

Human-Relevant Non-Animal Methodologies (NAMs)
Specialised Models Series: Cardiovascular & Respiratory
Pulmonary Fibrosis & Chronic Lung Disease Models
Reference 4.4 in Full Scope Roadmap

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Legal & Scientific Imperative for Universities

Section 2A of the *Animals (Scientific Procedures) Act 1986* imposes a statutory duty on the Secretary of State to ensure compliance with the principles of replacement, reduction, and refinement. The principle of replacement requires that, wherever a specialised, scientifically satisfactory method or testing strategy not entailing the use of protected animals is available, it must be used to replace the use of a regulated procedure.

Idiopathic Pulmonary Fibrosis (IPF) is a devastating, progressive, and irreversible interstitial lung disease characterised by chronic alveolar epithelial cell injury, aberrant myofibroblast activation, and pathological extracellular matrix (ECM) deposition. Preclinical drug candidate screening has historically relied on in vivo animal models of pulmonary fibrosis. However, the cellular microenvironment, mechanical stress dynamics, and immunological pathways of rodents differ fundamentally from human patients, making animal models non-predictive of clinical outcomes.

At the physiological, mechanical, and molecular levels, human and rodent systems diverge during fibrotic pathology:

- **Reversibility Mismatch (Spontaneous Resolution):** The standard rodent model of pulmonary fibrosis relies on a single intratracheal instillation of the chemotherapeutic agent bleomycin. While this induces rapid alveolar damage and subsequent collagen deposition in rodents, the fibrotic response is transient and spontaneously resolves within 28 days. In stark contrast, human clinical IPF is progressive, non-resolving, and fatal, meaning anti-fibrotic drug candidates that succeed by merely accelerating natural rodent resolution routinely fail to show efficacy in human clinical trials.
- **Mechanical Strain and Breathing Dynamics:** Pulmonary fibrosis is highly regulated by mechanical tension. In a healthy human lung, cyclical breathing stretches alveolar epithelial cells, maintaining tissue homeostasis. In diseased lungs, stiffened fibrotic tissue concentrates mechanical stress at the boundary of active fibrotic foci, driving further myofibroblast activation through stretch-induced transforming growth factor-beta 1 (TGF- β 1) activation. Rodents, with rapid breathing rates (up to 350 breaths per minute) and vastly different chest-wall compliance, cannot replicate these human-specific localised mechanical strain dynamics.
- **Species-Specific Cellular Cascades:** Human fibrotic remodeling is driven by a unique secretome of matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and localised cytokines. Rodent fibroblasts express highly distinct receptor densities and cytokine profiles. Consequently, major pathways targeted by anti-fibrotic candidates fail to translate because rodent inflammatory cascades do not mirror the progressive, non-inflammatory epithelial-mesenchymal transition (EMT) seen in human clinical IPF.
- **Animal Welfare and High Severity:** The intratracheal bleomycin model represents one of the highest-severity procedures permitted under regulated animal research. Animals experience progressive, severe respiratory distress, hypoxia, dramatic weight loss, and chronic inflammatory pain. This intense systemic distress compromises the scientific validity of the model, as systemic stress-induced corticosteroid release acts as a major, uncontrolled confounding variable.

Transitioning to human-relevant Non-Animal Methodologies (NAMs)—such as mechanically active breathing alveolar chips, multi-cellular human fibrotic organoids, and ex vivo human precision-cut lung slices (PCLS)—is a dual scientific and statutory mandate for UK academic research.

4.4 Pulmonary Fibrosis & Breathing-on-Chip Models

This section covers human-relevant biological, tissue-engineered, and computational platforms designed to model progressive alveolar wall stiffening, stretch-induced TGF- β 1 activation, extracellular matrix deposition kinetics, and microvascular barrier breakdown.

