

Human-Relevant Non-Animal Methodologies (NAMs)
*Specialised Models Series: **Cardiovascular & Respiratory***
Vascular / Endothelial Models
Reference 4.3 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.

Vascular disorders—including atherosclerosis, deep vein thrombosis (DVT), stroke, and systemic endothelial dysfunction during sepsis or viral infection—represent major targets in drug discovery. Historically, safety and efficacy testing has relied on *in vivo* animal models of vascular injury and thrombosis. However, the human vascular endothelium and coagulation systems exhibit profound, species-specific physiological and molecular differences that make animal models fundamentally non-predictive of human clinical outcomes.

At the physiological, mechanical, and molecular levels, human and rodent vascular systems diverge:

- **Coagulation Cascade Mismatch:** Human and rodent coagulation factors differ drastically in their activation kinetics and baseline plasma concentrations. For example, rodents possess significantly higher baseline levels of clotting factors VIII, IX, and X, alongside higher platelet counts (up to five-fold higher than humans). This hypercoagulable baseline in rodents routinely masks the efficacy of novel human anti-thrombotic or anti-platelet therapies, leading to false negatives during safety and efficacy screening.
- **Shear Stress and Mechanotransduction:** Endothelial cells are highly sensitive to fluid shear stress, translating frictional drag into biochemical signals via specialised mechanoreceptors (such as PECAM-1 and VE-cadherin). Rodent blood vessel geometry and blood flow rates produce wall shear stresses that are magnitudes higher than those in human vessels, fundamentally altering endothelial cell alignment, nitric oxide (NO) production, and vascular remodelling.
- **Atherosclerosis Resistance:** Wild-type rodents do not naturally develop atherosclerosis due to highly distinct lipid and lipoprotein profiles (rodents transport cholesterol primarily via high-density lipoproteins, HDL, rather than low-density lipoproteins, LDL). To force plaque formation, researchers must use genetically modified animals (such as ApoE-deficient mice) subjected to high-fat diets, which still fail to capture the complex, inflammatory human plaque rupture and thrombus dynamics.
- **Vascular Injury Severity:** Traditional *in vivo* models of vascular damage and thrombosis—such as the ferric chloride (FeCl₃)-induced carotid artery injury, mechanical cuff placement, or the tail-bleeding assay—inflict immense, high-severity suffering. These procedures cause rapid vessel necrosis, systemic ischemic pain, and localised tissue trauma, which create massive physiological stress that acts as an uncontrolled confounding variable.

Transitioning to human-relevant Non-Animal Methodologies (NAMs)—such as perfused microfluidic vessels-on-chips, shear-stress-enabled thrombosis-on-chips, and bioprinted microvascular networks—is a dual scientific and statutory mandate for UK academic research.

4.3 Vascular, Endothelial & Thrombosis-on-Chip Models

This section covers human-relevant biological, tissue-engineered, and computational platforms designed to model microvascular perfusion, endothelial barrier dysfunction, leukocyte extravasation, and real-time microvascular thrombosis.

Landmark Regulatory & Scientific Momentum

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

- **Perfused Microfluidic Vessel-on-Chip Platforms:** Recreate human vascular physiology by culturing human primary or iPSC-derived endothelial cells inside microfluidic channels under continuous fluid flow, triggering physiological alignment, nitric oxide release, and tight-junction barrier formation.
- **Shear-Stress-Enabled Thrombosis-on-Chips:** Simulate dynamic platelet activation and thrombus formation by perfusing whole human blood through microchannels coated with human collagen and tissue factor, allowing real-time tracking of platelet adhesion, aggregation, and fibrin deposition.
- **3D Bioprinted Microvascular Networks:** Fabricate complex, multi-cellular vascular channels using human endothelial cells, smooth muscle cells, and pericytes embedded within biomimetic hydrogels, enabling the study of angiogenesis and vascular permeability.

