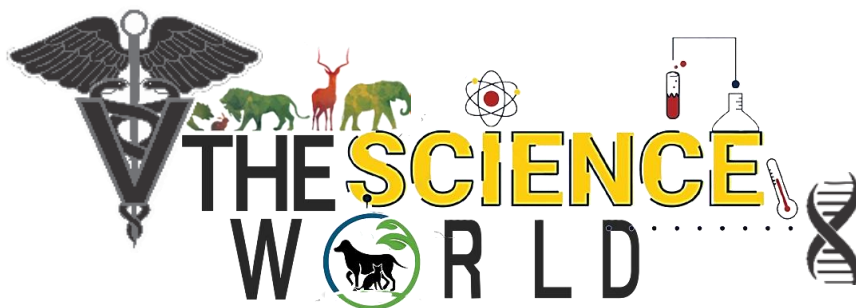




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Experimental Models of Atherosclerosis: From Traditional Induction to advanced Translational Systems

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Abstract

Atherosclerosis (AS) remains the leading cause of global morbidity and mortality. A significant bottleneck in cardiovascular drug development is the "translational gap," where therapies successful in preclinical models fail in human clinical trials. This failure often stems from the inability of standard models to replicate the complex, multi-factorial pathophysiology of human vulnerable plaques. This review details the evolution of atherosclerosis modeling, ranging from traditional dietary and genetic manipulations to cutting-edge bioengineered 3D tissue models and large-animal translational platforms. By aligning specific therapeutic mechanisms with high-fidelity pathological models, researchers can significantly de-risk clinical translation.

1. Introduction: The Pathological Benchmark

The fundamental "ground truth" of human atherosclerosis is rooted in the Response-to-Retention hypothesis. Sub-endothelial retention of Low-Density Lipoproteins (LDL) by modified proteoglycans triggers oxidative modification, chronic inflammation, and immune cell recruitment.

To achieve translational viability, experimental models must move beyond simple lipid-loading and aim to replicate the features of the human "vulnerable plaque," which include:

- Large Necrotic Core: Accumulation of apoptotic debris and cholesterol crystals.
- Thin Fibrous Cap: A fragile layer of collagen and smooth muscle cells (SMCs).
- Intra-plaque Neovascularization: Fragile, leaky micro vessels arising from the vasa vasorum.
- Spontaneous Rupture: Mechanical failure leading to luminal thrombosis, myocardial infarction (MI), and stroke.



2. Traditional Methods for Inducing Atherosclerosis

2.1 Dietary Induction Models

Feeding animals a high-fat/high-cholesterol (HFHC) diet causes hyperlipidaemia, leading to LDL accumulation and foam cell formation.

- Formulations: Western diets (21% fat, 0.15–0.25% cholesterol) and Paigen diets (15% fat, 1.25% cholesterol, 0.5% cholic acid). Note: Cholic acid is highly atherogenic but causes severe liver toxicity in mice.
- Species: Rabbits, mice, rats, pigs, and non-human primates.
- Mechanism: Increased plasma LDL/VLDL → LDL oxidation (oxLDL) → Macrophage recruitment via scavenger receptors (e.g., CD36) → Foam cell formation.
- Pros/Cons: Simple and cost-effective, but lesions often differ from human plaques in location and composition (e.g., mice primarily develop lesions in the aortic root, whereas humans develop them in coronary arteries).

2.2 Genetic Models of Atherosclerosis

Genetic manipulation disrupts lipoprotein metabolism, accelerating plaque formation even on normal chow diets.

- ApoE Knockout (ApoE^{-/-}) Mice: The most widely used model. Develops severe hypercholesterolemia (high VLDL/chylomicrons) and spontaneous plaques rich in macrophages, validating the Inflammatory Hypothesis.
- LDL Receptor Knockout (LDLR^{-/-}) Mice: Impaired LDL clearance. Requires a Western diet to develop plaques. Closely mimics human familial hypercholesterolemia (FH).
- PCSK9-AAV Models: An emerging gold standard. Adeno-associated virus (AAV) delivers a gain-of-function PCSK9 mutation (e.g., D374Y) to wild-type mice. This induces hypercholesterolemia rapidly without time-consuming germline breeding, significantly reducing R&D timelines.
- Additional Insight: Recent advancements include CRISPR/Cas9-generated *Ldlr* mutant rats, which provide larger blood volumes for pharmacokinetic studies compared to mice.



2.3 Mechanical and Surgical Induction

These methods create endothelial injury or altered hemodynamics (disturbed flow), accelerating localized plaque formation.

- Partial Carotid Ligation (PCL): Alters shear stress from laminar to oscillatory, causing rapid endothelial dysfunction and plaques within 2–3 weeks.
- Tandem Stenosis: Creates two points of narrowing to generate high tensile stress, closely simulating the mechanical instability of human plaques.
- Cuff Placement & Wire Injury: Direct endothelial denudation promoting massive smooth muscle cell (SMC) proliferation and neointimal hyperplasia.

2.4 Chemical and Metabolic Induction

Used to mimic the compounding risk factors of metabolic syndrome.

- Streptozotocin (STZ): Induces type 1 diabetes. Hyperglycemia promotes advanced glycation end-product (AGE) formation and vascular inflammation.
- L-Arginine / L-NAME: Modulates nitric oxide (NO) pathways to induce systemic hypertension and endothelial dysfunction.
- Combination Models: STZ-induced diabetic mice fed a Western diet represent a highly translational model for diabetic macrovascular complications.

