Integrating mechanical cues with engineered platforms to explore cardiopulmonary development and disease

Donia W. Ahmed[1,*], Madeline K. Eiken[1,*], Samuel J. DePalma[1], Adam S. Helms[2], Rachel L. Zemans[3], Jason R. Spence[4], Brendon M. Baker[1], Claudia Loebel[1,5]

[1]Department of Biomedical Engineering, University of Michigan, Lurie Biomedical Engineering Building, 1101 Beal Avenue, Ann Arbor, MI 48109, USA
[2]Division of Cardiovascular Medicine, University of Michigan, Ann Arbor, MI 48109, USA
[3]Department of Internal Medicine - Division of Pulmonary Sciences and Critical Care Medicine – Gastroenterology, University of Michigan, 109 Zina Pitcher Place, Ann Arbor, MI 48109, USA
[4]Department of Internal Medicine – Gastroenterology, University of Michigan, 109 Zina Pitcher Place, Ann Arbor, MI 48109, USA
[5]Department of Materials Science & Engineering, University of Michigan, North Campus Research Complex, 2800 Plymouth Rd, Ann Arbor, MI 48109, USA
*Equal contribution

Summary

Mechanical forces provide critical biological signals to cells during healthy and aberrant organ development as well as during disease processes in adults. Within the cardiopulmonary system, mechanical forces, such as shear, compressive, and tensile forces, act across various length scales, and dysregulated forces are often a leading cause of disease initiation and progression such as in bronchopulmonary dysplasia and cardiomyopathies. Engineered *in vitro* models have supported studies of mechanical forces in a number of tissue and disease-specific contexts, thus enabling new mechanistic insights into cardiopulmonary development and disease. This review first provides fundamental examples where mechanical forces operate at multiple length scales to ensure precise lung and heart function. Next, we survey recent engineering platforms and tools that have provided new means to probe and modulate mechanical forces across *in vitro* and *in vivo settings*. Finally, the potential for interdisciplinary collaborations to inform novel therapeutic approaches for a number of cardiopulmonary diseases are discussed.

Introduction

Mechanical forces are integral to tissue function and morphogenetic processes, and aberrant tissue mechanics have been linked to disease and developmental defects. Within the lung and heart, mechanical forces including shear, tension, and compression occur continuously as air and blood circulate through the cardiopulmonary system. Additionally, integral to tissue function is the ability of cells to perceive mechanical forces and other environmental cues that modulate their function and differentiation.[1–5] As such, mechanical forces regulate tissue function at different scales, ranging from macroscale (i.e., tissue) to micro/nanoscale (i.e., cellular/subcellular). The importance of mechanical forces in tissue development and disease has led to significant interest and efforts in engineering tools that may enable deeper mechanistic understanding of how mechanical forces define biological signaling. One of the goals is often to recreate and mimic mechanical forces (e.g., compression, tension and shear) that cells and their surrounding extracellular matrix components experience. Although there are many excellent examples where such platforms have been integrated into fundamental biology, the challenge ahead is to more widely integrate engineered models of tissue function and disease into cardiopulmonary biology. This review article first aims to establish the current understanding of mechanical forces in cardiopulmonary tissue development and disease. We will highlight examples of engineering tools that recapitulate mechanical forces at different length scales ranging from tissue to subcellular level. Finally, emerging concepts are highlighted in the context of collaborative studies, leveraging the potential of interdisciplinary collaborations and informing therapeutic approaches for a number of cardiopulmonary diseases. The goal of this review is to emphasize the importance of mechanical forces in studies of cardiopulmonary development and disease including towards drug development and testing. Although not a comprehensive

review of the vast literature that is available on engineered in vitro culture models, we highlight a set of studies towards improving integration of mechanical cues into cell culture.

Physical laws govern tissue self-organization and morphogenesis

Tissue morphogenesis involves the process of cell specification, organization, and the resulting shaping of the developing tissue, which often involves changes in cell number, shape, contractility, and position as well as concurrent changes in the surrounding matrix. Critical for these constituent processes are dynamic and spatially heterogeneous mechanical forces (Figure 1).[2] For example, fluid pressure either through active surface tension (i.e., cohesive forces of a liquid's surface that allows it to resist external forces) or passive shear stress (e.g., due to frictional forces generate by liquid flow) can shape tissue architecture, where cells sense these forces and respond to actively shape tissues through local proliferation/apoptosis or collective motion. Detailed descriptions of these processes are described in several excellent reviews.[3,5,6] Below, we provide a general description of key morphogenetic mechanical signals that are critical in probing mechanisms of tissue function and dysfunction.

Fluid pressure and tension (Figure 1-i): Fluid flow regulates embryonic development, and its influence is seen as early as during the establishment of left-right asymmetry after gastrulation. Following the posterior positioning of motile cilia, the established right-to-left directional fluid flow is thought to be sensed by mechanoresponsive cilia, leading to distinct genes expressed on each side of the embryo (e.g., Krüppel-like factor, Klf2a, paired like homeodomain 2 (PITX2), Nodal nodal growth differentiation factor).[7] Blood flow is also crucial for sculpting the heart. For example, during cardiac looping, the heart tube forms a C-shape like structure, resulting in variations in flow rates and pressure which induces the pharyngeal arch to grow in diameter while others degenerate. In adults, pressure gradients and flow rates are tightly regulated to maintain cardiac tissue function and tissue oxygenation.[6] Similarly, fluid pressure regulates the formation of the early tracheal and bronchial buds and subsequent alveologenesis. Upon birth, the lungs undergo a rapid transition from oxygenation via the placenta to direct breathing of air. For example, thyroid transcription factor 1 (TTF-1) promotes transcription of surfactant proteins that regulate surface tension homeostasis in the lung.[8] Whether the lung is mature enough to adapt to air depends on the sufficient production of pulmonary surfactant, which is required to lower surface tension and prevent atelectasis.[9,10]

Volumetric growth or contraction (Figure 1-ii): Morphogenetic changes are generally based on local volumetric shrinkage or expansion, either through programmed cell shrinkage or death, or cell hypertrophy or proliferation. In addition, local degradation or accumulation of ECM as well as gradients of signaling molecules (e.g., retinoic acid (RA), bone morphogenetic proteins (BMP-4), fibroblast growth factors (FGF)) often act to reinforce cell-mediated changes in tissue shape. Interaction between cells and their surrounding matrix further enables the generation of cellular forces. Cytoskeletal contractility and coordinated transmission of active forces across multiple cells are critical for both cardiac and pulmonary development.[11,12] For example, current understanding is that contractile forces of regional myofibroblasts leads to secondary septation during the final stages of lung maturation, and thus facilitates the increase in alveolar epithelial surface area.[13,14] Throughout this process, cellular activity is guided through changes in transcription factors such as homeoprotein NKX2.1, GATA6 and hepatocyte nuclear factor-3 bone-morphogenetic protein 4 (BMP-4) to mediate epithelial proliferation and differentiation.[15] Within the heart, early heart tube formation is driven by carefully orchestrated endodermal actomyosin contractions[16,17], and impaired contractility impacts normal myofilament growth and maturation.[18] Thus, contractile force itself is a major input for normal cellular maturation from an early developmental stage. During organ level development, an equilibrium is attained, as myosin heavy chain contractility in ventricular cardiomyocytes restricts cellular growth, counteracting hemodynamic forces that induce cellular elongation, helping to define the curvature of the ventricle.[19]

Cellular contacts (Figure 1-iii): Collective motion of multiple cells in development is often associated with the process of jamming and fluidization. Cellular jamming describes the phenomenon of collective cell migration that increases epithelial rearrangement and plasticity to allow for tissue elongation or branching.[20] High compressive stresses experienced by cells in a jammed state facilitates tissue remodeling. As tissues are sculpted, cells transition from a fluid-like unjammed phase to a solid-like jammed phase, which leads to the formation of regional

tissue structures.[21] For example, in vertebrae body axis elongation, jammed and unjammed tissue regions provide a gradient of mechanical stress to guide morphogenetic flow and unidirectional tissue extension.[22–24] During lung alveologenesis, transient unjamming of the lung mesenchyme enables the growth of epithelial layers and local airway branching.[20,25] The underlying molecular events of jamming and unjamming are associated with several signaling cascades and downstream pathways including extracellular signal–regulated kinase (ERK) 1/2, mitogen-activated protein kinase (MAPK).[26] Within the heart as cells become overcrowded, more contractile cells will delaminate from the myocardial wall, seeding cardiac trabeculations.[27] In lung disorders like asthma, bronchospasms are caused by disrupted jamming transitions, leading to a change in cell shape and alignment.[23]

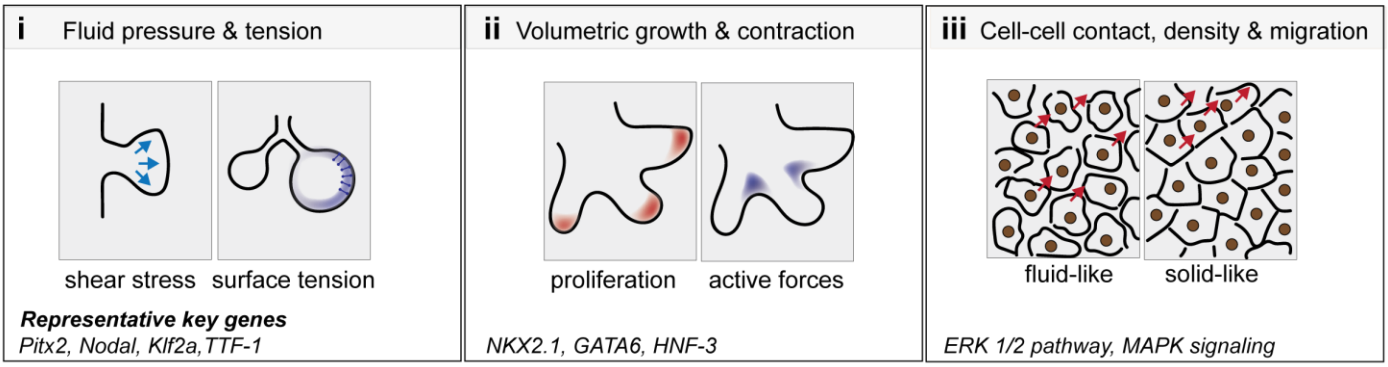

Figure 1 Physical laws control cardiopulmonary tissue shaping, transcriptional programming, and function. (i) Increase in hydrostatic pressure in the epithelial lumen and adequate surface tension determines tissue shape during development and growth; **(ii)** Tissue size and shape further result from volume expansion by local cell proliferation and contraction through active cellular forces; **(iii)** Multicellular collective motions within a tissue are often accompanied with cell unjamming (fluid-like) while jamming (solid-like) is required for sculpting tissue structures.

Mechanical forces in cardiopulmonary diseases

Recent advances in understanding how mechanical forces guide cardiopulmonary development have provided new insights into their contributions to congenital and acquired diseases. Both active and passive forces are known to regulate disease development and progression. Here, we describe examples of mechanical forces acting at different length scales that progress the pathogenesis of cardiopulmonary organs (Figure 2).

Tissue level

Lung development is described in several stages, including the embryonic, pseudoglandular, canalicular, saccular, and alveolar stage. Although this process starts early in fetal development, the alveolar stage is not completed until several years after birth. Mechanical forces are thought to directly contribute to all stages of lung development, and defects in the generation of forces underlie many types of congenital diseases (Figure 2A-i). The formation of the initial lung buds from the ventral foregut endoderm is followed by branching morphogenesis, a series of stereotypic bifurcations that establish the arborized airways.[28] At this embryonic stage, the developing lung is filled with amniotic fluid which results in positive transmural pressure.[29] Insufficient amniotic fluid (oligohydramnios) often leads to pulmonary hypoplasia, indicating that mechanical forces exerted by positive transmural pressures are essential throughout development.[30,31] Indeed, retinoic acid (RA) signaling, known to be locally regulated during lung development, is activated by transmural pressure, resulting in airway epithelial branching during the pseudoglandular stage.[32,33] In addition, animal models of pulmonary hypoplasia, altered fibroblast growth factor 10 (FGF 10) expression suggests a critical role in the regulation of fluid pressure and branching morphogenesis defects.[34–36] As branching continues into the canalicular stage, the distal tips of the branches bifurcate until the formation of terminal saccules that then mature into alveoli.[28] During distal sac formation the magnitude of mechanical forces exerted onto alveolar epithelial cells specifies their fate and function.[37] For example, cells exposed to heightened mechanical tension generated by the movement of amniotic fluid differentiate into flat alveolar epithelial cells while those shielded from these forces ultimately differentiate

into cuboidal alveolar progenitor cells.[37] Similarly, mechanical tension induced by regional pneumonectomy led to local activation of YAP/TAZ nuclear translocation and subsequent alveolar regeneration (Figure 2A-ii).[38] However, when exposed to excessive positive pressure such as during mechanical ventilation, the premature infant lung can undergo abnormal growth and deformations which disrupt airway epithelia. Here, the activation of mechanoreceptors such as TRPV4 increases the release of inflammatory cytokines in the developing lung.[39] Another example is the natural capacity of the lungs to elastically recoil inwards which is essential for lung deflation (Figure 2A-iii), but if compromised such as due to proteolytic destruction (e.g., due to inflammation) of alveolar ECM components (collagen, elastin) in lung disorders like emphysema, the alveolar walls become weakened. As a result, nonphysiologically high mechanical forces cause local failure of alveolar walls, further contributing to the progression of disease, which is also observed in fibrotic disorders.[40] Notably, in both emphysema and COPD, α1-antitrypsin deficiency is one of the major causes for increased elastolytic enzymes that degrade the elastic fiber network. Mechanical forces during cardiac development and growth follow similar mechanisms where local compressive stresses and directed buckling guide cardiac looping and blood flow guided by genes such as PITX2 (Figure 2B-i).[41,42] The heart tube is fixed at the two poles and as it grows it begins buckling as a result of left/right asymmetries that form longitudinally beginning in the first stages of cardiac looping.[43] Additionally, during late stages of development, abnormal alignment of the heart chambers, deficient remodeling of the inner curvature of the loop, or enlargement of the atrioventricular canal all can contribute to congenital heart defects such as misalignment of the heart chambers or ventricular septal defects.[43,44] After cardiac looping, the heart tube folds in on itself to form trabeculations, pieces of muscle fibers that extend into the heart chamber and are responsible for early contractility of the heart. Critical for this process of trabecular compaction are synchronized cardiomyocyte division and cytoskeletal contractility.[27] If either process is disrupted, abnormal compaction during development results in left ventricular noncompaction, a subtype of cardiomyopathy. In noncompaction cardiomyopathy, a relatively thinned compacted myocardial layer with excessive trabeculation in the left ventricle may lead to contractile dysfunction and heart failure.[45] In addition, blood flow, shear stress, and wall tension (via blood pressure) are all critical to normal cardiomyocyte growth and replication during development – if these parameters are altered in any developing cardiac chamber, its development is perturbed. For example, hypoplastic left heart syndrome (maldevelopment of the left-sided chambers) results from either severe congenital aortic valve stenosis (reducing flow out of the left ventricle) or severe congenital mitral valve stenosis (reducing flow into the left ventricle).[46,47] Similarly, alteration of normal flow through the right heart (e.g. pulmonary atresia or tricuspid valve atresia) causes dysregulation right ventricular development and hypoplastic right ventricle.[48] Following birth, continuous blood flow and pressure on cardiomyocytes remain critical for cardiomyocyte maintenance, and the effects of perturbations in these parameters accumulate over years of life, potentially leading to heart failure (Figure 2B-ii).[49] For example, increased volume through the right heart occurs with intracardiac shunting from septal defects, eventually leading to right-sided chamber enlargement and dysfunction. Increased pressure afterload from hypertension leads to cardiomyocyte hypertrophy, which eventually becomes maladaptive (the most common cause of heart failure in adults).[48]