Core Specialised Vascular Platforms

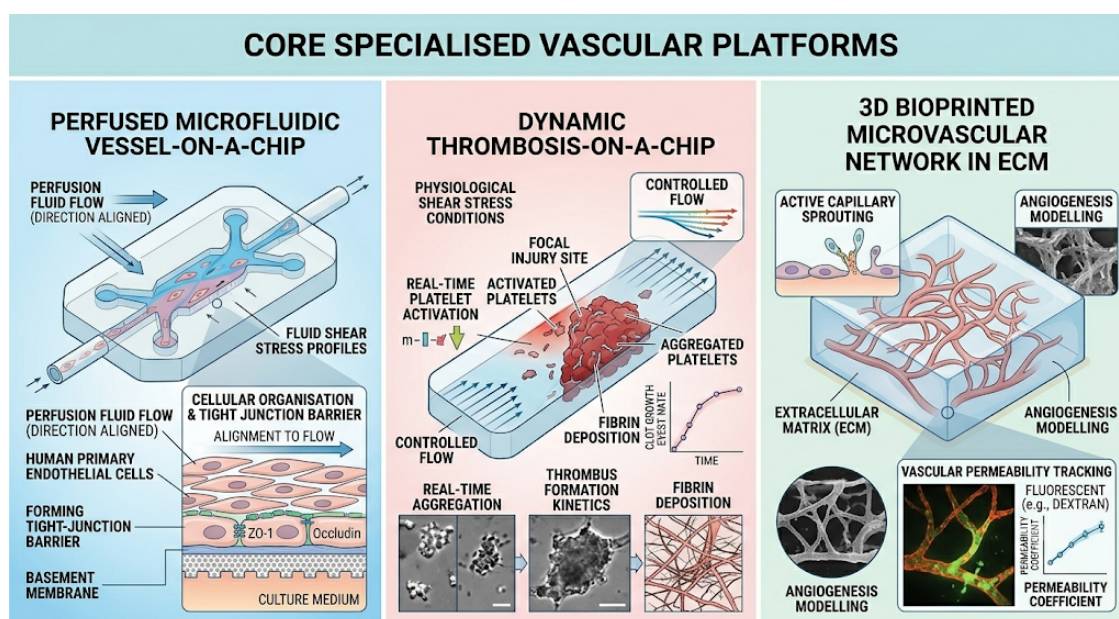


Figure 1: Core Specialised Vascular Platforms — Perfused microfluidic vessel-on-a-chip showing human primary endothelial cells aligned in the direction of fluid flow, forming a tight-junction barrier over a basement membrane (*left*); dynamic thrombosis-on-a-chip platform showing real-time human platelet activation, aggregation, and fibrin deposition under physiological shear stress (*centre*); and a 3D bioprinted microvascular network embedded within an extracellular matrix, showcasing active capillary sprouting and vascular permeability tracking (*right*).

Table 1: Specialised Biological & Tissue-Engineered (In Vitro) Vascular Platforms

Platform / Model	Description	Key Applications	Benefits for Universities & 3Rs
Perfused Vessel-on-Chip	Microfluidic channels lined with human endothelial cells exposed to continuous fluid flow and physiological shear stress.	Modelling endothelial barrier permeability, leukocyte adhesion/extravasation during inflammation, and drug transport.	Directly replaces invasive rodent mesenteric and cremaster muscle intravital imaging studies used to track leukocyte rolling.
Thrombosis-on-Chip	Microfluidic channels coated with human extracellular matrix proteins and tissue factor, perfused with whole human blood.	Real-time monitoring of platelet adhesion, clot kinetics, fibrin polymerisation, and testing novel anti-platelet and anti-thrombotic drugs.	Eliminates the need for high-severity ferric chloride (FeCl_3)-induced carotid artery injury and tail-bleeding assays.
Atherosclerosis-on-Chip	Co-culture of human endothelial cells and smooth muscle cells in microchannels subjected to disturbed blood flow and LDL perfusion.	Recapitulating human-specific lipid accumulation, foam cell formation, vascular calcification, and inflammatory plaque development.	Replaces the long-term, high-fat diet regimens and mechanical vascular cuff surgeries in transgenic ApoE-deficient mice.
3D Bioprinted Microvascular Networks	High-precision biofabricated 3D constructs containing hollow, self-assembled capillary channels embedded within hydrogels.	Evaluating angiogenesis, tumour-induced vascularisation, pericyte-endothelial structural crosstalk, and systemic drug distribution.	Bypasses highly painful animal corneal pocket neovascularisation assays and chorioallantoic membrane (CAM) studies.

Cross-Cutting & Enabling Platforms for Vascular Research

These platforms support multiple cardiovascular, inflammatory, and pharmacokinetic research areas and are widely applicable across small-molecule target discovery, safety pharmacology, and clinical trial simulations. While physical vessels-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.

