

Human-Relevant Non-Animal Methodologies (NAMs)
*Specialised Models Series: **Cardiovascular & Respiratory***
Cardiovascular Models
Reference 4.1 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.

Cardiovascular diseases and drug-induced cardiotoxicity represent some of the highest hurdles in clinical translation and regulatory drug approval. Historically, preclinical safety and efficacy pipelines have relied on in vivo animal models, ranging from transgenic rodents to surgically mutated canines and non-human primates. However, the mammalian cardiac system exhibits profound species-specific differences that make animal models structurally and functionally non-predictive of human clinical outcomes.

At the physiological and molecular levels, human and rodent hearts differ fundamentally in their electrophysiological and biochemical handling:

- **Heart Rate Mismatch:** Rodent hearts beat at approximately 600 bpm compared to the human resting heart rate of 60–80 bpm, altering the kinetic demands of every biochemical cascade.
- **Calcium Handling Divergence:** Human cardiomyocytes rely predominantly on the sarcoplasmic reticulum (Ca²⁺-ATPase, SERCA2a) and the sodium-calcium exchanger (NCX) for relaxation, whereas rodent cardiomyocytes utilise distinct, rapid calcium reuptake pathways.
- **Myosin Heavy Chain (MHC) Isoforms:** Adult human hearts express the slow β-MHC isoform, whereas adult rodent ventricles are dominated by the fast α-MHC isoform, fundamentally altering contractile velocity, myofibrillar ATPase activity, and pharmacological sensitivity.
- **Ion Channel Discrepancies:** Key cardiac repolarisation channels, such as the human ether-à-go-go-related gene (hERG) potassium channel, differ dramatically in expression and structure between species, causing animal models to routinely yield false negatives during cardiotoxicity and QT-prolongation screening.

Transitioning to human-relevant Non-Animal Methodologies (NAMs)—such as microphysiological heart-on-chips, bioprinted cardiac scaffolds, fluid-ejecting organoid chambers, and in silico haemodynamic digital twins—is a dual scientific and statutory mandate for UK academic research.

4.1 Cardiovascular & Ejection-Fraction MPS

This section covers human-relevant biological, tissue-engineered, and computational platforms designed to model myocardial contractility, action potential propagation, sarcomere alignment, and multi-chambered fluid ejection mechanics.

Landmark Regulatory & Scientific Momentum

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

- **Biomechanically Active Heart-on-Chip Platforms:** Recreate human myocardial physiology by applying continuous electrical pacing and cyclical mechanical stretching to aligned human iPSC-derived cardiomyocytes, capturing contractility and electrophysiological responses under physiological workloads.
- **Novoheart Mini-Heart / MyHeart™ Platforms:** Recapitulate clinical cardiac pump kinetics, stroke volume, and active ejection fractions using fluid-ejecting, stem-cell-derived 3D cardiac chambers ("heart-in-a-jar") to screen for therapeutics in heart failure.
- **Ex Vivo Human Heart Perfusion Systems (Langendorff):** Maintain explanted human donor hearts under oxygenated perfusion, preserving native 3D architecture, metabolic profiles, and intact electrophysiology to validate medical devices and novel pharmacotherapeutics in real human tissue without live-animal testing.