Cellular level

At the cellular level, spatiotemporal patterning, cell contractility and jamming/unjamming tissue phase transitions are all critical to tissue morphogenesis but can also contribute to disease development and/or progression in adults. For example, alveologenesis is directed by spatially patterned fibroblast populations including secondary crest myofibroblasts (SCMF) that are highly contractile (Figure 2C-i). SCMF generated forces have been proposed to locally deform the developing alveoli.[13] Recent reports have also begun to investigate the role of genes like insulin-like growth factor 1 (IGF)1 and SRY-box transcription factor 9 (Sox-9) in maintaining contractile properties of lung fibroblasts and nuclear YAP activity necessary for alveologenesis.[50] However, models to study the effects of mechanical loads and molecular mechanisms on fetal lung are still limited.[32] On the other hand, hypercontractile cardiomyocytes, for example, arising from mutations in β-cardiac myosin, lead to hypertrophic signaling and widespread, aberrant remodeling of the myocardium (cardiomyopathy).[51] Local increases in compressive stresses on the bronchial epithelium have been shown to cause the typical buckling of the airway walls and epithelial cell jamming (Figure 2C-ii).[23] In asthma, cellular jamming is delayed and inefficient, suggesting an immature epithelial phenotype that is perpetuated by increased mechanical loading during

bronchospasm.[23,31,52] Dysregulation of integrity (integrin β6) and cytoskeletal remodeling genes (DPYSL3, FERMT1) have been reported indicators of severe asthma, highlighting the role of this migratory cell phenomenon.[53–55]

The role of cellular forces in fibrotic remodeling is perhaps one of the most appreciated mechanisms leading to both pulmonary and cardiac fibrosis.[56] In the lung, activated myofibroblasts contribute to fibrosis through aberrant collagen deposition, leading to impaired gas exchange and matrix stiffening causing difficulties in breathing.[57] More recently, cell division cycle 42 (CDC42)-deficient, injury-induced alveolar loss has been proposed to lead to sustained alveolar mechanical tension which was shown to inhibit alveolar epithelial cell-induced repair and thus led to progressive fibrosis, probably due to the accumulation of pre-fibrotic epithelial cells and secretion of transforming growth factor beta 1 (TGFβ1, Figure 2C-iii).[58,59] In addition, upregulation of ECM genes such as collagen type XVII alpha 1 chain (COL17A1) and matrix metalloproteinase-1 (MMP1) increases matrix remodeling that alters cellular mechanosensing of matrix composition and stiffness that promote TGFβ1 expression seen in fibrotic disease.[60]

Similarly, in the heart, excessive contractility due to sarcomere gene variants that increase myofilament sliding velocity and consequently heighten wall tension also lead to myofibroblast activation.[61] In contrast, reduced force transmission due to sarcomeric variants associated with hypo-contractility leads to ineffective transmission of force to the extracellular matrix, limited protection from mechanical stress, and thus subsequent thinning of the myocardial wall.[62,63] Consequently, cardiomyocytes become hypertrophic in response to increases in contractile demand, and the additional stress feeds into cardiac fibroblast proliferation and fibrotic matrix deposition (Figure 2C-iv).[64–66] Thus, the fibrotic response appears to be a convergent pathway stemming from chronic, abnormal levels of cardiac contractility and wall stress, similarly mediated by connective tissue growth factor (CTGF) and TGFβ1-activated myofibroblasts regardless of initial etiology (hypertrophic or dilated cardiomyopathy subtype).[67]

Subcellular level

Fibrotic remodeling is one example where bidirectional signaling between cells and ECM critically regulates disease initiation and progression. The predominant class of mechanical linkers between cells and ECM are integrins. Although essential for tissue morphogenesis and maturation, integrin expression can rapidly change in disease. For example, within the distal lung, $\alpha 5\beta 6$ integrins are expressed by alveolar epithelial cells and are known to be essential for epithelial maturation.[68] However, repetitive alveolar injury has been shown to increase expression of $\alpha 5$ domains in epithelial cells. Here, increased cellular traction through $\alpha 5\beta 1$ integrins is one of the key mechanisms in the activation of the pro-fibrotic growth factors, including through the release of TGFβ from its latent complex sequestered within the ECM.[69] Similarly, activation of latent TGFβ1 in heart tissue is directly linked with increased contractility of myofibroblasts through integrins $\alpha v\beta 5$ and $\alpha v\beta 3$.[69–71] Cardiac cells also actively interact with the ECM through vinculin, one of the key proteins of focal adhesions. Vinculin localization is regulated by active cell forces as in during cardiomyocyte contractility and serves as key initiation points for sarcomerogenesis, myofibril assembly, and cortical cytoskeletal organization.[72–74] There are numerous genes implicated in this process[75] although most of the primary sensors of mechanical stress within the heart are not known. Alterations in vinculin or other focal adhesion proteins (e.g., filamin C) have also been shown to contribute to dilated cardiomyopathies and pulmonary fibrosis.[76,77]

Transmission of extracellular mechanical signals occurs through several mechanisms. YAP/TAZ signaling is perhaps the most well-studied mechanotransduction pathway during cardiopulmonary development and disease.[78–82] Inactivation of YAP/TAZ has been observed in underdeveloped lungs of diseased infants with bronchopulmonary dysplasia, presumably due to reduced contractility.[50] During heart development, atrial expansion activates YAP/TAZ nuclear translocation due to increased mechanical strain on the endocardium through VE-cadherin.[83] Additionally, increased YAP expression and decreased inhibitory phosphorylation at Ser127 have been observed in both tissue samples from patients with hypertrophic cardiomyopathy (HCM) and mouse models of myocardial infarction.[84] In addition to YAP/TAZ, mechanical signals are transmitted through Piezo1-dependent Ca^{2+} influx and ATP release. Aberrant Piezo-1 activation has been linked to cardiac hypertrophy, cardiopulmonary fibrosis and pulmonary arterial hypertension.[85,86] Notably, increased Piezo-1 activity has been shown to promote degradation of VE-cadherin, thus contributing to pressure-induced

pulmonary edema.[87,88] In addition, lung epithelial cells sense mechanical stress through autocrine loops (Figure 2D-i). For example, compressive stresses on the epithelium during bronchoconstriction provoke shedding of epidermal growth factor (EGF) into the intracellular space and subsequent binding of EGF receptors necessary for mechanosignalling.[24,89,90] In the heart, individual cardiomyocytes are connected to each other through intercalated discs (desmosomes, adherens and gap junctions) that transduce contractile force between cells (Figure 2D-ii).[91]

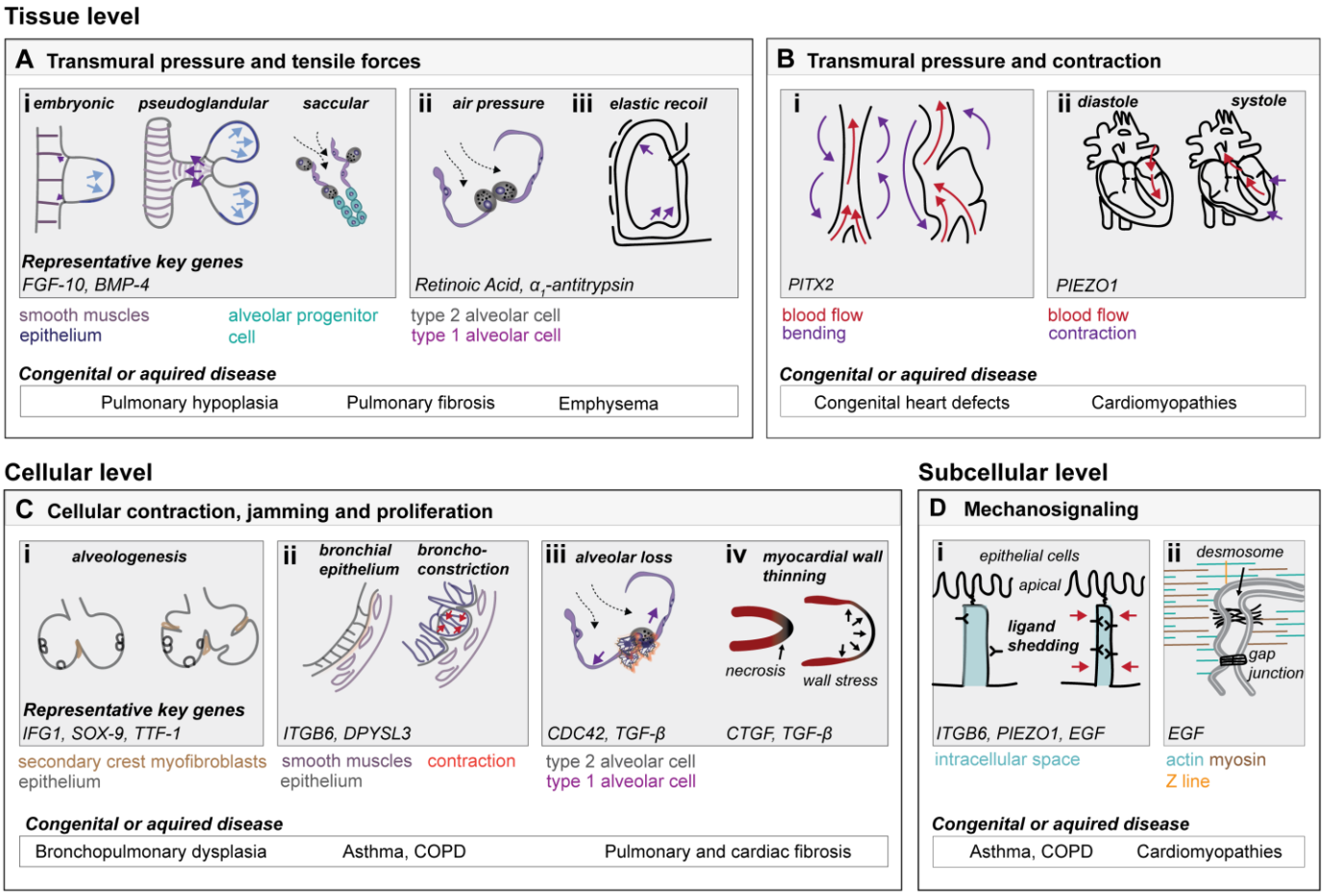

Figure 2 Mechanical forces during cardiopulmonary tissue morphogenesis and associated disease A (i) Positive transmural pressures regulate lung development during embryonic, pseudoglandular and saccular stages; **(ii)** In young adults, increase in mechanical tension initiates alveolar regeneration through differentiation of type 2 alveolar epithelial (AT2) cells to type 1 alveolar epithelial (AT1) cells; **(iii)** Elastic recoil of the lung is essential for deflation of the lung during expiration. **B (i)** Local compressive stresses and directed buckling guide cardiac looping and blood flow; **(ii)** Continuous blood flow and increasing pressure on cardiomyocytes is critical for myocardial growth and maintenance. **C (i)** Local contractility of secondary crest myofibroblasts deforms and shapes alveoli during development; **(ii)** Jamming to unjamming transition of bronchial epithelial cells during bronchoconstriction; **(iii)** Injury-induced alveolar leads to sustained increase in mechanical tension leading to accumulation of pre-fibrotic alveolar epithelial cells and fibrotic remodeling; **(iv)** Thinning of the myocardial walls upon myocardial injury. **D (i)** Compressive stresses on the bronchial epithelium shrink the lateral intercellular space that promotes ligand shedding responsible for downstream mechanotransduction. **(ii)** Dysfunction of adherens, desmosomal and gap junctions in cardiomyocytes leads to defective mechanical coupling and cardiomyopathies

Engineering tools to study mechanical forces *in vitro*

Engineering tools, ranging from relatively simple biomaterial cell culture platforms to more complex systems such organs-on-a-chip devices, have provided insights into how mechanical forces regulate cell signaling and function *in vitro*. There have been several excellent reviews on the development of engineering tools for cell and tissue engineering.[92–95] In this section, we highlight some key engineering approaches towards studying cardiopulmonary development and disease with a focus on accessible techniques with high potential for translation into biology-focused laboratories.