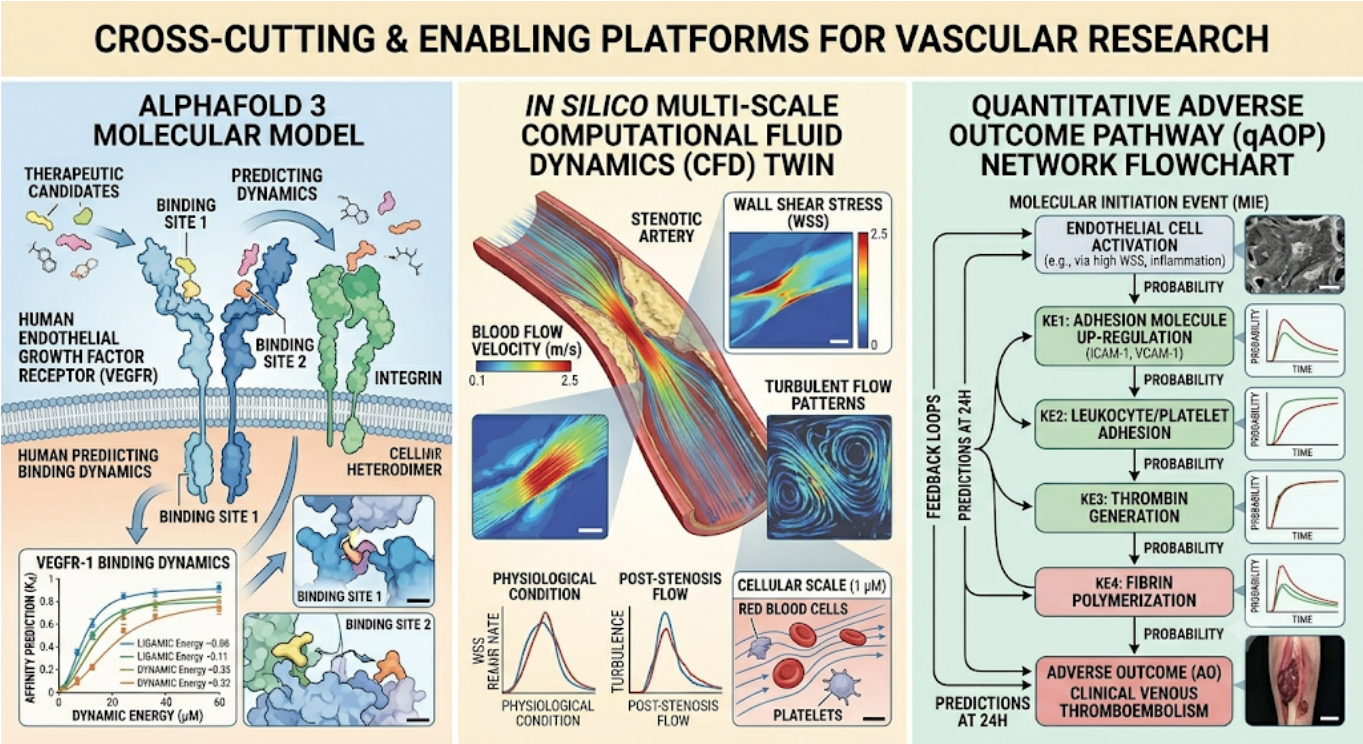


Figure 2: Cross-Cutting & Enabling Platforms for Vascular Research — AlphaFold 3 molecular model predicting the binding dynamics of therapeutic candidates interacting with human vascular endothelial growth factor receptors (VEGFR) and integrins (*left*); in silico multi-scale computational fluid dynamics (CFD) twin simulating blood flow velocity, wall shear stress, and turbulent flow patterns across stenotic human arteries (*centre*); and a quantitative Adverse Outcome Pathway (qAOP) network flowchart linking molecular initiation events (such as endothelial cell activation) to clinical venous thromboembolism (*right*).

Table 2: Genetic, Computational & Framework-Driven Platforms

Platform / Model	Description	Key Applications	Benefits for Universities & 3Rs
In silico Hemodynamic Simulators	Computational fluid dynamics (CFD) models mapping patient-specific vascular geometry, blood flow velocity, and wall shear stress.	Predicting plaque vulnerability sites, calculating localised drug delivery doses, and optimising coronary stent design.	Bypasses ex vivo animal vascular perfusion assays and invasive surgical stenotic models in dogs or non-human primates.
Molecular Dynamics & Receptor Docking	AI-driven structural predictions mapping the interactions of anti-coagulants with human thrombin, Factor Xa, and platelet receptors.	Screening compound libraries for off-target vascular toxicity, predicting drug binding affinity, and structural lead optimisation.	Replaces animal-tissue-derived platelet aggregation studies and radioactive ligand-binding assays using animal membranes.
Patient-Derived iPSC Endothelial Models	Reprogrammed patient-specific iPSCs differentiated into endothelial cells and smooth muscle cells, assembled into vascular networks.	Precision modelling of genetic vascular diseases (e.g., CADASIL, hereditary hemorrhagic telangiectasia) and testing gene therapies.	Bypasses the need for breeding and maintaining specialised transgenic rodent strains with human-restricted genetic vascular mutations.
qAOP-Integrated Vascular Screeners	Quantitative Adverse Outcome Pathways linking molecular initiation events (e.g., endothelial injury) to clinical thrombosis.	Regulatory safety assessment, environmental chemical vascular 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.