Landmark Regulatory & Scientific Momentum

Significant progress has been made in the development and application of human-relevant respiratory fibrosis NAMs:

- **Cyclic-Stretch Alveolar-Capillary Breathing Chips:** Recreate the mechanical microenvironment of the human alveolus by co-culturing epithelial and endothelial cells on flexible membranes under vacuum-driven cyclic stretch, allowing researchers to study how mechanical strain accelerates fibrotic tissue remodelling.
- **Multi-Cellular Alveolar Fibrotic Organoids:** Recapitulate the complex, non-resolving cellular crosstalk of human pulmonary fibrosis by co-culturing primary human alveolar epithelial cells (hAECs), lung fibroblasts, and alveolar macrophages in self-assembling 3D micro-tissues.
- **Ex Vivo Human Precision-Cut Lung Slices (PCLS):** Maintain viable sections of human donor lung tissue containing intact alveolar architecture, enabling real-time pharmacological profiling of progressive collagen deposition and therapeutic tissue remodelling in native human tissue.

Core Specialised Respiratory Platforms

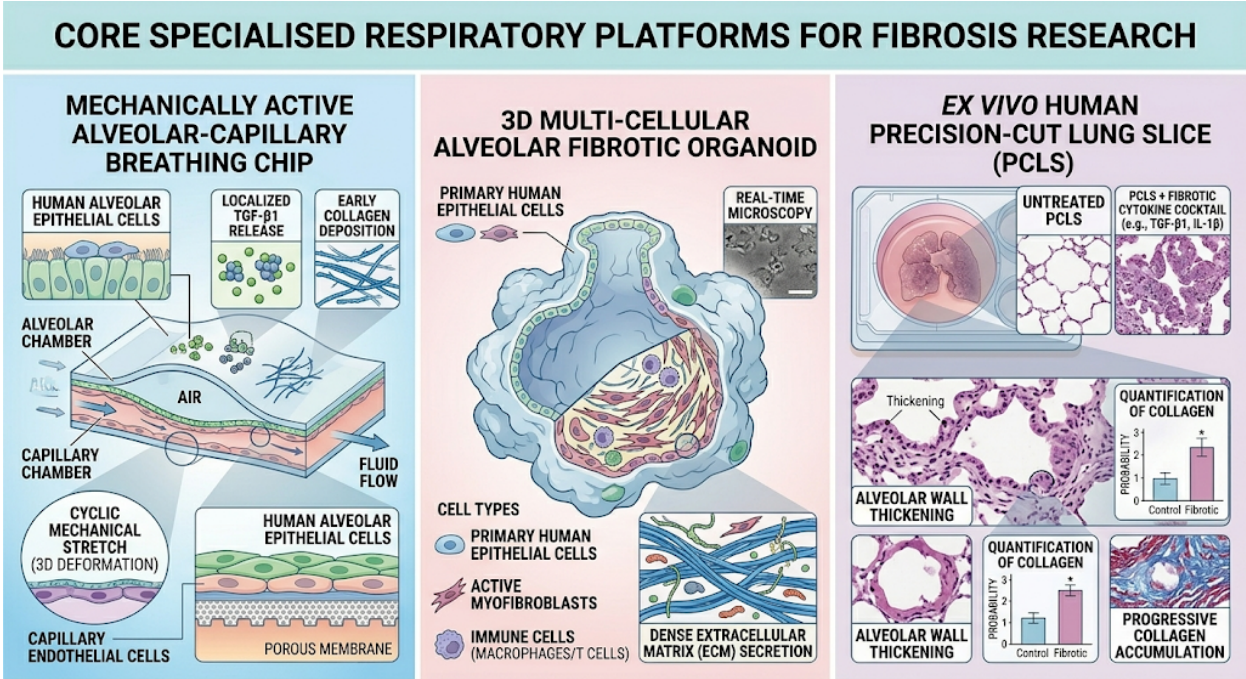


Figure 1: Core Specialised Respiratory Platforms — Mechanically active alveolar-capillary breathing chip showing co-cultured human alveolar epithelial cells and capillary endothelial cells on a flexible membrane under cyclic stretch, showing localised TGF- β 1 release and early collagen deposition (*left*); 3D multi-cellular alveolar fibrotic organoid co-culture containing primary human epithelial cells, active myofibroblasts, and immune cells secreting dense extracellular matrix (*centre*); and ex vivo human precision-cut lung slice (PCLS) treated with a fibrotic cytokine cocktail, showcasing alveolar wall thickening and progressive collagen accumulation (*right*).