Core Specialised Cardiovascular Platforms

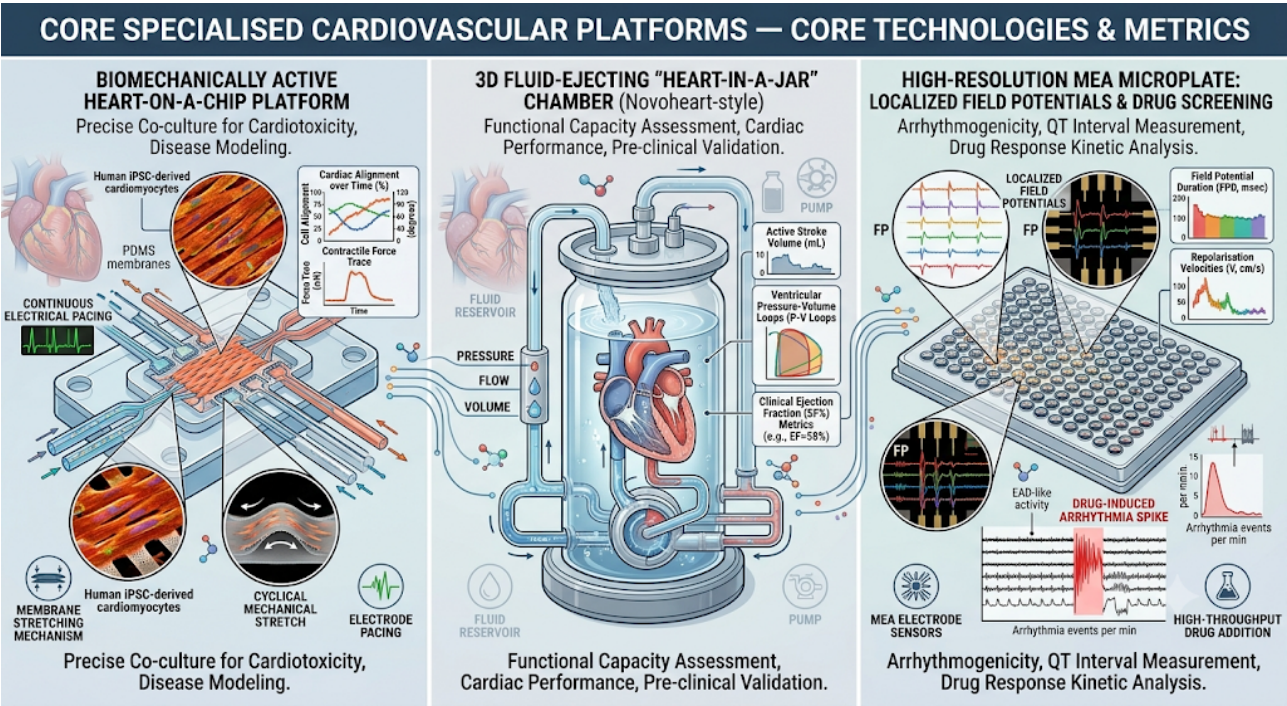


Figure 1: Core Specialised Cardiovascular Platforms — Biomechanically active heart-on-a-chip platform showing human iPSC-derived cardiomyocytes aligned on flexible PDMS membranes under continuous electrical pacing and cyclical mechanical stretch (*left*); 3D fluid-ejecting "heart-in-a-jar" chamber (Novoheart-style) measuring active stroke volume, ventricular pressure-volume loops, and clinical ejection fraction metrics (*centre*); and a high-resolution multi-electrode array (MEA) microplate recording localised field potential durations, repolarisation velocities, and drug-induced arrhythmia spikes (*right*).

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

Platform / Model	Description	Key Applications	Benefits for Universities & 3Rs
Biomechanically Active Heart-on-Chip	Microfluidic devices co-culturing aligned human iPSC-derived cardiomyocytes, endothelial cells, and cardiac fibroblasts under electrical pacing and cyclical mechanical strain.	High-throughput cardiotoxicity screening, drug-induced QT prolongation profiling, and modelling mechanical stress-induced hypertrophic remodelling.	Directly replaces chronic telemetry-equipped canine and rodent studies used to assess drug-induced cardiac conduction and structural safety.
Novoheart Mini-Heart / Ejection-Fraction MPS	Fluid-ejecting, stem-cell-derived 3D human cardiac chambers suspended over pressure-volume micro-sensors ("heart-in-a-jar").	Measuring active stroke volume, clinical ejection fraction, pressure-volume (P-V) loops, and evaluating therapies for Heart Failure (HFpEF).	Eliminates the need for high-severity rodent surgical heart failure models (e.g., transverse aortic constriction or coronary ligation).
Ex Vivo Human Heart Perfusion (Langendorff)	Whole explanted non-transplantable human donor hearts maintained in a viable state via computerised perfusion of nutrient-rich, oxygenated fluids.	Evaluating whole-organ haemodynamics, cardiac device biocompatibility, ischaemic-reperfusion injury, and real-time pharmacokinetic organ clearance.	Bypasses large-animal (pig, sheep, canine) open-chest surgical models, providing direct translation in actual human organ tissue.
Advanced 3D Myocardial Scaffolds	High-precision biofabricated 3D constructs containing structured micro-channels, extracellular matrix proteins, and primary cardiac cell lineages.	Assessing localised myocardial infarction healing, stem cell graft-host integration, long-term biocompatibility, and systemic vascular transport.	Replaces invasive animal biomaterial transplantation studies and longitudinal epicardial patch testing in live animals.