Engineered cell culture models

There has been an increasing interest in using synthetic or natural extracellular matrix-derived biomaterials for cell culture. Recent advances in material engineering have further enabled spatiotemporal control over biomaterial properties to dynamically and on-demand modulate mechanical forces that cells experience *in vivo.* One example is hydrogels, water-swollen polymer networks, that are tunable in their mechanical properties including viscous behavior and elastic moduli (Figure 3A).[96,97] Hydrogels that offer tunability over their modulus have been useful in identifying the role of elasticity and mechanical memory in pulmonary fibroblast differentiation into myofibroblasts.[98,99] To integrate dynamic changes of ECM mechanics, several approaches have been introduced to either stiffen or soften engineered hydrogels in the presence of adherent cells.[100] Examples include hydrolytically and enzymatically degradable polymers or crosslinkers that induce hydrogel softening over time in response to reaction with water or cell-secreted proteases,[101] In contrast, on-demand stiffening or softening can be achieved through engineering polymers that respond to external stimuli, such as pH, temperature, electric or magnetic fields or biological targets.[102] A relatively well-established example is the use of photosensitive crosslinkers to stiffen or soften hydrogels in response to light exposure.[103–105] Such hydrogels have been used to study pulmonary fibroblast function in responses to ECM stiffening such as observed during pulmonary fibrosis.[106] Towards mimicking the increasing ECM modulus of developing chick hearts, chemistries with slow crosslinking kinetics to induce time-dependent hydrogel stiffening enhanced the maturity and calcium signaling of cultured cardiomyocytes.[107,108] Thus, such dynamic hydrogels are useful towards recapitulating changes in mechanical stresses within the lung and heart.[104,109,110]

In addition to changing elastic moduli, hydrogels have been designed to provide control over tissue architecture (Figure 3B). For example, mimicking the curved geometry of alveoli may direct cellular organization and dynamics.[111–113] An approach to engineering curvature has been the use of polycarbonate membranes that are thermoformed into microwells, enabling physiologically relevant co-culture of epithelial and endothelial cells on opposite sides of a thin polymer films.[114] Hydrogels are also being applied towards mimicking the 3D architecture of pulmonary tissues such as the curvature present within bronchioles by leveraging mechanical instabilities between two hydrogel layers; this technique further allows for combination with magnetic particles to induce dynamic changes to scaffold formation and be used to study cell response to changing patterns relevant to disease conditions such as asthma.[115] In addition, microsphere hydrogels have been used to recreate the 3D architecture of alveolar tissue. Here, recent notable approaches include the use of sacrificial microspheres, which are pre-seeded with epithelial cells prior to embedding into another 3D hydrogel containing fibroblasts.[116] Another elegant study embedded epithelial cells in photodegradable microspheres within a 3D hydrogel, which then formed a monolayer within the voids after light-induced degradation.[117] These 3D models are powerful in mimicking aspects of the 3D architecture of the alveoli. However, the light absorption of the hydrogels renders cells relatively inaccessible and may be challenging to retrieve the cells for downstream analyses. In addition, fibrous microstructure is a ubiquitous feature of interstitial ECM that typically is lacking in engineered hydrogels. In recent work, composite hydrogels incorporating synthetic cell-adhesive fibers into soft hydrogels allowed for robust activation of myofibroblasts in 3D.[118]

For cardiomyocytes, the geometry of individual cells can be constrained using micropatterning on elastic substrates to investigate intrinsic relationships among cell shape, myofibrillar alignment, and contractile force. These techniques have been used to demonstrate that the optimal myofibrillar alignment and force generation occurs when the cell aspect ratio mimics that of cardiomyocytes in the in vivo heart (i.e. ~7:1), both in rat cardiomyocytes and in human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs).[119–121] This observation further scaled to 7:1 micropatterned multicellular iPSC-CM derived cardiac muscle bundles

micropatterned on elastic silicone polymers (elastic modulus ~8 kPa).[18] This system revealed a feedforward set of interactions among elongated geometry, myofibrillar alignment, uniaxial contractile direction, and contractile force during cardiomyocyte development.

Hydrogels for cell culture provide control over mechanical signals including viscoelasticity and geometry. In addition, tools that incorporate dynamic mechanical forces such as stretch through mechanical tension may be relevant to the cardiopulmonary system. Given that cyclic stretch is highly relevant to the cardiopulmonary system, introducing dynamic mechanical stimulation has been the focus of several recent strategies (Figure 3C). For example, cyclic stretch of cells seeded atop a silicone elastomer was used to determine the role of Piezo-1 in lung development[122] or studying pharmacokinetics under more physiological conditions, an important step forward for drug testing.[123] Although less controlled, explanted lung tubes have also been stretched directly in a customized device supporting the hypothesis that mechanical forces determine cell shape and mitotic spindle formation in the developing lung.[124] When performed in zebrafish heart models, stretching showed that cardiac contractility directs myofibril development.[125] In general, *in vitro* dynamic stretching of iPSCs-derived cardiomyocytes has been used in several studies to improve overall tissue function, and contributed to our understanding of how mechanical stretch feeds into in contractile myofibril function.[126–129] In the engineering of 3D cardiac tissue, a myriad of techniques has been reported, including flexible pillar systems (Figure 3C). Most investigators currently utilize variants of an elastomeric pillar system, wherein tissues assemble longitudinally between two flexible pillars that deflect under tissue forces, thereby providing a direct read-out of tissue function. The influence of extracellular matrix topography has also been investigated in cardiac tissue formation by patterning different organizations of a collagen layer atop polymeric materials. Surfaces with higher matrix alignment increased cardiac fibroblast differentiation and cardiomyocyte maturation, demonstrating the importance of extracellular organization matrix *vitro*.[18,73,130,131] As passive tension and afterload are critical for heart function, recent microfabrication strategies have been described to mimic physiological tissue loading. For example, formation of cardiomyocyte microtissues between two elastic pillars enables studying the impact of passive load on tissue maturation or dysfunction.[132–134] In these studies, using pillars with higher elastic moduli, heightened passive tension or afterload increased iPSC-derived cardiomyocyte tissue maturation. However, when the elastic moduli of the pillars were too high, the contractile function of cardiomyocytes diminished, providing an engineered system to study the effect of pathological afterload.[132–134] Finally, pacing of young iPSC-derived cardiomyocytes via electrical stimulation has been shown to markedly increase levels of tissue maturity.[135] However, engineers are often faced with limited experience with generating and culturing iPSC-derived cardiomyocytes. To address this companies including '*FUJIFILM Cellular Dynamics*' offer cardiomyocytes differentiated from human iPS cells (iCell Cardimyocytes). An important limitation of micropillars is the need for engineering techniques such as photolithography that may be inaccessible to non-engineering labs. Here, emerging companies such as '*4D cell.com' and 'curibio.com'* offer a range of organ-in-a-well® and engineered heart tissue platform technologies that may help address this limitation.

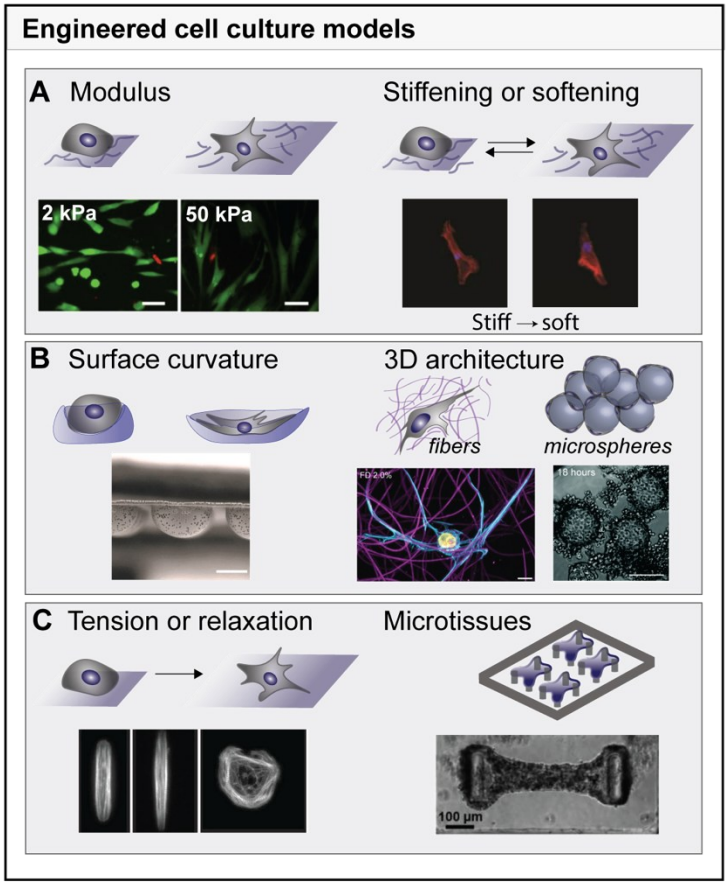

Figure 3 Applications of engineered cell culture models: A Polymeric hydrogels engineered to recapitulate the modulus and dynamics of tissue stiffening and softening. Representative fluorescent images of fibroblasts cultured on 1 (left) and 50 kPa (right) polyacrylamide hydrogels for 2 days (scale bars 100 µm, left) and maximum projections of single cells stained for f-actin (red) and nuclei (blue) (scale bar 50 µm, right). **B** Recreation of tissue surface curvature and 3D architecture using molds and biofabrication techniques. Representative images showing SEM image of a close-up of non-porous and porous microwells (scale bar 100 µm, left), high-resolution confocal *z-stack* projections of representative cells in samples with no, intermediate, and high fiber density (F-actin (cyan), nuclei (yellow), dexVS fibers (magenta), scale bar 10 µm, middle), and representative bright field images of lung epithelial cells (A549) progressively covering fibronectin-loaded microspheres (scale bar 200 µm, right). **C** Mimicking the dynamics of tissue using hydrogels atop stretching devices to mimic tension and relaxation (e.g., Lifeact-labeled actin in myofibrils in live hPSC-cardiomyocytes, scale bar 20 µm, or microposts to engineer 3D microtissues from neonatal rat cardiomyocytes to stimulate tissue contraction (e.g., representative brightfield image of cardiac microtissues).

Adapted with permission from the following: **A** Asano S et al., Physiol Rep 2017[97], Rosales AM et al., Angew. Chem. Int. Ed., 2017[104], **B** Baptista D et al., Biomaterials 2021[114], Lewis JR et al., Biomater. Sci. 2015[117], Matera DL et al., *ACS* Biomater. Sci. Eng. *2019*[194], **C** Ribeiro AJS et al., PNAS 2015[120], Boudou T et al., Tissue Eng Part A 2012[132].

Organ-level engineering tools

Advances in understanding how mechanical forces regulate the interplay between multiple cell types and guide changes in tissue architecture has also pushed the field of organ engineering. Lung models may involve *ex vivo* culture of explanted or sliced lungs as well as organ-on-chip model. For example, a microfluidic chest cavity was

generated using embryonic mouse lungs to determine how transmural pressure regulates lung development and branching morphogenesis (Figure 4A).[32] Precision-cut lung slices (PCLS) have been used to interrogate epithelial migration and cell clustering occurring during alveologenesis (Figure 4B).[136] However, these *ex vivo* lung culture models are generally limited to a few days. Thus, "lung-on-a-chip," models providing longer-term culture durations have been invaluable. For example, microfluidic devices have been used in probing mechanisms of alveolar-capillary barrier function during breathing (Figure 4C). These are often microfluidics-based silicone models, and may include multiple cell types and mimic the cyclic mechanical stretching resulting from breathing.[137] Recent advances in lung-on-chip models include a microdiaphragm that mimics the biaxial geometry of mechanical stretching and an air-liquid interface of the *in vivo* lung alveolus.[138] Towards engineering heart-on-chip models, high precision fabrication techniques such as two-photon 3D printing have been leveraged to recapitulate ventricular fluidic pumping.[139] Alternatively, fibrous polycaprolactone (PCL) scaffolds have been used to create organized cardiomyocyte tissues with a shape similar to that of a rat ventricle.[140] These systems have allowed further allowed significant advances in connecting multiple tissue-chip systems such as via a shared endothelium (Figure 4C).[141,142]

Another aspect of engineered organ-on-chip systems is the ability to interrogate how fluid pressure and flow direct cardiopulmonary development and disease. For example, vasculature-on-a-chip platforms have been developed to measure the impact of physiologic and pathologic shear stress on platelet aggregation, endothelial cell phenotype, and initiation of atherosclerosis.[143–145] Such systems further enable modeling arterial hypertension by mimicking the effect of blood pressure and wall shear stress on endothelial cells and thus provide a platform for drug testing and discovery.[146–148] Similarly, microfluidic devices have been used to induce shear flow across alveolar epithelial cells which was found to regulate surfactant secretion.[149] Towards larger scale experiments, perfusion bioreactors have been developed for cardiopulmonary cell and tissue culture. For example, cardiac perfusion bioreactors increase oxygen delivery and distribution across the cultured tissue, [150] which has been shown to improve the maturity and contractility of rat cardiomyocytes.[151] Similar to microfluidic devices, bioreactors are technically more challenging than standard cell culture, curtailing their widespread adoption.

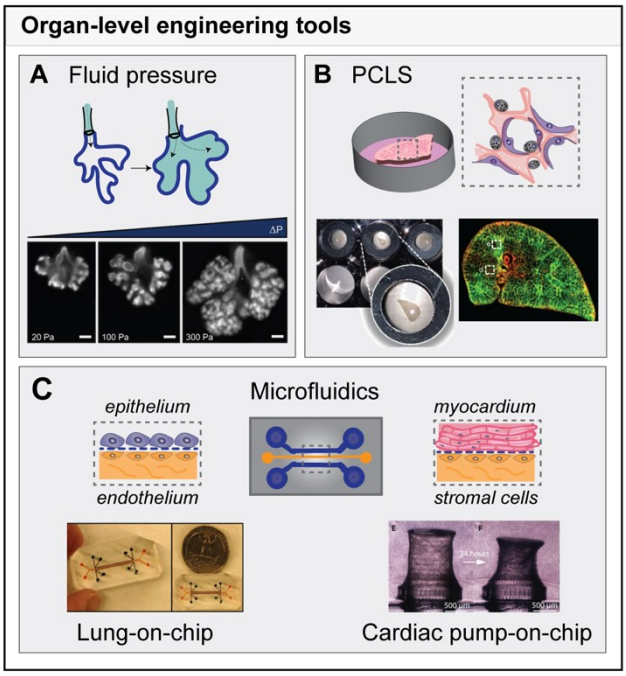

Figure 4 Applications of organ-level engineering tools: A microfluidic chest cavity models to study the role of transpulmonary pressures. Lung explants cultured at different pressure differences and immunostained for E-cadherin (scale bars: 200 μm); **B** precision-cut-lung slices (PCLS) to recreate the lung microenvironment *ex vivo*. PCLS in 24-well plate and representative widefield, single plane z-stack image of PCLS; **C** Organ-on-chip devices with representative images of lung-on-chip and cardiac pump-on-chip to mimic aspects of the lung and heart microenvironment *in vitro*.