Table 1: Specialised Biological & Tissue-Engineered (In Vitro) Respiratory Platform

Platform / Model	Description	Key Applications	Benefits for Universities & 3Rs
Breathing Alveolar-Capillary Fibrosis Chip	Co-culture of human alveolar epithelial cells and microvascular endothelial cells on flexible membranes subjected to cyclic mechanical stretch and inflammatory cytokines.	Modelling stretch-induced epithelial injury, localised TGF- β 1 activation, alveolar-capillary barrier breakdown, and fibrotic myofibroblast transition.	Directly replaces highly distressing rodent bleomycin inhalation exposure models and chronic particulate matter inhalation assays.
3D Alveolar Fibrotic Organoids	Self-organising 3D micro-tissues combining primary human alveolar epithelial cells, lung fibroblasts, and macrophages in biomimetic extracellular matrices.	Screening high-throughput anti-fibrotic compound libraries, evaluating cell-cell signaling cascades, and measuring progressive matrix deposition.	Replaces the long-term breeding of transgenic rodent strains designed to study human-restricted pulmonary inflammatory pathways.
Ex Vivo Human PCLS Fibrosis Model	Thin, viable slices of human donor lung tissue exposed to a cocktail of pro-fibrotic cytokines (TGF- β 1, PDGF, TNF- α) to induce progressive, localised matrix remodelling.	Real-time tracking of human-specific tissue contraction, alveolar wall thickening, collagen cross-linking, and anti-fibrotic drug efficacy.	Replaces invasive, high-severity allergen and chemical-sensitisation protocols used to induce chronic fibrogenesis in rodents.
Bioprinted Fibrotic Airway Scaffolds	High-precision biofabricated 3D constructs containing structured micro-channels, primary lung cells, and collagen-dense hydrogels.	Evaluating long-term, localised drug delivery kinetics across stiffened extracellular matrices and tracking chronic tissue-remodelling progression.	Bypasses highly invasive large-animal (sheep, pig) focal bronchial scar-induction and tracheal tissue grafting studies.

Cross-Cutting & Enabling Platforms for Respiratory Research

These platforms support multiple cardiorespiratory, pharmacokinetic, and environmental toxicology research areas and are widely applicable across small-molecule target discovery, safety profiling, and clinical trial simulations. While physical breathing-on-chips capture micro-structural niches, these cross-cutting digital and hybrid systems provide the quantitative, predictive pipelines required to validate high-maturity NAMs for regulatory-accepted, "fit-for-purpose" safety profiling.