Cross-Cutting & Enabling Platforms for Cardiovascular Research

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

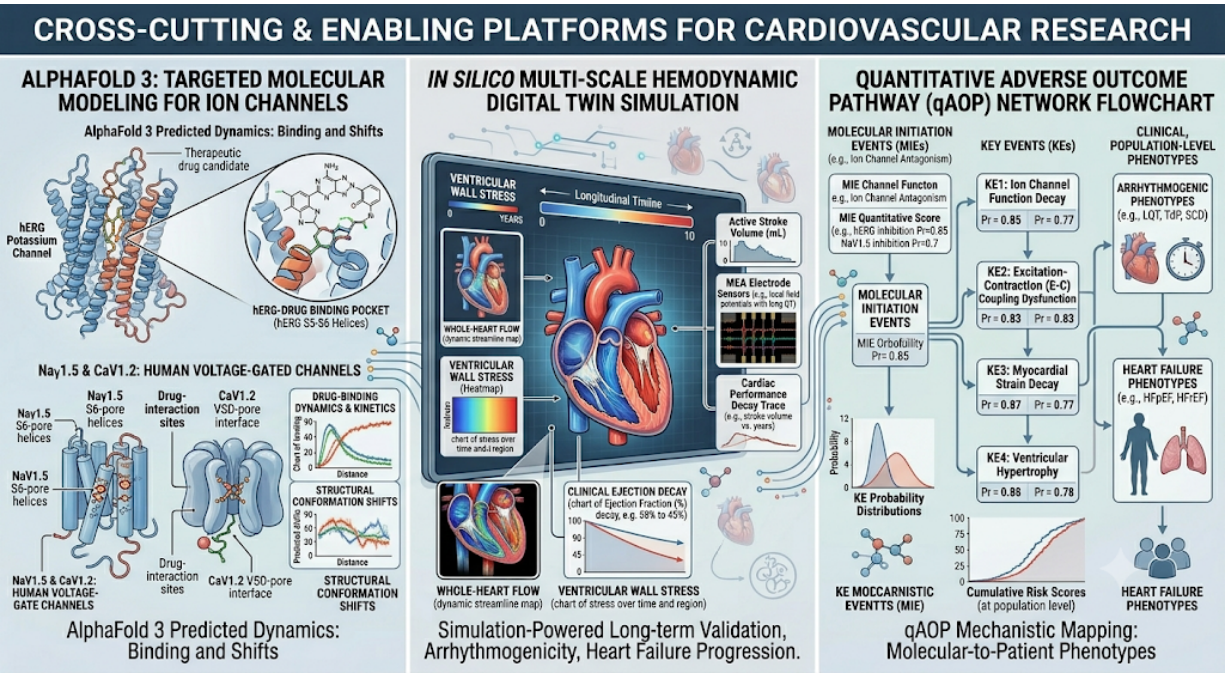


Figure 2: Cross-Cutting & Enabling Platforms for Cardiovascular Research — AlphaFold 3 molecular model predicting the binding dynamics and structural conformation of therapeutic candidates interacting with human voltage-gated ion channels (e.g., hERG, Na_v1.5, and Ca_v1.2) (*left*); in silico multi-scale hemodynamic digital twin simulating whole-heart blood flow dynamics, ventricular wall stress, and clinical ejection decay over longitudinal horizons (*centre*); and a quantitative Adverse Outcome Pathway (qAOP) network flowchart linking molecular initiation events (such as ion channel antagonism) to clinical, population-level arrhythmogenic and heart failure phenotypes (*right*).