Adapted with permission from the following: **A** Nelson C.M et al., Development 2017[32], **B** Akram et al., Nat Commun 2019[136], **C** Huh et al., Science 2010,[137] Michas et al., Sci. Adv. 2022.[139]

Measuring mechanical forces

Studying how cells respond to mechanical signals requires tools that enable quantification of the forces that cells reciprocally exert and perceive (Figure 5A). Collagen contraction assays were an early and primitive force measurement established in 1979[152], and are still widely used to measure fibroblast contractility, including in a recent study on myofibroblast contractility in alveologenesis.[14] However, these measurements are not precise enough to extract single cell traction forces. Measuring displacement of an underlying mechanically defined hydrogel matrix via traction force microscopy (TFM) provides far more accurate spatial characterization of local cellular forces. Typically, matrices are made from polyacrylamide or silicone-based elastomers with embedded fluorescent beads. For example, TFM was critical in quantifying contractility of alveolar fibroblasts during alveologenesis and jamming transitions of bronchial epithelial cells derived from healthy and asthmatic patients.[13,23] TFM has also been used to quantify contractile force in micropatterned cardiomyocytes.[119–121] Given that TFM requires fluorescent-bead containing elastomers it is currently primarily used in engineering labs, and is relatively inaccessible to biology labs without collaborators. In addition, atomic force microscopy (AFM) nano-indentation with a flexible cantilever allows for quantitative measurements of cardiac microtissue contraction including the beat rate and force as well as the spatial stiffness of fibrotic tissues (Figure 5B).[153,154] However, AFM can be technically challenging. Alternatively, mechanical forces can also be estimated from simple

measurements of cardiac muscle bundle displacements on deformable 2D substrates or silicone post displacements for 3D tissues.[18]

Towards measuring subcellular forces, molecular sensors that take advantage of fluorescence resonance energy transfer (FRET) have been developed, including sensors for cell-ECM interactions and intracellular mechanosignalling (Figure 5C).[155] For example, within the lung, FRET-based sensors have been used to measure cellular traction forces and their colocalization to Piezo 1[156] or cell-cell forces mediated by cadherin in response to mechanical tension on pulmonary endothelial cells.[157] Similar sensors have also been useful in quantifying intracellular forces such as those generated by myosin within cardiomyocytes.[158] Although a powerful tool, the efficiency of FRET-based sensors is often limited by high signal-to-noise ratios. Thus, computational simulations have been developed to predict cellular responses to mechanical forces and cell force generation and transmission in complex, multicellular tissues (Figure 5D). For example, modeling of cell jamming/unjamming transitions contributed to the current understanding of mechanical forces in asthma.[21,23,159] Additionally, computational fluid dynamics analyses have been used to model blood flow in the heart[160] and airflow through the lungs. Such studies not only contribute to our understanding of healthy and diseased tissue but also have clinical implications for drug design and delivery.[161] Finite element analysis simulations are another tool to predict the role of mechanical forces such as during mucosal buckling in asthma and COPD and potential drug applications.[162] Integrated computational modeling and experimental approaches are both critical for understanding the role of mechanical forces in cardiopulmonary development and disease. For example, our understanding of branching morphogenesis in the developing lung has been established through a combination of mathematical and experimental models.[163–165] It is likely that future findings will likewise depend on hand-in-hand advances in modeling and experimental approaches.[166–169]

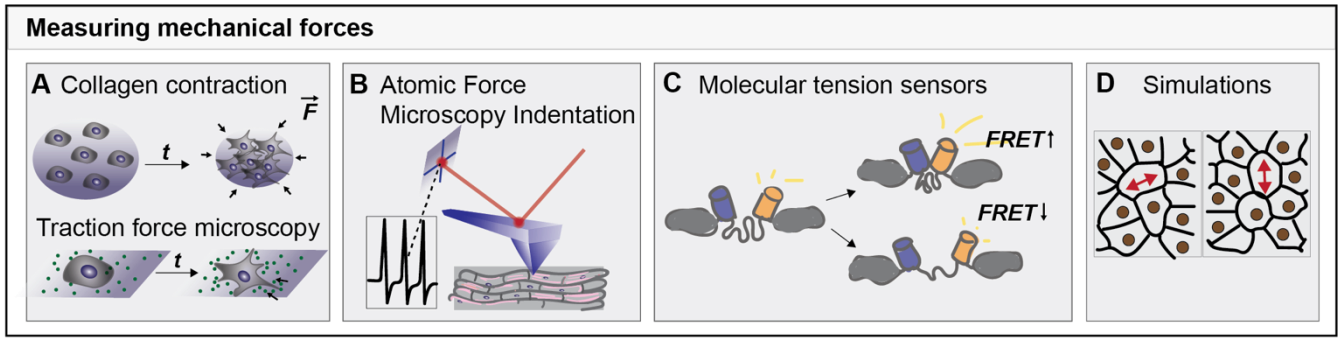

Figure 5 Applications of tools to measure mechanical forces: A collagen contraction and traction force microscopy (TFM) assays to quantify collective and single cell forces; **B** atomic force microscopy (AFM) nano-indentation to measure cardiac beat rate and force; **C** fluorescence resonance energy transfer (FRET) to measure subcellular forces; **D** computational simulations to calculate and predict directional forces such as during the jamming/unjamming transition.

Conclusion and Future Opportunities

In this review article, we discussed the role of mechanical forces in cardiac and pulmonary development and disease. These insights have often relied on the development and application of new engineering tools. Although many of these tools are already well-established in the field, we believe that there is a need for increased accessibility and continued improvement of such tools to enable widespread adoption amongst non-engineers. Chemically defined hydrogels are an excellent example of an already widely accessible tool that has provided numerous insights into (sub)cellular responses to matrix stiffness and cell contractile forces.[96] Based on various natural and synthetic polymer backbones, hydrogels have been used to mimic tissue elasticity[103], adhesion properties[170,171], or growth factor composition.[172] While commercially available hydrogels are readily accessible, their widespread adoption remains still limited. Concerns often include their applicability for downstream analyses such as imaging, or incompatibility with established protocols for gene and protein expression. In addition, commercially available hydrogels often require additional modifications to facilitate cell adhesion (e.g.,

coating with matrix proteins), making them less amenable to off-the-shelf use. Here, pre-formed hydrogels or hydrogel kits containing cell-adhesive domains may ease the transition from tissue culture plastic to hydrogels of varying elastic moduli (e.g., CytoSoft®, Advanced Biomatrix). Towards broader applicability of hydrogel platforms, materials that are easily fabricated without the need for expensive specialized equipment will be crucial. Beyond static hydrogel platforms, devices that apply stretch to cells have started to become widely available for studies of cell maturation and mechanosignaling. Several systems including the Flexcell® tension system[125,173] as well as the Emulate® system can apply cyclic uniaxial or biaxial tension to cells cultured atop silicone substrates. Although more complex systems exist, the simplicity of the designs and their commercial availability and cost are critical inputs into the adoption of such approaches among non-engineering laboratories.

A potential opportunity for growth is a better integration of engineering tools in biological assays such as the measurement of intracellular forces. Collagen contraction assays provide insight into cellular contractility but are limited to collective cellular forces. In contrast, traction force microscopy is based on the displacement of fluorescent beads to measure forces generated by single cells. While this technique uses materials and tools that are easily accessible, fabricating the fluorescent bead-containing hydrogels often requires collaboration with an engineering laboratory. Here, commercially available hydrogels for TFM and bead-tracking software may be beneficial for the integration of TFM into biology laboratories. In addition, advanced TFM techniques such as light tracking of matrix fibers or tissue tracking without the need of fluorescent beads may enable an even broader adoption of the technique,[174] including the measurement of forces in PCLS and within 3D tissues.[133,175] Taken together, continued communication and collaborations between biologists and engineers is essential to improving and implementing engineering tools into biological workflows. Pre-formed hydrogels are just one example of minimally engineered tools that are relatively easy to be implemented and are offered for a range of applications such as microwells for cost-effective and reproducible organoid culture.[176,177] In addition, other strategies may include workshops and seminars that are tailored for the needs of non-engineers to access and learn how to implement engineering tools into their research questions.

Finally, that mechanical forces are critical as a therapeutic target has been highlighted throughout the review. It is worth noting several drugs that target aberrant tissue mechanics have successfully been implemented in the clinic. For example, treatment with the protease inhibitor α1 antitrypsin (GLASSIA) is commonly used to prevent the continued destruction of alveolar ECM in emphysema patients with α1 antitrypsin deficiency.[178] Exogenous surfactant, either animal-derived or synthetic, is supplied to infants with bronchopulmonary dysplasia to prevent alveolar collapse.[179,180] Another FDA-approved drug is Entresto® which includes a neprilysin inhibitor used to treat patients with chronic heart failure.[181] Inhibition of neprilysin prevents the cleavage of atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) that are downstream integrators of mechanical stress.[182] In addition, drugs such as Mavacamten and Aficamten that inhibit myosin and reduce hypercontractility, are commonly used for the treatment of patients with hypertrophic cardiomyopathy.[183–185] Another FDA-approved drug is pirfenidone, an anti-fibrotic drug that is known to act on multiple fibrogenic pathways;[186] and may be applicable to other fibrotic diseases such as cardiac fibrosis.[187,188] however, the exact mechanisms are still unclear. For example, studies have shown that pirfenidone treatment reduces collagen deposition by activated in fibroblasts leading to an increase in lung tissue compliance in patients with pulmonary fibrosis.[189] Other studies have demonstrated that pirfenidone acts through inhibiting TGFβ signaling pathways, including SMAD.[190] Interestingly, approaches to reduce the release of latent TGFβ through the monoclonal antibody against αvβ6 integrins (BG00011) were successful in static cell culture studies[191] but have ultimately failed in clinical trials.[192,193] Thus, such an example of unsuccessful translation into patients may highlight the need for integrating dynamic mechanical forces into biological studies. In fact, engineered beating heart tissue platforms are now being used for screening new drugs for cardiotoxicity (e.g., *Valeo Health*, *Comprehensive In Vitro Proarrythmia Assay (CiPA)).* Taken together, these examples and new directions of engineered tools in drug testing underline the significance in understanding the potential therapeutic applications to countervail the effects of mechanical cues on cardiopulmonary diseases.

Acknowledgements

This work was partially supported by funding from the NIH (R00-HL151670 to C.L.) and the American Lung Association (IA-939940 to C.L.), National Science Foundation Graduate Research Fellowship (to M.K.E), and the University of Michigan Rackham Merit Fellowship and Cellular Biotechnology Training Program (to D.W.A.).

1. Grotberg, J.B. (2011). Respiratory fluid mechanics. Physics of Fluids *23*, 021301. 10.1063/1.3517737.

2. Stooke-Vaughan, G.A., and Campàs, O. (2018). Physical control of tissue morphogenesis across scales. Curr Opin Genet Dev *51*, 111–119. 10.1016/j.gde.2018.09.002.

3. Heisenberg, C.P., and Bellaïche, Y. (2013). Forces in Tissue Morphogenesis and Patterning. Cell *153*, 948–962. 10.1016/J.CELL.2013.05.008.

4. Varner, V.D., and Nelson, C.M. (2014). Cellular and physical mechanisms of branching morphogenesis. Development *141*, 2750–2759. 10.1242/dev.104794.

5. Nelson, C.M., and Gleghorn, J.P. (2012). Sculpting Organs: Mechanical Regulation of Tissue Development. Annu Rev Biomed Eng *14*, 129–154. 10.1146/annurev-bioeng-071811-150043.

6. Chugh, M., Munjal, A., and Megason, S.G. (2022). Hydrostatic pressure as a driver of cell and tissue morphogenesis. Semin Cell Dev Biol *131*, 134–145. 10.1016/j.semcdb.2022.04.021.

7. Daems, M., Peacock, H.M., and Jones, E.A. V. (2020). Fluid flow as a driver of embryonic morphogenesis. Development *147*. 10.1242/dev.185579.

8. Wert, S.E., Dey, C.R., Blair, P.A., Kimura, S., and Whitsett, J.A. (2002). Increased Expression of Thyroid Transcription Factor-1 (TTF-1) in Respiratory Epithelial Cells Inhibits Alveolarization and Causes Pulmonary Inflammation. Dev Biol *242*, 75–87. 10.1006/dbio.2001.0540.

9. Krens, S.F.G., Veldhuis, J.H., Barone, V., Čapek, D., Maître, J.-L., Brodland, G.W., and Heisenberg, C.-P. (2017). Interstitial fluid osmolarity modulates the action of differential tissue surface tension in progenitor cell segregation during gastrulation. Development *144*, 1798–1806. 10.1242/dev.144964.

10. Major, R.J., and Irvine, K.D. (2006). Localization and requirement for Myosin II at the dorsal-ventral compartment boundary of theDrosophila wing. Developmental Dynamics *235*, 3051–3058. 10.1002/dvdy.20966.

11. Tang, D.D., and Gerlach, B.D. (2017). The roles and regulation of the actin cytoskeleton, intermediate filaments and microtubules in smooth muscle cell migration. Respir Res *18*, 54. 10.1186/s12931-017-0544-7.

12. Lindsey, S.E., Butcher, J.T., and Yalcin, H.C. (2014). Mechanical regulation of cardiac development. Front Physiol *5*. 10.3389/fphys.2014.00318.

13. Zepp, J.A., Morley, M.P., Loebel, C., Kremp, M.M., Chaudhry, F.N., Basil, M.C., Leach, J.P., Liberti, D.C., Niethamer, T.K., Ying, Y., et al. (2021). Genomic, epigenomic, and biophysical cues controlling the emergence of the lung alveolus. Science (1979) *371*. 10.1126/science.abc3172.

14. Li, R., Li, X., Hagood, J., Zhu, M.-S., and Sun, X. (2020). Myofibroblast contraction is essential for generating and regenerating the gas-exchange surface. Journal of Clinical Investigation *130*, 2859–2871. 10.1172/JCI132189.

15. Minoo, P. (2000). Transcriptional regulation of lung development: emergence of specificity. Respir Res *1*, 7. 10.1186/rr20.

16. Kidokoro, H., Yonei-Tamura, S., Tamura, K., Schoenwolf, G.C., and Saijoh, Y. (2018). The heart tube forms and elongates through dynamic cell rearrangement coordinated with foregut extension. Development. 10.1242/dev.152488.