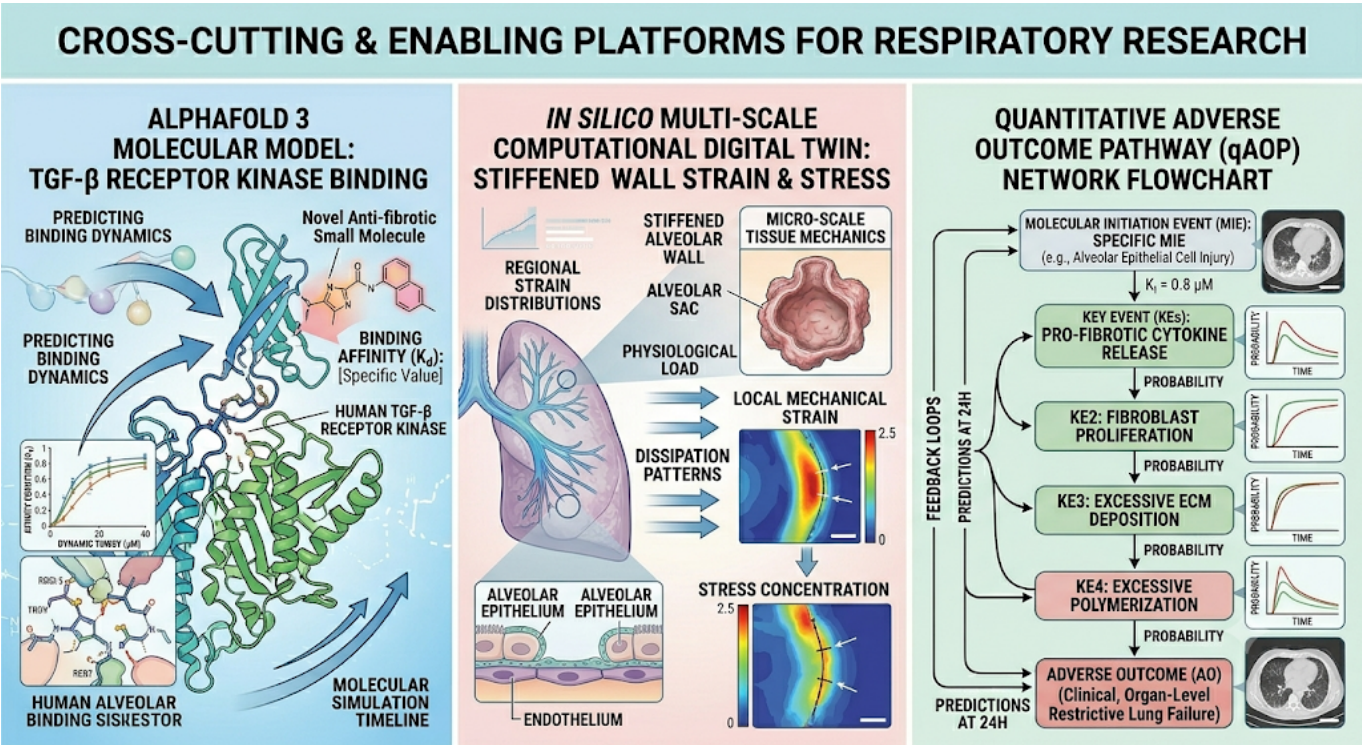


Figure 2: Cross-Cutting & Enabling Platforms for Respiratory Research — AlphaFold 3 molecular model predicting the binding dynamics of novel anti-fibrotic small molecules interacting with human TGF- β receptor kinase domains (*left*); in silico multi-scale computational digital twin simulating local mechanical strain and stress concentration across stiffened alveolar walls (*centre*); and a quantitative Adverse Outcome Pathway (qAOP) network flowchart linking molecular initiation events (such as alveolar epithelial cell injury) to clinical, organ-level restrictive lung failure (*right*).

Table 2: Genetic, Computational & Framework-Driven Platforms

Platform / Model	Description	Key Applications	Benefits for Universities & 3Rs
In Silico Alveolar Stretch Simulators	Finite element computational models mapping alveolar geometry, wall stiffness, localised tissue strain, and mechanical stress concentrations.	Predicting sites of mechanical-strain-driven fibrogenesis, calculating regional tissue dosing, and optimising anti-fibrotic therapy targeting.	Bypasses ex vivo animal vascular and airway perfusion assays and mechanical ventilation injury models in live rats.
Molecular Dynamics & Receptor Docking	AI-driven structural predictions mapping the interactions of anti-fibrotics with human TGF- β receptors, integrins, and fibroblast receptors.	High-throughput virtual screening of candidate compound libraries, predicting binding affinity, and structural lead optimisation.	Replaces animal-tissue-derived cell membrane preparation protocols traditionally used for radioactive ligand-binding assays.
qAOP-Integrated Fibrosis Screeners	Quantitative Adverse Outcome Pathways linking molecular initiation events (e.g., lysosomal stress) to chronic organ-level restrictive failure.	Regulatory safety assessment, environmental chemical toxicity screening, and high-throughput target prioritisation.	Integrates multiple in vitro data points into a legally defensible weight-of-evidence Replacement package for Project Licence applications.
Patient-Derived iPSC Alveolar Models	Reprogrammed patient-specific iPSCs differentiated into alveolar type II cells and assembled into diseased, multi-cellular alveolar assembloids.	Precision modelling of familial interstitial lung diseases, screening gene therapies, and validating cell-replacement therapeutics.	Bypasses the need for breeding and maintaining specialised transgenic rodent strains with human-restricted genetic respiratory mutations.