Table 2: Cross Cutting & Enabling Platforms in Cardiovascular Research

Platform / Model	Description	Key Applications	Benefits for Universities & 3Rs
AlphaFold & Molecular Dynamics Simulators	Advanced AI-driven structural predictions mapping the interactions of small molecules with human cardiac ion channels (hERG, Na _v 1.5, Ca _v 1.2).	Screening drug candidates for off-target cardiotoxicity, predicting QT prolongation risk, and optimising ligand-receptor binding profiles.	Replaces animal-derived tissue harvesting and membrane preparation protocols traditionally used for radioactive ligand-binding assays.
Virtual Heart Haemodynamic Digital Twins	Multi-scale mathematical and fluid-dynamic models simulating ventricular geometry, blood flow turbulence, and mechanical wall stress.	Simulating systemic blood flow, predicting therapeutic outcomes in virtual patient cohorts, and optimising surgical device structural parameters.	A system-wide in silico framework that replaces highly invasive canine and non-human primate haemodynamics and chronic pacing models.
In Silico Electrophysiological AP Simulators	Computational models simulating cardiomyocyte action potential (AP) propagation, ion channel kinetics, and local field potential decay.	High-throughput virtual screening of candidate compound safety and predicting drug-induced Torsades de Pointes.	Directly replaces ex vivo animal Purkinje fibre action potential recording and in vivo canine pro-arrhythmia assays.
qAOP-Integrated Cardiovascular Screeners	Quantitative Adverse Outcome Pathways linking molecular initiation events (e.g., hERG block) to organ-level cardiovascular failure.	Regulatory safety assessment, environmental chemical cardiotoxicity 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 industrial 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 Cardiophysiology & Microengineering:** Training programmes should bridge the gap between bench-top cellular biology, fluidic microengineering, and complex electrophysiology data analysis. Researchers require dual proficiency in maintaining long-term fluidic circuit pacemakers and leveraging the computational pipelines necessary to interpret high-density microelectrode array data.
- **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 tissue differentiation, cardiac organoid maintenance, and AI-driven structural computational modelling are increasingly critical for modern cardiovascular 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	Cardiac organoid culture & soft robotic heart fabrication	Direct replacement of animal-based heart function assays
Computational	Biomechanical pressure/flow waveform modelling	Bypasses invasive animal hemodynamic monitoring
Regulatory	Fit-for-Purpose validation of human-centric NAMs	Streamlines compliance with ASPA 1986 replacement mandate
Bioinformatics	Patient-specific data integration for digital twins	Eliminates animal-based drug toxicity and efficacy testing

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 Cardiac MPS Correcting Rodent hERG / Cardiotoxicity False Negatives

- **Background:** A novel therapeutic candidate designed for a metabolic indication demonstrated excellent safety profiles and zero cardiotoxicity across extensive in vivo rodent screening. However, during Phase I clinical trials, human volunteers experienced severe QT-interval prolongation, putting them at risk of fatal arrhythmias (Torsades de Pointes) and forcing the immediate termination of the program.
- **The Paradigm Shift:** A university cardiovascular safety lab re-evaluated the failed candidate using a human-relevant cardiac microphysiological system (MPS). The human-relevant platform correctly predicted the clinical risk, demonstrating that rodent cardiac models are false-negative indicators for human-restricted potassium channel blockades.
- **The Methodology:** Researchers cultured human iPSC-derived cardiomyocytes on multi-channel microelectrode arrays (MEAs). They applied the candidate compound and quantified localised field potential duration (FPD), calcium transient velocities, and beat-rate variability. The human system detected a significant, dose-dependent prolongation of the FPD corresponding to human hERG channel block, which mouse channels (IKr) do not mirror.
- **University & Legal Takeaway:** These results demonstrate how human-relevant cardiac MPS can successfully replace animal cardiotoxicity assays, proving to the Home Office under ASPA 1986 that preclinical animal safety testing was non-predictive.