17. Hosseini, H.S., Garcia, K.E., and Taber, L.A. (2017). A new hypothesis for foregut and heart tube formation based on differential growth and actomyosin contraction. Development. 10.1242/dev.145193.

18. Tsan, Y.-C., DePalma, S.J., Zhao, Y.-T., Capilnasiu, A., Wu, Y.-W., Elder, B., Panse, I., Ufford, K., Matera, D.L., Friedline, S., et al. (2021). Physiologic biomechanics enhance reproducible contractile development in a stem cell derived cardiac muscle platform. Nat Commun *12*, 6167. 10.1038/s41467-021-26496-1.

19. Auman, H.J., Coleman, H., Riley, H.E., Olale, F., Tsai, H.-J., and Yelon, D. (2007). Functional Modulation of Cardiac Form through Regionally Confined Cell Shape Changes. PLoS Biol *5*, e53. 10.1371/journal.pbio.0050053.

20. Mitchel, J.A., Das, A., O'Sullivan, M.J., Stancil, I.T., DeCamp, S.J., Koehler, S., Ocaña, O.H., Butler, J.P., Fredberg, J.J., Nieto, M.A., et al. (2020). In primary airway epithelial cells, the unjamming transition is distinct from the epithelial-to-mesenchymal transition. Nat Commun *11*, 5053. 10.1038/s41467-020-18841-7.

21. Bi, D., Yang, X., Marchetti, M.C., and Manning, M.L. (2016). Motility-Driven Glass and Jamming Transitions in Biological Tissues. Phys Rev X *6*, 021011. 10.1103/PhysRevX.6.021011.

22. Mongera, A., Rowghanian, P., Gustafson, H.J., Shelton, E., Kealhofer, D.A., Carn, E.K., Serwane, F., Lucio, A.A., Giammona, J., and Campàs, O. (2018). A fluid-to-solid jamming transition underlies vertebrate body axis elongation. Nature *561*, 401–405. 10.1038/s41586-018-0479-2.

23. Park, J.-A., Kim, J.H., Bi, D., Mitchel, J.A., Qazvini, N.T., Tantisira, K., Park, C.Y., McGill, M., Kim, S.-H., Gweon, B., et al. (2015). Unjamming and cell shape in the asthmatic airway epithelium. Nat Mater *14*, 1040–1048. 10.1038/nmat4357.

24. Martinez, F.D., and Vercelli, D. (2013). Asthma. The Lancet *382*, 1360–1372. 10.1016/S0140-6736(13)61536-6.

25. Spurlin, J.W., Siedlik, M.J., Nerger, B.A., Pang, M.-F., Jayaraman, S., Zhang, R., and Nelson, C.M. (2019). Mesenchymal proteases and tissue fluidity remodel the extracellular matrix during airway epithelial branching in the embryonic avian lung. Development. 10.1242/dev.175257.

26. De Marzio, M., Kılıç, A., Maiorino, E., Mitchel, J.A., Mwase, C., O'Sullivan, M.J., McGill, M., Chase, R., Fredberg, J.J., Park, J.-A., et al. (2021). Genomic signatures of the unjamming transition in compressed human bronchial epithelial cells. Sci Adv 7. 10.1126/sciadv.abf1088.

27. Priya, R., Allanki, S., Gentile, A., Mansingh, S., Uribe, V., Maischein, H.-M., and Stainier, D.Y.R. (2020). Tension heterogeneity directs form and fate to pattern the myocardial wall. Nature *588*, 130–134. 10.1038/s41586-020-2946-9.

28. Conway, R.F., Frum, T., Conchola, A.S., and Spence, J.R. (2020). Understanding Human Lung Development through In Vitro Model Systems. BioEssays *42*, 2000006. 10.1002/bies.202000006.

29. Harding, R., and Hooper, S.B. (1996). Regulation of lung expansion and lung growth before birth. J Appl Physiol *81*, 209–224. 10.1152/jappl.1996.81.1.209.

30. Perlman, M., Williams, J., and Hirsch, M. (1976). Neonatal pulmonary hypoplasia after prolonged leakage of amniotic fluid. Arch Dis Child *51*, 349–353. 10.1136/adc.51.5.349.

31. Kotecha, S., Barbato, A., Bush, A., Claus, F., Davenport, M., Delacourt, C., Deprest, J., Eber, E., Frenckner, B., Greenough, A., et al. (2012). Congenital diaphragmatic hernia. European Respiratory Journal *39*, 820–829. 10.1183/09031936.00066511.

32. Nelson, C.M., Gleghorn, J.P., Pang, M.-F., Jaslove, J., Goodwin, K., Varner, V.D., Miller, E., Radisky, D.C., and Stone, H.A. (2017). Microfluidic chest cavities reveal that transmural pressure controls the rate of lung development. Development. 10.1242/dev.154823.

33. Jaslove, J.M., Goodwin, K., Sundarakrishnan, A., Spurlin, J.W., Mao, S., Košmrlj, A., and Nelson, C.M. (2022). Transmural pressure signals through retinoic acid to regulate lung branching. Development *149*. 10.1242/dev.199726.

34. Jones, M.R., Chong, L., and Bellusci, S. (2021). Fgf10/Fgfr2b Signaling Orchestrates the Symphony of Molecular, Cellular, and Physical Processes Required for Harmonious Airway Branching Morphogenesis. Front Cell Dev Biol *8*. 10.3389/fcell.2020.620667.

35. Danopoulos, S., Shiosaki, J., and Al Alam, D. (2019). FGF Signaling in Lung Development and Disease: Human Versus Mouse. Front Genet *10*. 10.3389/fgene.2019.00170.

36. Yuan, T., Volckaert, T., Chanda, D., Thannickal, V.J., and De Langhe, S.P. (2018). Fgf10 Signaling in Lung Development, Homeostasis, Disease, and Repair After Injury. Front Genet *9*. 10.3389/fgene.2018.00418.

37. Li, J., Wang, Z., Chu, Q., Jiang, K., Li, J., and Tang, N. (2018). The Strength of Mechanical Forces Determines the Differentiation of Alveolar Epithelial Cells. Dev Cell *44*, 297-312.e5. 10.1016/j.devcel.2018.01.008.

38. Liu, Z., Wu, H., Jiang, K., Wang, Y., Zhang, W., Chu, Q., Li, J., Huang, H., Cai, T., Ji, H., et al. (2016). MAPK-Mediated YAP Activation Controls Mechanical-Tension-Induced Pulmonary Alveolar Regeneration. Cell Rep *16*, 1810–1819. 10.1016/j.celrep.2016.07.020.

39. Hillman, N.H., Kallapur, S.G., Pillow, J.J., Moss, T.J.M., Polglase, G.R., Nitsos, I., and Jobe, A.H. (2010). Airway Injury From Initiating Ventilation in Preterm Sheep. Pediatr Res *67*, 60–65. 10.1203/PDR.0b013e3181c1b09e.

40. Suki, B., Sato, S., Parameswaran, H., Szabari, M. V., Takahashi, A., and Bartolák-Suki, E. (2013). Emphysema and Mechanical Stress-Induced Lung Remodeling. Physiology *28*, 404–413. 10.1152/physiol.00041.2013.

41. Le Garrec, J.-F., Domínguez, J.N., Desgrange, A., Ivanovitch, K.D., Raphaël, E., Bangham, J.A., Torres, M., Coen, E., Mohun, T.J., and Meilhac, S.M. (2017). A predictive model of asymmetric morphogenesis from 3D reconstructions of mouse heart looping dynamics. Elife *6*. 10.7554/eLife.28951.

42. Bayraktar, M., and MÃ¤nner, J. (2014). Cardiac looping may be driven by compressive loads resulting from unequal growth of the heart and pericardial cavity. Observations on a physical simulation model. Front Physiol *5*. 10.3389/fphys.2014.00112.

43. Houyel, L., and Meilhac, S.M. (2021). Heart Development and Congenital Structural Heart Defects. Annu Rev Genomics Hum Genet *22*, 257–284. 10.1146/annurev-genom-083118-015012.

44. Gittenberger-de Groot, A.C., Bartelings, M.M., Deruiter, M.C., and Poelmann, R.E. (2005). Basics of Cardiac Development for the Understanding of Congenital Heart Malformations. Pediatr Res *57*, 169–176. 10.1203/01.PDR.0000148710.69159.61.

45. Rojanasopondist, P., Nesheiwat, L., Piombo, S., Porter, G.A., Ren, M., and Phoon, C.K.L. (2022). Genetic Basis of Left Ventricular Noncompaction. Circ Genom Precis Med *15*. 10.1161/CIRCGEN.121.003517.

46. Wong, H.S., Wiputra, H., Tulzer, A., Tulzer, G., and Yap, C.H. (2022). Fluid Mechanics of Fetal Left Ventricle During Aortic Stenosis with Evolving Hypoplastic Left Heart Syndrome. Ann Biomed Eng *50*, 1158–1172. 10.1007/s10439-022-02990-5.

47. Gobergs, R., Salputra, E., and Lubaua, I. (2016). Hypoplastic left heart syndrome: a review. Acta Med Litu *23*, 86–98. 10.6001/actamedica.v23i2.3325.

48. Pascall, E., and Tulloh, R.M. (2018). Pulmonary hypertension in congenital heart disease. Future Cardiol *14*, 343–353. 10.2217/fca-2017-0065.

49. Sedmera, D., Pexieder, T., Rychterova, V., Hu, N., and Clark, E.B. (1999). Remodeling of chick embryonic ventricular myoarchitecture under experimentally changed loading conditions. Anat Rec *254*, 238–252. 10.1002/(SICI)1097-0185(19990201)254:2<238::AID-AR10>3.0.CO;2-V.

50. He, H., Snowball, J., Sun, F., Na, C.-L., and Whitsett, J.A. (2021). IGF1R controls mechanosignaling in myofibroblasts required for pulmonary alveologenesis. JCI Insight *6*. 10.1172/jci.insight.144863.

51. Vander Roest, A.S., Liu, C., Morck, M.M., Kooiker, K.B., Jung, G., Song, D., Dawood, A., Jhingran, A., Pardon, G., Ranjbarvaziri, S., et al. (2021). Hypertrophic cardiomyopathy β-cardiac myosin mutation (P710R) leads to hypercontractility by disrupting super relaxed state. Proceedings of the National Academy of Sciences *118*. 10.1073/pnas.2025030118.

52. Park, J.-A., Fredberg, J.J., and Drazen, J.M. (2015). Putting the Squeeze on Airway Epithelia. Physiology *30*, 293–303. 10.1152/physiol.00004.2015.

53. Tsai, Y.-H., Parker, J.S., Yang, I. V., and Kelada, S.N.P. (2018). Meta-analysis of airway epithelium gene expression in asthma. European Respiratory Journal *51*, 1701962. 10.1183/13993003.01962-2017.

54. Pascoe, C.D., Obeidat, M., Arsenault, B.A., Nie, Y., Warner, S., Stefanowicz, D., Wadsworth, S.J., Hirota, J.A., Jasemine Yang, S., Dorscheid, D.R., et al. (2017). Gene expression analysis in asthma using a targeted multiplex array. BMC Pulm Med *17*, 189. 10.1186/s12890-017-0545-9.

55. Modena, B.D., Bleecker, E.R., Busse, W.W., Erzurum, S.C., Gaston, B.M., Jarjour, N.N., Meyers, D.A., Milosevic, J., Tedrow, J.R., Wu, W., et al. (2017). Gene Expression Correlated with Severe Asthma Characteristics Reveals Heterogeneous Mechanisms of Severe Disease. Am J Respir Crit Care Med *195*, 1449–1463. 10.1164/rccm.201607-1407OC.

56. Bochaton-Piallat, M.-L., Gabbiani, G., and Hinz, B. (2016). The myofibroblast in wound healing and fibrosis: answered and unanswered questions. F1000Res *5*, 752. 10.12688/f1000research.8190.1.

57. Herrera, J., Henke, C.A., and Bitterman, P.B. (2018). Extracellular matrix as a driver of progressive fibrosis. Journal of Clinical Investigation *128*, 45–53. 10.1172/JCI93557.

58. Wu, H., Yu, Y., Huang, H., Hu, Y., Fu, S., Wang, Z., Shi, M., Zhao, X., Yuan, J., Li, J., et al. (2020). Progressive Pulmonary Fibrosis Is Caused by Elevated Mechanical Tension on Alveolar Stem Cells. Cell *180*, 107-121.e17. 10.1016/j.cell.2019.11.027.

59. Jiang, P., Gil de Rubio, R., Hrycaj, S.M., Gurczynski, S.J., Riemondy, K.A., Moore, B.B., Omary, M.B., Ridge, K.M., and Zemans, R.L. (2020). Ineffectual Type 2 to Type 1 Alveolar Epithelial Cell

Differentiation in Idiopathic Pulmonary Fibrosis: Persistence of the KRT8hi Transitional State. Am J Respir Crit Care Med *201*, 1443–1447. 10.1164/rccm.201909-1726LE.

60. Evans, C.M., Fingerlin, T.E., Schwarz, M.I., Lynch, D., Kurche, J., Warg, L., Yang, I. V, and Schwartz, D.A. (2016). Idiopathic Pulmonary Fibrosis: A Genetic Disease That Involves Muco-ciliary Dysfunction of the Peripheral Airways. Physiol Rev *96*, 1567–1591. 10.1152/physrev.00004.2016.-Idiopathic.

61. Teekakirikul, P., Eminaga, S., Toka, O., Alcalai, R., Wang, L., Wakimoto, H., Nayor, M., Konno, T., Gorham, J.M., Wolf, C.M., et al. (2010). Cardiac fibrosis in mice with hypertrophic cardiomyopathy is mediated by non-myocyte proliferation and requires Tgf-β. Journal of Clinical Investigation *120*, 3520–3529. 10.1172/JCI42028.

62. Hankiewicz, J.H., Goldspink, P.H., Buttrick, P.M., and Lewandowski, E.D. (2008). Principal strain changes precede ventricular wall thinning during transition to heart failure in a mouse model of dilated cardiomyopathy. American Journal of Physiology-Heart and Circulatory Physiology *294*, H330–H336. 10.1152/ajpheart.01109.2007.

63. Schultheiss, H.-P., Fairweather, D., Caforio, A.L.P., Escher, F., Hershberger, R.E., Lipshultz, S.E., Liu, P.P., Matsumori, A., Mazzanti, A., McMurray, J., et al. (2019). Dilated cardiomyopathy. Nat Rev Dis Primers *5*, 32. 10.1038/s41572-019-0084-1.