Regulatory Software & Strategy Frameworks

Regulatory acceptance of NAMs—referred to as "fit-for-purpose qualification"—is not a one-size-fits-all process. Researchers should utilise the following frameworks to ensure their computational and biological strategies align with the *Animals (Scientific Procedures) Act 1986* and industry best practices:

- **Context of Use (COU) Documentation:** Before deploying models, clearly define the specific scientific question the model is intended to address. This is critical for demonstrating that the NAM is reliable and suitable for its intended regulatory purpose.
- **Technical Characterisation:** Maintain comprehensive Standard Operating Procedures (SOPs) and study plans for both the biological NAM and any software used to analyse its output. This transparency is vital for successful project licence applications.
- **Early Regulatory Engagement:** Leverage institutional or external regulatory support to track "fit-for-purpose" milestones. Early engagement with the Home Office/ASRU is strongly encouraged to test the acceptability of a computational or NAM-based approach before full project submission.
- **Weight-of-Evidence (WoE) Frameworks:** Use integrated digital dashboards to aggregate data from both *in vitro* (NAM) and *in silico* (computational) sources. Building a strong "weight-of-evidence" argument is the most effective way to strengthen Replacement justifications when moving away from traditional animal models.

Skills, Training, and Capacity Building

Successful adoption of high-maturity Non-Animal Methodologies (NAMs) requires a strategic shift in workforce development. To ensure the long-term success of the platforms outlined in this series, universities should prioritise the following training initiatives to build institutional capacity:

- **Integrated Pulmonary Physiology & Microfluidics:** Training programmes should bridge the gap between bench-top respiratory biology, fluidic circuit engineering, and complex computational mechanics. Researchers require dual proficiency in maintaining long-term alveolar tissue barrier integrity under dynamic stretch and leveraging the finite element computational modelling software necessary to interpret stress concentrations.
- **Regulatory Competency:** Staff should receive formal training on "Fit-for-Purpose" qualification and the specific regulatory expectations of the Home Office/ASRU. This includes understanding how to document evidence for the 3Rs within formal project licence applications to ensure they meet legal standards.
- **Technological Literacy:** Skills in microfluidic device troubleshooting, primary stem-cell alveolar differentiation, multi-cellular organoid maintenance, and AI-driven receptor docking are increasingly critical for modern respiratory research. Universities should facilitate internal workshops or establish external partnerships to provide PhD students and postdoctoral fellows with hands-on experience in these specialised tools.
- **Ethics & Translation:** Education should focus on the scientific imperative of human-relevant research, emphasising why NAMs provide more predictive insights for clinical outcomes than traditional rodent models. This fosters a culture of excellence and ethical research design that is fully aligned with modern international standards.

Table 3: Skills • Training and Capacity Building

Capability Domain	Focus Area	Impact on 3Rs/Replacement
Technical	Advanced human-derived airway epithelial-interface culture	Replaces animal-based acute inhalation toxicology studies
Computational	AI-driven environmental stressor simulation	Bypasses non-predictive murine pulmonary inflammation assays
Regulatory	Fit-for-Purpose validation of environmental toxicology NAMs	Facilitates compliance with ASPA 1986 replacement mandate
Bioinformatics	Toxicogenomic analysis of particulate matter exposure	Eliminates animal-based pulmonary tissue sampling

Strategic Note: Building institutional expertise in high-maturity human-relevant environmental toxicology NAMs is essential for bridging the translational gap in pulmonary safety assessments. By training staff in the management of complex airway interfaces and digital exposure modelling, the institution fulfills its statutory duty under *ASPA 1986* to transition away from high-severity animal challenge studies toward fully predictive, human-centric research models.

Clinical Translation Case Studies

Case Study 1: Human Breathing-on-Chip Correcting Rodent Bleomycin False Positives

- **Background:** A novel anti-inflammatory and anti-fibrotic candidate drug demonstrated spectacular success during preclinical evaluation, showing 100% resolution of pulmonary scarring and airway stiffness in the standard rat bleomycin-induced lung injury model. However, when progressed to human clinical trials, the therapeutic candidate demonstrated zero clinical efficacy, and human patients experienced progressive, unchecked lung stiffness, forcing the sudden termination of the trial.
- **The Paradigm Shift:** An academic respiratory laboratory re-evaluated the drug candidate using a human alveolar-capillary breathing-on-chip. The human-relevant platform correctly predicted the clinical failure, proving that rodent models are false-positive indicators driven by spontaneous, non-human resolving mechanisms.
- **The Methodology:** Researchers cultured human primary alveolar epithelial cells and lung microvascular endothelial cells on a flexible membrane in a microfluidic device, applying cyclic mechanical stretch and a pro-fibrotic cytokine cocktail. While the therapeutic candidate reduced early acute inflammation, it completely failed to block progressive, stretch-activated myofibroblast differentiation and subsequent collagen deposition in the human system, mirroring the clinical trial outcome.
- **University & Legal Takeaway:** This study highlights how human mechanically active breathing-on-chips successfully replace animal pulmonary injury models, proving to the Home Office under ASPA 1986 that the rodent preclinical data had been actively misleading.

