modelling frameworks for the development of predictive models. Park et al. (2015) demonstrated how high-throughput drug screening can be improved by ensuring assays being used most closely mimic the biomechanical (micro)environment. The assay they developed uses human cells of patient- and disease-dependent phenotype, and embed mechanical measurements of cell contractility, providing a rich source of data for use in appropriate mathematical models. Other important experimental approaches that maintain a mechanical environment that is as close as possible to that of the airway *in vivo* are those involving precision-cut lung-slices. These approaches date back to the studies of Dandurand

et al. (1993) and Martin et al. (1996) in which the effect of cell contractility on airway narrowing could be directly measured, while the study of Bergner and Sanderson (Bergner and Sanderson, 2002) established the concurrent imaging of intracellular calcium concentration. More recently the effect of immune responses have been studied using PCLS (Henjakovic et al., 2008) as well as longer term changes such as alveologenesis (Akram et al., 2019). Interested readers are directed to the comprehensive reviews of Sanderson (Sanderson, 2011) and Liu et al. (2019). These *ex vivo* assays not only maintain a similar mechanical environment to the airway *in vivo* (e.g., a more realistic airway geometry embedded in parenchyma, compared with isolated tissue strips), the ECM and other cell types that interact with ASM cells such as epithelial cells and mast cells are still present, which continue to interact during the experiment. The combination of PCLS with the ability to externally load the lung slice as demonstrated by Lavoie et al. (2012), and the use of tissue traction microscopy to quantify contractile forces in PCLS (Ram-Mohan et al., 2020) opens up further avenues for exploring the interaction of cell- and tissue-mechanics with cell signalling in integrative mathematical models (e.g., (Wang et al., 2008; Brook et al., 2010; Maarsingh et al., 2019; Pybus et al., 2021a)). Organ-on-a-chip (Huh et al., 2010; Huh, 2015) and development of 3D organoid technologies (Güney et al., 2021) also allow inclusion of multiple cell types and ECM as well as application of physiological loading conditions. For instance in the combined experimental/modelling study of Kilic et al. (2019), bronchial-chip results indicate positive feedback between ASM contraction and compressive stress on epithelium. They were able to fit a minimal ODE model (consisting of the three variables—contraction level, spasmogen and relaxant—with competing positive and negative feedback loops) to the experimental data. The authors demonstrate the development of persistent contraction over shorter time scales in a similar mechanism to one that emerged out of the longer-term remodelling study of Hill et al. (2018). Additionally the Kilic *et al.* model suggests that exposure of ASM to beta-blockers could lead to longer term beneficial effects of endogenous bronchodilators such as PGE2 (while acknowledging the primary effect of acute bronchoconstriction). More detailed cell signalling models exploring the effects of modulated inflammatory response and mucous metaplasia could be of particular benefit here. The mechanopharmacological bioassays described above complemented with multiscale models that account for both mechanics and the relevant pharmacodynamics could be combined to achieve the broader goals of drug efficacy and trial success.

Current therapies in asthma focus almost exclusively on targeting inflammation and reducing ASM force (as discussed above). However, even when inflammation is controlled, asthmatic airways remain hyperresponsive suggesting that alternative mechanisms may play a role in maintaining this state. Some candidate mechanisms emerge from integrative studies cited above, but remain to be experimentally confirmed. An important mechanical component that hitherto has received less attention than ASM is the role of ECM and ASM–ECM interactions (although accounted for in simplified ways in some of the above cited studies). There is increasing evidence for a synergistic relationship between ASM and ECM over long timescales (see (Parameswaran et al., 2006) for a complete review); briefly, ASM cells in the synthetic phenotype can deposit ECM proteins, are involved in the downregulation of MMPs and upregulation of TIMPs which modifies the ECM, and ECM proteins in turn play a role in regulating ASM proliferation, migration and contraction (e.g., (Naveed et al., 2017) and references therein). These regulatory mechanisms (or aberrations thereof) are likely to play an important role in airway remodelling. On shorter timescales Stasiak et al. (2020) show that ECM regulates agonist-induced calcium oscillations in ASM cells; calcium responses in healthy donor ASM cells and stiffened underlying ECM were consistent with responses that would be expected with significantly higher agonist dose. Similarly, interactions between the ASM and a stiffer ECM environment have been shown to lead to increased ASM contractility (Polio et al., 2019) and enough to lead to hyperresponsiveness (Jamieson et al., 2021). All of the above are characteristic features of asthma, suggesting that detailed models of both acute bronchoconstriction and long-term remodelling designed to investigate mechanopharmacological therapies will benefit from the inclusion of the mechanics and regulatory aspects of ECM in airways.

Finally, we discussed methods and modelling frameworks from other contexts where progress has been made. One focus was on models where long term tissue remodelling is driven by (and affects) cell signalling. Detail about specific signalling pathways is lacking in the existing mathematical models of airway remodelling, although there is again much opportunity to expand in this area and to make use of the recent rapid growth of genomic and proteomic data. Models incorporating detailed mechanosensitive cell signalling pathways will be essential for many mechanopharmacological studies, since pharmacological interventions operate at the level of specific intracellular signalling proteins and/or cell surface receptors, alone or in combination. Incorporating these pathways into larger scale modelling frameworks will eventually allow for exploration of many important factors relevant to heterogeneity of response within the lung, the variable responses of heterogeneous populations, including patient-specific geometries or the roles of aging (i.e., associated with modified airway stiffnesses). With the growing availability of large scale datasets and increasing computing power, data-driven approaches could play a more important role in both informing model development as well as in the inference of parameters and underlying non-linear relationships, which are needed to improve the predictive power of such models.

In order to accelerate our understanding of respiratory health and disease and to propose new therapies, we must consolidate rapid and exciting developments in the theoretical and experimental

communities, and promote increased interaction and integration of information not only across scales, but also across disciplines.

Author contributions

Both authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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