64. Helms, A.S., Tang, V.T., O'Leary, T.S., Friedline, S., Wauchope, M., Arora, A., Wasserman, A.H., Smith, E.D., Lee, L.M., Wen, X.W., et al. (2020). Effects of MYBPC3 loss-of-function mutations preceding hypertrophic cardiomyopathy. JCI Insight *5*. 10.1172/jci.insight.133782.

65. Toepfer, C.N., Wakimoto, H., Garfinkel, A.C., McDonough, B., Liao, D., Jiang, J., Tai, A.C., Gorham, J.M., Lunde, I.G., Lun, M., et al. (2019). Hypertrophic cardiomyopathy mutations in *MYBPC3* dysregulate myosin. Sci Transl Med *11*. 10.1126/scitranslmed.aat1199.

66. Harvey, P.A., and Leinwand, L.A. (2011). Cellular mechanisms of cardiomyopathy. Journal of Cell Biology *194*, 355–365. 10.1083/jcb.201101100.

67. Chaffin, M., Papangeli, I., Simonson, B., Akkad, A.-D., Hill, M.C., Arduini, A., Fleming, S.J., Melanson, M., Hayat, S., Kost-Alimova, M., et al. (2022). Single-nucleus profiling of human dilated and hypertrophic cardiomyopathy. Nature *608*, 174–180. 10.1038/s41586-022-04817-8.

68. Munger, J.S., Huang, X., Kawakatsu, H., Griffiths, M.J.D., Dalton, S.L., Wu, J., Pittet, J.-F., Kaminski, N., Garat, C., Matthay, M.A., et al. (1999). A Mechanism for Regulating Pulmonary Inflammation and Fibrosis: The Integrin αvβ6 Binds and Activates Latent TGF β1. Cell *96*, 319–328. 10.1016/S0092-8674(00)80545-0.

69. Wipff, P.J., Rifkin, D.B., Meister, J.J., and Hinz, B. (2007). Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. J Cell Biol *179*, 1311–1323. 10.1083/JCB.200704042.

70. Sarrazy, V., Koehler, A., Chow, M.L., Zimina, E., Li, C.X., Kato, H., Caldarone, C.A., and Hinz, B. (2014). Integrins αvβ5 and αvβ3 promote latent TGF-β1 activation by human cardiac fibroblast contraction. Cardiovasc Res *102*, 407–417. 10.1093/cvr/cvu053.

71. Hatano, N., Itoh, Y., and Muraki, K. (2009). Cardiac fibroblasts have functional TRPV4 activated by 4α-phorbol 12,13-didecanoate. Life Sci *85*, 808–814. 10.1016/j.lfs.2009.10.013.

72. Chopra, A., Kutys, M.L., Zhang, K., Polacheck, W.J., Sheng, C.C., Luu, R.J., Eyckmans, J., Hinson, J.T., Seidman, J.G., Seidman, C.E., et al. (2018). Force Generation via β-Cardiac Myosin, Titin, and α-

Actinin Drives Cardiac Sarcomere Assembly from Cell-Matrix Adhesions. Dev Cell *44*, 87-96.e5. 10.1016/j.devcel.2017.12.012.

73. DePalma, S.J., Davidson, C.D., Stis, A.E., Helms, A.S., and Baker, B.M. (2021). Microenvironmental determinants of organized iPSC-cardiomyocyte tissues on synthetic fibrous matrices. Biomater Sci *9*, 93–107. 10.1039/D0BM01247E.

74. Kaushik, G., Spenlehauer, A., Sessions, A.O., Trujillo, A.S., Fuhrmann, A., Fu, Z., Venkatraman, V., Pohl, D., Tuler, J., Wang, M., et al. (2015). Vinculin network–mediated cytoskeletal remodeling regulates contractile function in the aging heart. Sci Transl Med *7*. 10.1126/scitranslmed.aaa5843.

75. Hoshijima, M. (2006). Mechanical stress-strain sensors embedded in cardiac cytoskeleton: Z disk, titin, and associated structures. American Journal of Physiology-Heart and Circulatory Physiology *290*, H1313–H1325. 10.1152/ajpheart.00816.2005.

76. Jordan, E., Peterson, L., Ai, T., Asatryan, B., Bronicki, L., Brown, E., Celeghin, R., Edwards, M., Fan, J., Ingles, J., et al. (2021). Evidence-Based Assessment of Genes in Dilated Cardiomyopathy. Circulation *144*, 7–19. 10.1161/CIRCULATIONAHA.120.053033.

77. White, M.J., Raczy, M.M., Budina, E., Yuba, E., Solanki, A., Shim, H.-N., Zhang, Z.J., Gray, L.T., Cao, S., Alpar, A.T., et al. (2022). Engineered collagen-targeting therapeutics reverse lung and kidney fibrosis in mice. BioRXiv.

78. Lin, C., Yao, E., Zhang, K., Jiang, X., Croll, S., Thompson-Peer, K., and Chuang, P.-T. (2017). YAP is essential for mechanical force production and epithelial cell proliferation during lung branching morphogenesis. Elife *6*. 10.7554/eLife.21130.

79. Isago, H., Mitani, A., Mikami, Y., Horie, M., Urushiyama, H., Hamamoto, R., Terasaki, Y., and Nagase, T. (2020). Epithelial expression of YAP and TAZ is sequentially required in lung development. Am J Respir Cell Mol Biol *62*, 256–266. 10.1165/RCMB.2019-0218OC/SUPPL_FILE/DISCLOSURES.PDF.

80. Lin, C., Yao, E., and Chuang, P.-T. (2015). A conserved MST1/2–YAP axis mediates Hippo signaling during lung growth. Dev Biol *403*, 101–113. 10.1016/j.ydbio.2015.04.014.

81. von Gise, A., Lin, Z., Schlegelmilch, K., Honor, L.B., Pan, G.M., Buck, J.N., Ma, Q., Ishiwata, T., Zhou, B., Camargo, F.D., et al. (2012). YAP1, the nuclear target of Hippo signaling, stimulates heart growth through cardiomyocyte proliferation but not hypertrophy. Proceedings of the National Academy of Sciences *109*, 2394–2399. 10.1073/pnas.1116136109.

82. Wang, J., Liu, S., Heallen, T., and Martin, J.F. (2018). The Hippo pathway in the heart: pivotal roles in development, disease, and regeneration. Nat Rev Cardiol *15*, 672–684. 10.1038/s41569-018-0063-3.

83. Bornhorst, D., Xia, P., Nakajima, H., Dingare, C., Herzog, W., Lecaudey, V., Mochizuki, N., Heisenberg, C.-P., Yelon, D., and Abdelilah-Seyfried, S. (2019). Biomechanical signaling within the developing zebrafish heart attunes endocardial growth to myocardial chamber dimensions. Nat Commun *10*, 4113. 10.1038/s41467-019-12068-x.

84. Wang, P., Mao, B., Luo, W., Wei, B., Jiang, W., Liu, D., Song, L., Ji, G., Yang, Z., Lai, Y.-Q., et al. (2014). The alteration of Hippo/YAP signaling in the development of hypertrophic cardiomyopathy. Basic Res Cardiol *109*, 435. 10.1007/s00395-014-0435-8.

85. Liao, J., Lu, W., Chen, Y., Duan, X., Zhang, C., Luo, X., Lin, Z., Chen, J., Liu, S., Yan, H., et al. (2021). Upregulation of Piezo1 (Piezo Type Mechanosensitive Ion Channel Component 1) Enhances the Intracellular Free Calcium in Pulmonary Arterial Smooth Muscle Cells From Idiopathic Pulmonary

Arterial Hypertension Patients. Hypertension *77*, 1974–1989. 10.1161/HYPERTENSIONAHA.120.16629.

86. Yu, Z.-Y., Gong, H., Kesteven, S., Guo, Y., Wu, J., Li, J.V., Cheng, D., Zhou, Z., Iismaa, S.E., Kaidonis, X., et al. (2022). Piezo1 is the cardiac mechanosensor that initiates the cardiomyocyte hypertrophic response to pressure overload in adult mice. Nature Cardiovascular Research *1*, 577–591. 10.1038/s44161-022-00082-0.

87. Friedrich, E.E., Hong, Z., Xiong, S., Zhong, M., Di, A., Rehman, J., Komarova, Y.A., and Malik, A.B. (2019). Endothelial cell Piezo1 mediates pressure-induced lung vascular hyperpermeability via disruption of adherens junctions. Proc Natl Acad Sci U S A *116*, 12980–12985. 10.1073/PNAS.1902165116/SUPPL_FILE/PNAS.1902165116.SAPP.PDF.

88. Zhong, M., Wu, W., Kang, H., Hong, Z., Xiong, S., Gao, X., Rehman, J., Komarova, Y.A., and Malik, A.B. (2020). Alveolar stretch activation of endothelial Piezo1 protects adherens junctions and lung vascular barrier. Am J Respir Cell Mol Biol *62*, 168–177. 10.1165/RCMB.2019-0024OC/SUPPL_FILE/DISCLOSURES.PDF.

89. Tschumperlin, D.J., Dai, G., Maly, I. V, Kikuchi, T., Laiho, L.H., Mcvittie, A.K., Haley, K.J., Lilly, C.M., So, P.T.C., Lauffenburger, D.A., et al. (2004). Mechanotransduction through growth-factor shedding into the extracellular space.

90. Wang, Y., Maciejewski, B.S., Soto-Reyes, D., Lee, H.-S., Warburton, D., and Sanchez-Esteban, J. (2009). Mechanical stretch promotes fetal type II epithelial cell differentiation via shedding of HB-EGF and TGF-α. J Physiol *587*, 1739–1753. 10.1113/jphysiol.2008.163899.

91. Noorman, M., van der Heyden, M.A.G., van Veen, T.A.B., Cox, M.G.P.J., Hauer, R.N.W., de Bakker, J.M.T., and van Rijen, H.V.M. (2009). Cardiac cell–cell junctions in health and disease: Electrical versus mechanical coupling. J Mol Cell Cardiol *47*, 23–31. 10.1016/j.yjmcc.2009.03.016.

92. Rajendran, A.K., Sankar, D., Amirthalingam, S., Kim, H.D., Rangasamy, J., and Hwang, N.S. (2023). Trends in mechanobiology guided tissue engineering and tools to study cell-substrate interactions: a brief review. Biomater Res *27*, 55. 10.1186/s40824-023-00393-8.

93. Shakoor, A., Gao, W., Zhao, L., Jiang, Z., and Sun, D. (2022). Advanced tools and methods for single-cell surgery. Microsyst Nanoeng *8*, 47. 10.1038/s41378-022-00376-0.

94. Puryear III, J.R., Yoon, J.-K., and Kim, Y. (2020). Advanced Fabrication Techniques of Microengineered Physiological Systems. Micromachines (Basel) *11*, 730. 10.3390/mi11080730.

95. Kim, D.-H., Wong, P.K., Park, J., Levchenko, A., and Sun, Y. (2009). Microengineered Platforms for Cell Mechanobiology. Annu Rev Biomed Eng *11*, 203–233. 10.1146/annurev-bioeng-061008-124915.

96. Caliari, S.R., and Burdick, J.A. (2016). A practical guide to hydrogels for cell culture. Nat Methods *13*, 405–414. 10.1038/NMETH.3839.

97. Asano, S., Ito, S., Takahashi, K., Furuya, K., Kondo, M., Sokabe, M., and Hasegawa, Y. (2017). Matrix stiffness regulates migration of human lung fibroblasts. Physiol Rep *5*, e13281. 10.14814/phy2.13281.

98. Raczkowska, J., Orzechowska, B., Patryas, S., Awsiuk, K., Kubiak, A., Kinoshita, M., Okamoto, M., Bobrowska, J., Stachura, T., Soja, J., et al. (2020). Effect of Substrate Stiffness on Physicochemical Properties of Normal and Fibrotic Lung Fibroblasts. Materials *13*, 1–17. 10.3390/MA13204495.

99. Balestrini, J.L., Chaudhry, S., Sarrazy, V., Koehler, A., and Hinz, B. (2012). The mechanical memory of lung myofibroblasts. Integrative Biology *4*, 410–421. 10.1039/C2IB00149G.

100. Burgstaller, G., Oehrle, B., Gerckens, M., White, E.S., Schiller, H.B., and Eickelberg, O. (2017). The instructive extracellular matrix of the lung: basic composition and alterations in chronic lung disease. European Respiratory Journal *50*, 1601805. 10.1183/13993003.01805-2016.

101. Guvendiren, M., and Burdick, J. (2015). Hydrogels with dynamically tunable properties. In Integrative Mechanobiology: Micro- and Nano-Techniques in Cell Mechanobiology, Y. Sun, D.-H. Kim, and C. A. Simmons, eds. (Cambridge University Press), pp. 90–109.

102. Rosales, A.M., and Anseth, K.S. (2016). The design of reversible hydrogels to capture extracellular matrix dynamics. Nat Rev Mater *1*, 15012. 10.1038/natrevmats.2015.12.

103. Guvendiren, M., and Burdick, J.A. (2012). Stiffening hydrogels to probe short- and long-term cellular responses to dynamic mechanics. Nat Commun *3*. 10.1038/ncomms1792.

104. Rosales, A.M., Vega, S.L., DelRio, F.W., Burdick, J.A., and Anseth, K.S. (2017). Hydrogels with Reversible Mechanics to Probe Dynamic Cell Microenvironments. Angewandte Chemie *129*, 12300–12304. 10.1002/ange.201705684.

105. Stowers, R.S., Allen, S.C., Suggs, L.J., and Anseth, K.S. (2015). Dynamic phototuning of 3D hydrogel stiffness. Proc Natl Acad Sci U S A *112*, 1953–1958. 10.1073/pnas.1421897112.

106. Petrou, C.L., D'Ovidio, T.J., Bölükbas, D.A., Tas, S., Brown, R.D., Allawzi, A., Lindstedt, S., Nozik-Grayck, E., Stenmark, K.R., Wagner, D.E., et al. (2020). Clickable decellularized extracellular matrix as a new tool for building hybrid-hydrogels to model chronic fibrotic diseases *in vitro*. J Mater Chem B *8*, 6814–6826. 10.1039/D0TB00613K.