Case Study 2: Human Alveolar Spheroids Replacing High-Severity Intratracheal Bleomycin Instillation in Rodents

- **Background:** Traditional research into idiopathic pulmonary fibrosis (IPF) target validation relies on injecting rats or mice intratracheally with bleomycin. This high-severity procedure induces severe acute lung necrosis, progressive asphyxiation, chronic systemic pain, and massive weight loss, with high mortality rates occurring before tissue harvesting.
- **The Paradigm Shift:** A university research department implemented multi-cellular 3D human alveolar spheroids, completely replacing the need for high-severity animal pulmonary injury models for target validation.
- **The Methodology:** The team co-cultured primary human alveolar epithelial cells (hAECs) with human lung fibroblasts and primary alveolar macrophages within 3D hydrogels. By introducing pro-fibrotic cytokines, they induced a non-resolving fibrotic state characterised by progressive extracellular matrix stiffening and myofibroblast activation. They measured collagen accumulation and tested novel therapeutic small molecules using non-invasive, real-time fluorescent biosensors, validating key drug targets in human-predictive tissue without animal suffering.
- **University & Legal Takeaway:** This study showcases a highly predictive human alternative that completely replaces high-severity animal protocols, aligning perfectly with Home Office and ASRU expectations for the application of high-maturity Replacement models.

Executive Summary & Compliance Dashboard

Executive Summary

The assessment of environmental pollutants and pulmonary toxicity has long relied on high-severity animal inhalation challenge studies, which often fail to replicate human-specific airway barrier responses and toxicogenomic sensitivity. These legacy animal models provide poor predictive value for human clinical outcomes due to species-specific anatomical and physiological differences in the respiratory tract. This roadmap mandates a transition to high-fidelity, human-relevant NAMs—specifically human-derived airway epithelial interfaces and AI-driven toxicogenomic modeling—to replace animal-based testing paradigms. This strategic shift ensures all environmental and pulmonary research is scientifically precise, ethically robust, and fully compliant with the Section 2A statutory duty of the *ASPA 1986*.

Compliance Dashboard

Focus Area	Primary Animal Model	NAM Replacement Technology	ASPA 1986 Status
Pulmonary Toxicology	Acute animal inhalation studies	Airway Epithelial Interfaces & AI Toxicogenomics	High

Maturity Matrix

- Phase 1 (Exploratory):** Audit current reliance on animal-based inhalation toxicology and pulmonary stressor models.
- Phase 2 (Pilot):** Integrate human-derived airway epithelial cultures and environmental stressor simulation platforms.
- Phase 3 (Integration):** Implement AI-driven toxicogenomic analysis to predict human-specific pulmonary responses.
- Phase 4 (Standardisation):** Replace 100% of high-severity pulmonary animal challenge models with qualified, human-relevant NAMs.

Final Compliance Statement

All proposed models satisfy the Section 2A statutory duty of *ASPA 1986*. By adopting these methodologies, the institution positions itself at the forefront of the global transition toward human-centric, cruelty-free medical science.

Integration Context: Roadmap Conclusion

The modules detailed in this roadmap—from the pulmonary toxicology platforms explored in this section to the broader integrated cardiopulmonary and neurological frameworks established throughout this series—demonstrate that replacement is an integrated institutional strategy. By mapping these specialised NAM-based toxicology models into a unified regulatory framework, the institution moves decisively toward a standardised, human-centric research paradigm. This systemic integration is the essential prerequisite for fulfilling the *ASPA 1986* statutory duty, ensuring all research is scientifically accurate, ethically aligned, and regulatory compliant.