Case Study 2: Novoheart Mini-Heart Platform Replacing High-Severity Rodent Left-Ventricle Ligation Models

- **Background:** Preclinical evaluation of therapeutics for heart failure with preserved ejection fraction (HFpEF) historically relies on surgically mutating rodents. Animals undergo permanent ligation of the left anterior descending coronary artery or transverse aortic constriction to induce high-severity hypertrophic remodelling, resulting in severe physical distress, chronic dyspnoea, and progressive organ failure.
- **The Paradigm Shift:** An academic research group implemented a fluid-ejecting, 3D human cardiac organoid chamber ("heart-in-a-jar"), completely replacing the need for high-severity animal surgical models for target validation.
- **The Methodology:** The team generated 3D hollow mini-heart chambers using human iPSC-derived cardiomyocytes, fibroblasts, and endothelial cells. They mounted the constructs in microfluidic chambers connected to pressure-volume sensors. They validated the therapeutic lead by measuring cardiac output, stroke volume, and the restoration of clinical ejection fractions under chronic endothelin-1-induced hypertrophic stress, 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

Cardiovascular research has historically relied on *in vivo* rodent models, such as Langendorff preparations or invasive surgical hemodynamic monitoring, which often fail to replicate human-specific cardiac electrophysiology, contractile mechanics, and vascular responses. These animal models present significant translational limitations, including species-specific differences in heart rate regulation and ion channel expression. This roadmap mandates a transition to high-fidelity, human-relevant NAMs—specifically patient-derived cardiac organoids, "heart-on-a-chip" microfluidic platforms, and computational digital twins—to ensure research is scientifically accurate, 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
Cardiovascular	Invasive rodent hemodynamic assays	Cardiac Organoids & Heart-on-a-Chip	High

See Appendix: Animal-Free Matrix for the long term strategic implementation roadmap

Maturity Matrix

Phase 1 (Exploratory): Audit current animal use in cardiovascular research and hemodynamic monitoring.

Phase 2 (Pilot): Integrate human iPSC-derived cardiac organoids and microfluidic platforms.

Phase 3 (Integration): Implement biophysical computational modelling to simulate human-specific cardiovascular dynamics.

Phase 4 (Standardisation): Replace 100% of invasive animal hemodynamic testing and heart-function assays 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 fundamental cardiovascular platforms explored in this series to the broader neurobiological and psychiatric frameworks established previously—demonstrate that replacement is an integrated institutional strategy. By mapping these specialised NAM-based cardiovascular 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.

Appendix
Strategic Roadmap: Transitioning to Animal-Free Models

The transition toward animal-free methodologies in the cardiovascular and respiratory series is supported by the requirement under the Animals (Scientific Procedures) Act (ASPA) 1986 to utilise viable non-animal alternatives. This matrix summarises the current readiness of these platforms and outlines the essential milestones required to establish them as robust regulatory tools.

Animal-Free Maturity Summary Matrix

Platform / Method	Maturity Level	Regulatory Context of Use (CoU)	Critical Milestone for Advancement
Biomechanically Active Heart-on-Chip	Maturing	Direct replacement for canine/rodent haemodynamic studies.	Standardisation of multi-site reproducibility protocols.
Ex Vivo (Langendorff) Perfusion	Thriving	Validation of human-specific ischaemic/graft outcomes.	Maintaining human-tissue supply chain logistics.
AlphaFold & Molecular Dynamics	Thriving	Regulatory safety screening and Torsades de Pointes prediction.	Integration into standard IND/ NDA filing packages.
Cardiac Organoids & Soft Robotics	Developing	Longitudinal safety testing; hypertrophy modelling.	Demonstration of "fit-for-purpose" sensitivity against human toxicants.
In Silico "Digital Twins"	Emerging	Patient-specific therapeutic/ surgical outcome prediction.	Establishing independent verification/validation (V&V) standards.

Implementation Priorities

- **Regulatory Confidence:** Future efforts must prioritise the transition of platforms from "Developing" to "Maturing" by generating transparent "fit-for-purpose" validation datasets.
- **Operational Integration:** Platforms designated as "Thriving" should be formalised within institutional research pipelines to serve as the primary Weight of Evidence (WoE) for safety assessments.
- **Technical Verification:** Establishing rigorous V&V standards remains the primary hurdle for "Emerging" technologies like Digital Twins, necessitating cross-disciplinary collaboration between engineers and clinical researchers.

This strategic framework provides the necessary documentation to demonstrate a commitment to both high-quality science and the progressive replacement of animal models in line with current regulatory expectations.