3. The Evolution of In Vitro and Ex Vivo Models

3.1 Overcoming 2D Limitations

Traditional 2D monolayers of endothelial cells (ECs) represent a strategic liability because they cannot simulate flow-mediated mechanotransduction. The emergence of human induced pluripotent stem cells (hiPSCs) allows for the study of Neuroimmune Cardiovascular Interfaces (NICIs)—exploring how inflammatory signals from plaques communicate with the CNS.

3.2 Advanced 3D Architectures

- Tissue-Engineered Blood Vessels (TEBVs): Utilizing 3D bioprinting or decellularized scaffolds, TEBVs mimic the tri-layered arterial structure (intima, media, adventitia). They are essential for studying early-stage AS and SMC migration.
- Vessels-on-a-Chip (VoC): Microfluidic devices that replicate precise hemodynamic forces (low vs. high shear stress).



- *Strategic Advantage:* VoCs allow high-throughput screening of nanoparticle-based drug delivery systems and real-time monitoring of leukocyte-endothelial interactions without the systemic noise of live animals.

3.3 Ex Vivo Analysis: The Physiological Bridge

- **Perfusion Systems:** PDMS-embedded isolated vessel segments allow high-resolution confocal imaging of monocyte adhesion and receptor targeting efficiency over 24 hours with zero motion artifacts.
- **Aortic Ring Angiogenesis Assay:** Culturing aortic rings in hypoxic conditions (1% O₂) simulates the interior of an advanced plaque, triggering the formation of fragile microvessels that mimic intra-plaque neovascularization.
- *Limitation:* Tissue degradation restricts ex vivo models to short-term observations (24–72 hours).

4. In-vivo Rodent Models: The Plaque Rupture Breakthrough

Mice are naturally resistant to plaque rupture. For decades, this made them poor models for studying myocardial infarction (MI) and stroke. However, recent genetic crossbreeding has solved this.

The *ApoE^{-/-}Fbn1C1039G^{+/-}* Milestone

By crossing an ApoE knockout with a mouse carrying a mutation in the *Fbn1* gene (which causes elastin fragmentation, akin to Marfan syndrome), researchers created a model that consistently yields spontaneous plaque rupture.

- **The "Triple Threat":** Elastin fragmentation + Western diet + Hypoxia.
- **Biomarker:** These mice exhibit significantly higher iNOS (inducible nitric oxide synthase) expression, signaling a unique inflammatory state identical to human vulnerable plaques.
- **Utility:** This is the current gold standard for validating plaque-stabilizing therapies and anti-inflammatory drugs aimed at preventing acute coronary syndromes.

5. Large Animal and Translational Bridging Models

Rodents cannot replicate human vessel scale, making large animals indispensable for clinical hardware validation (stents, catheters) and medical imaging (CT, MRI, OCT).



Animal Model	Induction Method	Key Translational Features	Primary Limitation
Rabbits (WHHL / ApoE ^{-/-})	Genetic mutation + Diet	Human-like lipoprotein profile (ApoB-containing); large plaques.	Coronary arteries too small for standard clinical catheters.
Minipigs (D374Y-PCSK9)	DNA transposition / CRISPR	Premier platform for stent testing; human-scale coronary anatomy.	High cost, slow lesion development.
Non-Human Primates	Diet-induced	Develop coronary fibro-fatty plaques identical to humans.	Extreme cost, strict ethical regulations.

Additional Note on Pigs: The D374Y-PCSK9 gain-of-function minipig exhibits severe hypercholesterolemia and rapid atherosclerosis, bridging the gap between rodent genetics and human cardiology.

6. Strategic Selection Framework for Therapeutic Validation

To avoid Phase II clinical attrition, research leads must strictly align their therapeutic mechanism with the model's pathological fidelity.

Therapeutic Goal	Recommended Model	Pathological Justification
Lipid Lowering (e.g., PCSK9 inhibitors)	LDLR ^{-/-} Mouse, WHHL Rabbit, PCSK9-AAV Mouse	Directly addresses the Response-to-Retention hypothesis by focusing on LDL clearance and proteoglycan binding.
Anti-Inflammatory (e.g., Canakinumab analogs)	ApoE ^{-/-} Mouse	Highly receptive to cytokine expression modulation and macrophage infiltration mapping.
Plaque Stabilization	ApoE ^{-/-} Fbn1C1039G ^{+/-} Mouse	Only robust model of spontaneous fibrous cap rupture, thin-cap fibroatheroma (TCFA), and intra-plaque hemorrhage.



Nanomedicine Targeting	Vessels-on-a-Chip (VoC) & Ex Vivo Perfusion	Allows precise optimization of shear-dependent endothelial binding and nanoparticle permeability.
Device / Stent Testing (ISR)	D374Y-PCSK9 Minipig	Provides human-scale vascular diameters and analogous lesion topography required for clinical sensor calibration.

7. Future Perspectives

The future of atherosclerosis research relies on integrative, multi-factorial modeling. This includes:

1. Multi-Omics Integration: Combining spatial transcriptomics with proteomics in *Fbn1* models to map the exact cellular drivers of cap thinning.
2. Humanized Mouse Models: Mice engrafted with human immune cells to accurately test human-specific immunotherapies.
3. AI-Driven VoCs: Using machine learning algorithms to analyze microfluidic flow data and predict nanoparticle adhesion rates in human vasculature.

8. Conclusion

Experimental induction of atherosclerosis has evolved from simple cholesterol feeding in rabbits to sophisticated gene-edited, bioengineered vascular systems. While genetically modified mice remain fundamental workhorses for mechanistic studies, the advent of the *Fbn1* rupture model and advanced microfluidic VoCs has opened previously closed "black boxes" in pre-clinical research. By implementing a tiered modeling strategy—moving from high-throughput 3D chips to high-fidelity minipigs—researchers can ensure that novel cardiovascular therapies successfully bridge the translational gap.

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