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 Vascular Biology & Microfluidics:** Training programmes should bridge the gap between bench-top vascular biology, microfluidic circuit engineering, and complex hemodynamic data analysis. Researchers require dual proficiency in maintaining long-term endothelial perfusion and leveraging the computational fluid dynamics software necessary to interpret shear stress profiles.
- **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 endothelial differentiation, microvascular network maintenance, and AI-driven molecular docking are increasingly critical for modern vascular 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 & Capacity Building

Capability Domain	Focus Area	Impact on 3Rs/Replacement
Technical	Multi-organ MPS (Cardiopulmonary-on-a-chip)	Replaces systemic animal models for drug distribution
Computational	Integrated hemodynamic/aerosol modeling	Bypasses complex multi-system animal physiology assays
Regulatory	Cross-platform WoE package development	Streamlines multi-system drug safety approvals under ASPA 1986
Bioinformatics	Systems biology analysis of organ-organ crosstalk	Eliminates animal-based multi-organ toxicology screens

Strategic Note: This investment in human expertise is a key component of a robust, future-proof institutional roadmap, demonstrating to regulators that the institution is committed to the safe and effective operation of high-maturity NAMs.

Clinical Translation Case Studies

Case Study 1: Human Thrombosis-on-Chip Correcting Rodent Coagulation False Negatives

- **Background:** A novel anti-platelet therapeutic candidate targeting a specific human platelet receptor demonstrated excellent efficacy and zero bleeding liabilities across extensive in vivo rodent testing. However, during Phase I clinical trials, human volunteers experienced severe, unexpected bleeding complications and thrombocytopenia, forcing the immediate termination of the program.
- **The Paradigm Shift:** An academic research group re-evaluated the failed candidate using a human-relevant thrombosis-on-chip platform. The human platform correctly predicted the high clinical bleeding risk, demonstrating that rodent coagulation profiles fail to mirror human-specific platelet activation and glycoprotein receptor density.
- **The Methodology:** Researchers perfused whole human blood through microfluidic channels coated with human collagen and tissue factor under arterial shear stress. Upon introducing the therapeutic candidate, they used high-resolution digital imaging to track platelet adhesion, P-selectin expression, and clot retraction. The human system detected a profound, dose-dependent inhibition of platelet aggregation and severe clot destabilisation, which rodent blood assays had failed to detect.
- **University & Legal Takeaway:** This study highlights how human-relevant thrombosis-on-chip platforms can successfully replace animal bleeding and thrombosis models, proving to the Home Office under ASPA 1986 that the rodent preclinical data had been actively misleading.

Case Study 2: Perfused Plaque-on-Chip Replacing High-Severity Rodent Carotid Cuff Atherosclerosis Models

- **Background:** Preclinical evaluation of therapeutics for plaque stability and atherosclerosis historically relies on genetically modified ApoE-deficient mice. To force plaque rupture, these animals are subjected to high-fat diets and undergo surgical placement of a constrictive plastic cuff around the carotid artery. This high-severity procedure causes chronic arterial constriction, excruciating ischemic pain, vascular necrosis, and unexpected sudden death.
- **The Paradigm Shift:** A university research department implemented a perfused, multi-cellular plaque-on-chip platform, completely replacing the need for high-severity animal surgical models for target validation.
- **The Methodology:** The team co-cultured human vascular endothelial cells, smooth muscle cells, and primary human macrophages in a microfluidic channel under disturbed, oscillatory shear stress. They perfused the system with human LDL to induce lipid accumulation and foam cell formation, successfully recapitulating a classic human atherosclerotic plaque. They tracked plaque degradation and macrophage inflammatory responses in real time via integrated biosensors, testing therapeutic candidates under realistic human flow conditions without using live animals.
- **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

Modern drug discovery requires an understanding of systemic interactions between the cardiovascular and respiratory systems—a complexity that isolated *in vivo* animal models fail to capture. Systemic animal models are often restricted by species-specific feedback loops and physiological divergence, leading to translational bottlenecks in safety pharmacology. This roadmap mandates a transition to high-fidelity, human-relevant NAMs—specifically multi-organ "Cardiopulmonary-on-a-chip" microphysiological systems and systems biology computational modelling—to evaluate organ-organ crosstalk. This integrated approach ensures that research strategies are 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
Cardiopulmonary	Systemic multi-organ animal models	Integrated MPS & Systems Biology Modelling	High

Maturity Matrix

Phase 1 (Exploratory): Audit current reliance on systemic animal models for drug distribution studies.

Phase 2 (Pilot): Integrate multi-organ cardiopulmonary-on-a-chip microphysiological systems.

Phase 3 (Integration): Implement systems biology computational modelling of multi-organ crosstalk.

Phase 4 (Standardisation): Replace 100% of systemic multi-organ animal toxicology assays with integrated 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 integrated cardiopulmonary platforms explored in this series to the broader respiratory, cardiovascular, and neurological frameworks established previously—demonstrate that replacement is an integrated institutional strategy. By mapping these specialised NAM-based systemic 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.