107. Young, J.L., and Engler, A.J. (2011). Hydrogels with time-dependent material properties enhance cardiomyocyte differentiation in vitro. Biomaterials *32*, 1002–1009. 10.1016/j.biomaterials.2010.10.020.

108. Young, J.L., Kretchmer, K., Ondeck, M.G., Zambon, A.C., and Engler, A.J. (2015). Mechanosensitive Kinases Regulate Stiffness-Induced Cardiomyocyte Maturation. Sci Rep *4*, 6425. 10.1038/srep06425.

109. McGann, C.L., Levenson, E.A., and Kiick, K.L. (2013). Resilin-Based Hybrid Hydrogels for Cardiovascular Tissue Engineering. Macromol Chem Phys *214*, 203–213. 10.1002/macp.201200412.

110. Fu, L., Haage, A., Kong, N., Tanentzapf, G., and Li, H. (2019). Dynamic protein hydrogels with reversibly tunable stiffness regulate human lung fibroblast spreading reversibly. Chemical Communications *55*, 5235–5238. 10.1039/C9CC01276A.

111. Baptista, D., Teixeira, L., van Blitterswijk, C., Giselbrecht, S., and Truckenmüller, R. (2019). Overlooked? Underestimated? Effects of Substrate Curvature on Cell Behavior. Trends Biotechnol *37*, 838–854. 10.1016/j.tibtech.2019.01.006.

112. Callens, S.J.P., Uyttendaele, R.J.C., Fratila-Apachitei, L.E., and Zadpoor, A.A. (2020). Substrate curvature as a cue to guide spatiotemporal cell and tissue organization. Biomaterials *232*, 119739. 10.1016/j.biomaterials.2019.119739.

113. Assoian, R.K., Bade, N.D., Cameron, C. V., and Stebe, K.J. (2019). Cellular sensing of micron-scale curvature: a frontier in understanding the microenvironment. Open Biol *9*, 190155. 10.1098/rsob.190155.

114. Baptista, D., Teixeira, L.M., Birgani, Z.T., van Riet, S., Pasman, T., Poot, A., Stamatialis, D., Rottier, R.J., Hiemstra, P.S., Habibović, P., et al. (2021). 3D alveolar in vitro model based on epithelialized biomimetically curved culture membranes. Biomaterials *266*, 120436. 10.1016/j.biomaterials.2020.120436.

115. Roy, A., Zhang, Z., Eiken, M.K., Shi, A., Pena-Francesch, A., and Loebel, C. (2023). Programmable Tissue Folding Patterns in Structured Hydrogels. Advanced Materials. 10.1002/adma.202300017.

116. Caracena, T., Blomberg, R., Hewawasam, R.S., Fry, Z.E., Riches, D.W.H., and Magin, C.M. (2022). Alveolar epithelial cells and microenvironmental stiffness synergistically drive fibroblast activation in three-dimensional hydrogel lung models. Biomater Sci *10*, 7133–7148. 10.1039/D2BM00827K.

117. Lewis, K.J.R., Tibbitt, M.W., Zhao, Y., Branchfield, K., Sun, X., Balasubramaniam, V., and Anseth, K.S. (2015). In vitro model alveoli from photodegradable microsphere templates. Biomater Sci *3*, 821–832. 10.1039/c5bm00034c.

118. Matera, D.L., DiLillo, K.M., Smith, M.R., Davidson, C.D., Parikh, R., Said, M., Wilke, C.A., Lombaert, I.M., Arnold, K.B., Moore, B.B., et al. (2020). Microengineered 3D pulmonary interstitial mimetics highlight a critical role for matrix degradation in myofibroblast differentiation. Sci Adv *6*. 10.1126/sciadv.abb5069.

119. Ufford, K., Friedline, S., Tong, Z., Tang, V.T., Dobbs, A.S., Tsan, Y.-C., Bielas, S.L., Liu, A.P., and Helms, A.S. (2021). Myofibrillar Structural Variability Underlies Contractile Function in Stem Cell-Derived Cardiomyocytes. Stem Cell Reports *16*, 470–477. 10.1016/j.stemcr.2021.01.007.

120. Ribeiro, A.J.S., Ang, Y.-S., Fu, J.-D., Rivas, R.N., Mohamed, T.M.A., Higgs, G.C., Srivastava, D., and Pruitt, B.L. (2015). Contractility of single cardiomyocytes differentiated from pluripotent stem cells depends on physiological shape and substrate stiffness. Proceedings of the National Academy of Sciences *112*, 12705–12710. 10.1073/pnas.1508073112.

121. Bray, M.-A., Sheehy, S.P., and Parker, K.K. (2008). Sarcomere alignment is regulated by myocyte shape. Cell Motil Cytoskeleton *65*, 641–651. 10.1002/cm.20290.

122. Diem, K., Fauler, M., Fois, G., Hellmann, A., Winokurow, N., Schumacher, S., Kranz, C., and Frick, M. (2020). Mechanical stretch activates piezo1 in caveolae of alveolar type I cells to trigger ATP release and paracrine stimulation of surfactant secretion from alveolar type II cells. The FASEB Journal *34*, 12785–12804. 10.1096/fj.202000613RRR.

123. Doryab, A., Taskin, M.B., Stahlhut, P., Schröppel, A., Orak, S., Voss, C., Ahluwalia, A., Rehberg, M., Hilgendorff, A., Stöger, T., et al. (2021). A Bioinspired in vitro Lung Model to Study Particokinetics of Nano-/Microparticles Under Cyclic Stretch and Air-Liquid Interface Conditions. Front Bioeng Biotechnol *9*. 10.3389/fbioe.2021.616830.

124. Hu, Y., Wang, X., Hu, B., Mao, Y., Chen, Y., Yan, L., Yong, J., Dong, J., Wei, Y., Wang, W., et al. (2019). Dissecting the transcriptome landscape of the human fetal neural retina and retinal pigment epithelium by single-cell RNA-seq analysis. PLoS Biol *17*. 10.1371/journal.pbio.3000365.

125. Fukuda, R., Gunawan, F., Ramadass, R., Beisaw, A., Konzer, A., Mullapudi, S.T., Gentile, A., Maischein, H.-M., Graumann, J., and Stainier, D.Y.R. (2019). Mechanical Forces Regulate Cardiomyocyte Myofilament Maturation via the VCL-SSH1-CFL Axis. Dev Cell *51*, 62-77.e5. 10.1016/j.devcel.2019.08.006.

126. Bliley, J.M., Vermeer, M.C.S.C., Duffy, R.M., Batalov, I., Kramer, D., Tashman, J.W., Shiwarski, D.J., Lee, A., Teplenin, A.S., Volkers, L., et al. (2021). Dynamic loading of human engineered heart tissue enhances contractile function and drives a desmosome-linked disease phenotype. Sci Transl Med *13*. 10.1126/scitranslmed.abd1817.

127. Ruan, J.-L., Tulloch, N.L., Razumova, M. V., Saiget, M., Muskheli, V., Pabon, L., Reinecke, H., Regnier, M., and Murry, C.E. (2016). Mechanical Stress Conditioning and Electrical Stimulation Promote Contractility and Force Maturation of Induced Pluripotent Stem Cell-Derived Human Cardiac Tissue. Circulation *134*, 1557–1567. 10.1161/CIRCULATIONAHA.114.014998.

128. Ruan, J.-L., Tulloch, N.L., Saiget, M., Paige, S.L., Razumova, M. V., Regnier, M., Tung, K.C., Keller, G., Pabon, L., Reinecke, H., et al. (2015). Mechanical Stress Promotes Maturation of Human Myocardium From Pluripotent Stem Cell-Derived Progenitors. Stem Cells *33*, 2148–2157. 10.1002/stem.2036.

129. Knöll, R., Hoshijima, M., Hoffman, H.M., Person, V., Lorenzen-Schmidt, I., Bang, M.-L., Hayashi, T., Shiga, N., Yasukawa, H., Schaper, W., et al. (2002). The Cardiac Mechanical Stretch Sensor Machinery Involves a Z Disc Complex that Is Defective in a Subset of Human Dilated Cardiomyopathy. Cell *111*, 943–955. 10.1016/S0092-8674(02)01226-6.

130. Bugg, D., Bretherton, R., Kim, P., Olszewski, E., Nagle, A., Schumacher, A.E., Chu, N., Gunaje, J., DeForest, C.A., Stevens, K., et al. (2020). Infarct Collagen Topography Regulates Fibroblast Fate via p38-Yes-Associated Protein Transcriptional Enhanced Associate Domain Signals. Circ Res *127*, 1306–1322. 10.1161/CIRCRESAHA.119.316162.

131. Ribeiro, A.J.S., Ang, Y.-S., Fu, J.-D., Rivas, R.N., Mohamed, T.M.A., Higgs, G.C., Srivastava, D., and Pruitt, B.L. (2015). Contractility of single cardiomyocytes differentiated from pluripotent stem cells depends on physiological shape and substrate stiffness. Proceedings of the National Academy of Sciences *112*, 12705–12710. 10.1073/pnas.1508073112.

132. Boudou, T., Legant, W.R., Mu, A., Borochin, M.A., Thavandiran, N., Radisic, M., Zandstra, P.W., Epstein, J.A., Margulies, K.B., and Chen, C.S. (2012). A Microfabricated Platform to Measure and Manipulate the Mechanics of Engineered Cardiac Microtissues. Tissue Eng Part A *18*, 910–919. 10.1089/ten.tea.2011.0341.

133. Legant, W.R., Pathak, A., Yang, M.T., Deshpande, V.S., McMeeking, R.M., and Chen, C.S. (2009). Microfabricated tissue gauges to measure and manipulate forces from 3D microtissues. Proceedings of the National Academy of Sciences *106*, 10097–10102. 10.1073/pnas.0900174106.

134. Leonard, A., Bertero, A., Powers, J.D., Beussman, K.M., Bhandari, S., Regnier, M., Murry, C.E., and Sniadecki, N.J. (2018). Afterload promotes maturation of human induced pluripotent stem cell derived cardiomyocytes in engineered heart tissues. J Mol Cell Cardiol *118*, 147–158. 10.1016/j.yjmcc.2018.03.016.

135. Ronaldson-Bouchard, K., Ma, S.P., Yeager, K., Chen, T., Song, L., Sirabella, D., Morikawa, K., Teles, D., Yazawa, M., and Vunjak-Novakovic, G. (2018). Advanced maturation of human cardiac tissue grown from pluripotent stem cells. Nature *556*, 239–243. 10.1038/s41586-018-0016-3.

136. Akram, K.M., Yates, L.L., Mongey, R., Rothery, S., Gaboriau, D.C.A., Sanderson, J., Hind, M., Griffiths, M., and Dean, C.H. (2019). Live imaging of alveologenesis in precision-cut lung slices reveals dynamic epithelial cell behaviour. Nat Commun *10*, 1178. 10.1038/s41467-019-09067-3.

137. Huh, D., Matthews, B.D., Mammoto, A., Montoya-Zavala, M., Hsin, H.Y., and Ingber, D.E. (2010). Reconstituting Organ-Level Lung Functions on a Chip. Science (1979) *328*, 1662–1668. 10.1126/science.1189401.

138. Stucki, J.D., Hobi, N., Galimov, A., Stucki, A.O., Schneider-Daum, N., Lehr, C.-M., Huwer, H., Frick, M., Funke-Chambour, M., Geiser, T., et al. (2018). Medium throughput breathing human primary cell alveolus-on-chip model. Sci Rep *8*, 14359. 10.1038/s41598-018-32523-x.

139. Michas, C., Karakan, M.Ç., Nautiyal, P., Seidman, J.G., Seidman, C.E., Agarwal, A., Ekinci, K., Eyckmans, J., White, A.E., and Chen, C.S. (2022). Engineering a living cardiac pump on a chip using high-precision fabrication. Sci Adv *8*. 10.1126/sciadv.abm3791.

140. MacQueen, L.A., Sheehy, S.P., Chantre, C.O., Zimmerman, J.F., Pasqualini, F.S., Liu, X., Goss, J.A., Campbell, P.H., Gonzalez, G.M., Park, S.-J., et al. (2018). A tissue-engineered scale model of the heart ventricle. Nat Biomed Eng *2*, 930–941. 10.1038/s41551-018-0271-5.

141. Lai, B.F.L., Huyer, L.D., Lu, R.X.Z., Drecun, S., Radisic, M., and Zhang, B. (2017). InVADE: Integrated Vasculature for Assessing Dynamic Events. Adv Funct Mater *27*, 1703524. 10.1002/adfm.201703524.

142. Ronaldson-Bouchard, K., Teles, D., Yeager, K., Tavakol, D.N., Zhao, Y., Chramiec, A., Tagore, S., Summers, M., Stylianos, S., Tamargo, M., et al. (2022). A multi-organ chip with matured tissue niches linked by vascular flow. Nat Biomed Eng *6*, 351–371. 10.1038/s41551-022-00882-6.

143. Ma, Q., Ma, H., Xu, F., Wang, X., and Sun, W. (2021). Microfluidics in cardiovascular disease research: state of the art and future outlook. Microsyst Nanoeng *7*, 19. 10.1038/s41378-021-00245-2.

144. Kim, S., Kim, W., Lim, S., and Jeon, J. (2017). Vasculature-On-A-Chip for In Vitro Disease Models. Bioengineering *4*, 8. 10.3390/bioengineering4010008.

145. Moses, S.R., Adorno, J.J., Palmer, A.F., and Song, J.W. (2020). Vessel-on-a-chip models for studying microvascular physiology, transport, and function in vitro. American Journal of Physiology-Cell Physiology, ajpcell.00355.2020. 10.1152/ajpcell.00355.2020.

146. Li, L., Lv, X., Ostrovidov, S., Shi, X., Zhang, N., and Liu, J. (2014). Biomimetic Microfluidic Device for in Vitro Antihypertensive Drug Evaluation. Mol Pharm *11*, 2009–2015. 10.1021/mp5000532.

147. Na, J.-T., Hu, S.-Y., Xue, C.-D., Wang, Y.-X., Chen, K.-J., Li, Y.-J., Wang, Y., and Qin, K.-R. (2021). A microfluidic system for precisely reproducing physiological blood pressure and wall shear stress to endothelial cells. Analyst *146*, 5913–5922. 10.1039/D1AN01049B.

148. Cardoso, B.D., Castanheira, E.M.S., Lanceros-Méndez, S., and Cardoso, V.F. (2023). Recent Advances on Cell Culture Platforms for In Vitro Drug Screening and Cell Therapies: From Conventional to Microfluidic Strategies. Adv Healthc Mater *12*. 10.1002/adhm.202202936.

149. Mahto, S.K., Tenenbaum-Katan, J., Greenblum, A., Rothen-Rutishauser, B., and Sznitman, J. (2014). Microfluidic shear stress-regulated surfactant secretion in alveolar epithelial type II cells in vitro. American Journal of Physiology-Lung Cellular and Molecular Physiology *306*, L672–L683. 10.1152/ajplung.00106.2013.

150. Radisic, M., Marsano, A., Maidhof, R., Wang, Y., and Vunjak-Novakovic, G. (2008). Cardiac tissue engineering using perfusion bioreactor systems. Nat Protoc *3*, 719–738. 10.1038/nprot.2008.40.

151. Visone, R., Talò, G., Lopa, S., Rasponi, M., and Moretti, M. (2018). Enhancing all-in-one bioreactors by combining interstitial perfusion, electrical stimulation, on-line monitoring and testing within a single chamber for cardiac constructs. Sci Rep *8*, 16944. 10.1038/s41598-018-35019-w.

152. Bell, E., Ivarsson, B., and Merrill, C. (1979). Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. Proceedings of the National Academy of Sciences *76*, 1274–1278. 10.1073/pnas.76.3.1274.

153. Borin, D., Pecorari, I., Pena, B., and Sbaizero, O. (2018). Novel insights into cardiomyocytes provided by atomic force microscopy. Semin Cell Dev Biol *73*, 4–12. 10.1016/j.semcdb.2017.07.003.

154. Liu, F., and Tschumperlin, D.J. (2011). Micro-Mechanical Characterization of Lung Tissue Using Atomic Force Microscopy. Journal of Visualized Experiments. 10.3791/2911.

155. Liu, L., He, F., Yu, Y., and Wang, Y. (2020). Application of FRET Biosensors in Mechanobiology and Mechanopharmacological Screening. Front Bioeng Biotechnol *8*. 10.3389/fbioe.2020.595497.

156. Ellefsen, K.L., Holt, J.R., Chang, A.C., Nourse, J.L., Arulmoli, J., Mekhdjian, A.H., Abuwarda, H., Tombola, F., Flanagan, L.A., Dunn, A.R., et al. (2019). Myosin-II mediated traction forces evoke localized Piezo1-dependent Ca2+ flickers. Commun Biol *2*, 298. 10.1038/s42003-019-0514-3.

157. Daneshjou, N., Sieracki, N., van Nieuw Amerongen, G.P., Conway, D.E., Schwartz, M.A., Komarova, Y.A., and Malik, A.B. (2015). Rac1 functions as a reversible tension modulator to stabilize VE-cadherin trans-interaction. Journal of Cell Biology *208*, 23–32. 10.1083/jcb.201409108.

158. Hart, R.G., Kota, D., Li, F., Ramallo, D., Price, A.J., Otterpohl, K.L., Smith, S.J., Dunn, A.R., Liu, J., and Chandrasekar, I. (2019). Myosin II Tension Sensors Visualize Force Generation within the Actin Cytoskeleton in Living Cells. bioRXiv.

159. Farhadifar, R., Röper, J.-C., Aigouy, B., Eaton, S., and Jülicher, F. (2007). The Influence of Cell Mechanics, Cell-Cell Interactions, and Proliferation on Epithelial Packing. Current Biology *17*, 2095–2104. 10.1016/j.cub.2007.11.049.

160. Morris, P.D., Narracott, A., von Tengg-Kobligk, H., Silva Soto, D.A., Hsiao, S., Lungu, A., Evans, P., Bressloff, N.W., Lawford, P. V, Hose, D.R., et al. (2016). Computational fluid dynamics modelling in cardiovascular medicine. Heart *102*, 18–28. 10.1136/heartjnl-2015-308044.

161. Faizal, W.M., Ghazali, N.N.N., Khor, C.Y., Badruddin, I.A., Zainon, M.Z., Yazid, A.A., Ibrahim, N.B., and Razi, R.M. (2020). Computational fluid dynamics modelling of human upper airway: A review. Comput Methods Programs Biomed *196*, 105627. 10.1016/j.cmpb.2020.105627.

162. Wiggs, B.R., Hrousis, C.A., Drazen, J.M., and Kamm, R.D. (1997). On the mechanism of mucosal folding in normal and asthmatic airways. J Appl Physiol *83*, 1814–1821. 10.1152/jappl.1997.83.6.1814.

163. Miura, T. (2015). Models of lung branching morphogenesis. J Biochem *157*, 121–127. 10.1093/jb/mvu087.

164. Iber, D., and Menshykau, D. (2013). The control of branching morphogenesis. Open Biol *3*, 130088. 10.1098/rsob.130088.

165. Goodwin, K., and Nelson, C.M. (2020). Branching morphogenesis. Development *147*. 10.1242/dev.184499.

166. Brodland, G.W. (2015). How computational models can help unlock biological systems. Semin Cell Dev Biol *47–48*, 62–73. 10.1016/j.semcdb.2015.07.001.

167. Sharpe, J. (2017). Computer modeling in developmental biology: growing today, essential tomorrow. Development *144*, 4214–4225. 10.1242/dev.151274.

168. Niederer, S.A., Lumens, J., and Trayanova, N.A. (2019). Computational models in cardiology. Nat Rev Cardiol *16*, 100–111. 10.1038/s41569-018-0104-y.

169. Neelakantan, S., Xin, Y., Gaver, D.P., Cereda, M., Rizi, R., Smith, B.J., and Avazmohammadi, R. (2022). Computational lung modelling in respiratory medicine. J R Soc Interface *19*. 10.1098/rsif.2022.0062.

170. Massia, S.P., and Hubbell, J.A. (1991). An RGD Spacing of 440 nm Is Sufficient for Integrin a,ß3-mediated Fibroblast Spreading and 140 nm for Focal Contact and Stress Fiber Formation.

171. Clark, A.Y., Martin, K.E., García, J.R., Johnson, C.T., Theriault, H.S., Han, W.M., Zhou, D.W., Botchwey, E.A., and García, A.J. (2020). Integrin-specific hydrogels modulate transplanted human bone marrow-derived mesenchymal stem cell survival, engraftment, and reparative activities. Nat Commun *11*, 114. 10.1038/s41467-019-14000-9.

172. Jeon, O., Alt, D.S., Linderman, S.W., and Alsberg, E. (2013). Biochemical and physical signal gradients in hydrogels to control stem cell behavior. Advanced Materials *25*, 6366–6372. 10.1002/adma.201302364.

173. Scott, J.E., Yang, S.-Y., Stanik, E., and Anderson, J.E. (1993). Influence of Strain on [^{3} H]thymidine Incorporation, Surfactant-related Phospholipid Synthesis, and cAMP Levels in Fetal Type II Alveolar Cells. Am J Respir Cell Mol Biol *8*, 258–265. 10.1165/ajrcmb/8.3.258.

174. Stout, D.A., Bar-Kochba, E., Estrada, J.B., Toyjanova, J., Kesari, H., Reichner, J.S., and Franck, C. (2016). Mean deformation metrics for quantifying 3D cell-matrix interactions without requiring information about matrix material properties. Proc Natl Acad Sci U S A *113*, 2898–2903. 10.1073/pnas.1510935113.

175. Ram-Mohan, S., Bai, Y., Schaible, N., Ehrlicher, A.J., Cook, D.P., Suki, B., Stoltz, D.A., Solway, J., Ai, X., and Krishnan, R. (2020). Tissue traction microscopy to quantify muscle contraction within precision-cut lung slices. American Journal of Physiology-Lung Cellular and Molecular Physiology *318*, L323–L330. 10.1152/ajplung.00297.2019.

176. Loebel, C., Weiner, A.I., Eiken, M.K., Katzen, J.B., Morley, M.P., Bala, V., Cardenas-Diaz, F.L., Davidson, M.D., Shiraishi, K., Basil, M.C., et al. (2022). Microstructured Hydrogels to Guide Self-Assembly and Function of Lung Alveolospheres. Advanced Materials, 2202992. 10.1002/adma.202202992.

177. Brandenberg, N., Hoehnel, S., Kuttler, F., Homicsko, K., Ceroni, C., Ringel, T., Gjorevski, N., Schwank, G., Coukos, G., Turcatti, G., et al. (2020). High-throughput automated organoid culture via stem-cell aggregation in microcavity arrays. Nat Biomed Eng *4*, 863–874. 10.1038/s41551-020-0565-2.

178. Cazzola, M., Stolz, D., Rogliani, P., and Matera, M.G. (2020). a1-Antitrypsin deficiency and chronic respiratory disorders. European Respiratory Review *29*, 190073. 10.1183/16000617.0073-2019.

179. Polin, R.A., Carlo, W.A., Papile, L.-A., Polin, R.A., Carlo, W., Tan, R., Kumar, P., Benitz, W., Eichenwald, E., Cummings, J., et al. (2014). Surfactant Replacement Therapy for Preterm and Term Neonates With Respiratory Distress. Pediatrics *133*, 156–163. 10.1542/peds.2013-3443.

180. Soll, R. (1998). Synthetic surfactant for respiratory distress syndrome in preterm infants. Cochrane Database of Systematic Reviews *2010*. 10.1002/14651858.CD001149.

181. Fala, L. (2015). Entresto (Sacubitril/Valsartan): First-in-Class Angiotensin Receptor Neprilysin Inhibitor FDA Approved for Patients with Heart Failure. Am Health Drug Benefits *8*, 330–334.

182. Bozkurt, B., Nair, A.P., Misra, A., Scott, C.Z., Mahar, J.H., and Fedson, S. (2023). Neprilysin Inhibitors in Heart Failure. JACC Basic Transl Sci *8*, 88–105. 10.1016/j.jacbts.2022.05.010.

183. Palandri, C., Santini, L., Argirò, A., Margara, F., Doste, R., Bueno-Orovio, A., Olivotto, I., and Coppini, R. (2022). Pharmacological Management of Hypertrophic Cardiomyopathy: From Bench to Bedside. Drugs *82*, 889–912. 10.1007/s40265-022-01728-w.

184. Sebastian, S.A., Padda, I., Lehr, E.J., and Johal, G. (2023). Aficamten: A Breakthrough Therapy for Symptomatic Obstructive Hypertrophic Cardiomyopathy. American Journal of Cardiovascular Drugs *23*, 519–532. 10.1007/s40256-023-00599-0.

185. Spertus, J.A., Fine, J.T., Elliott, P., Ho, C.Y., Olivotto, I., Saberi, S., Li, W., Dolan, C., Reaney, M., Sehnert, A.J., et al. (2021). Mavacamten for treatment of symptomatic obstructive hypertrophic cardiomyopathy (EXPLORER-HCM): health status analysis of a randomised, double-blind, placebo-controlled, phase 3 trial. The Lancet *397*, 2467–2475. 10.1016/S0140-6736(21)00763-7.

186. Selvaggio, A.S., and Noble, P.W. (2016). Pirfenidone Initiates a New Era in the Treatment of Idiopathic Pulmonary Fibrosis. Annu Rev Med *67*, 487–495. 10.1146/annurev-med-120214-013614.

187. Aimo, A., Spitaleri, G., Nieri, D., Tavanti, L.M., Meschi, C., Panichella, G., Lupón, J., Pistelli, F., Carrozzi, L., Bayes-Genis, A., et al. (2022). Pirfenidone for Idiopathic Pulmonary Fibrosis and Beyond. Card Fail Rev *8*. 10.15420/cfr.2021.30.

188. Shah, P. V, Balani, P., Lopez, A.R., Nobleza, C.M.N., Siddiqui, M., and Khan, S. (2021). A Review of Pirfenidone as an Anti-Fibrotic in Idiopathic Pulmonary Fibrosis and Its Probable Role in Other Diseases. Cureus. 10.7759/cureus.12482.

189. Ruwanpura, S.M., Thomas, B.J., and Bardin, P.G. (2020). Pirfenidone: Molecular Mechanisms and Potential Clinical Applications in Lung Disease. Am J Respir Cell Mol Biol *62*, 413–422. 10.1165/rcmb.2019-0328TR.

190. Conte, E., Gili, E., Fagone, E., Fruciano, M., Iemmolo, M., and Vancheri, C. (2014). Effect of pirfenidone on proliferation, TGF-β-induced myofibroblast differentiation and fibrogenic activity of primary human lung fibroblasts. European Journal of Pharmaceutical Sciences *58*, 13–19. 10.1016/j.ejps.2014.02.014.

191. John, A.E., Graves, R.H., Pun, K.T., Vitulli, G., Forty, E.J., Mercer, P.F., Morrell, J.L., Barrett, J.W., Rogers, R.F., Hafeji, M., et al. (2020). Translational pharmacology of an inhaled small molecule αvβ6 integrin inhibitor for idiopathic pulmonary fibrosis. Nat Commun *11*, 4659. 10.1038/s41467-020-18397-6.

192. Raghu, G., Mouded, M., Prasse, A., Stebbins, C., Zhao, G., Song, G., Arefayene, M., Violette, S.M., Gallagher, D., and Gibson, K.F. (2022). Randomized Phase IIa Clinical Study of an Anti-$\alpha_v\beta_6$ Monoclonal Antibody in Idiopathic Pulmonary Fibrosis. Am J Respir Crit Care Med *206*, 1166–1168. 10.1164/rccm.202205-0868LE.

193. Raghu, G., Mouded, M., Chambers, D.C., Martinez, F.J., Richeldi, L., Lancaster, L.H., Hamblin, M.J., Gibson, K.F., Rosas, I.O., Prasse, A., et al. (2022). A Phase IIb Randomized Clinical Study of an Anti-$\alpha_v\beta_6$ Monoclonal Antibody in Idiopathic Pulmonary Fibrosis. Am J Respir Crit Care Med *206*, 1128–1139. 10.1164/rccm.202112-2824OC.

194. Matera, D.L., Wang, W.Y., Smith, M.R., Shikanov, A., and Baker, B.M. (2019). Fiber Density Modulates Cell Spreading in 3D Interstitial Matrix Mimetics. ACS Biomater Sci Eng *5*, 2965–2975. 10.1021/acsbiomaterials.9b00141.