3D in vitro human atherosclerosis model for high-throughput drug screening

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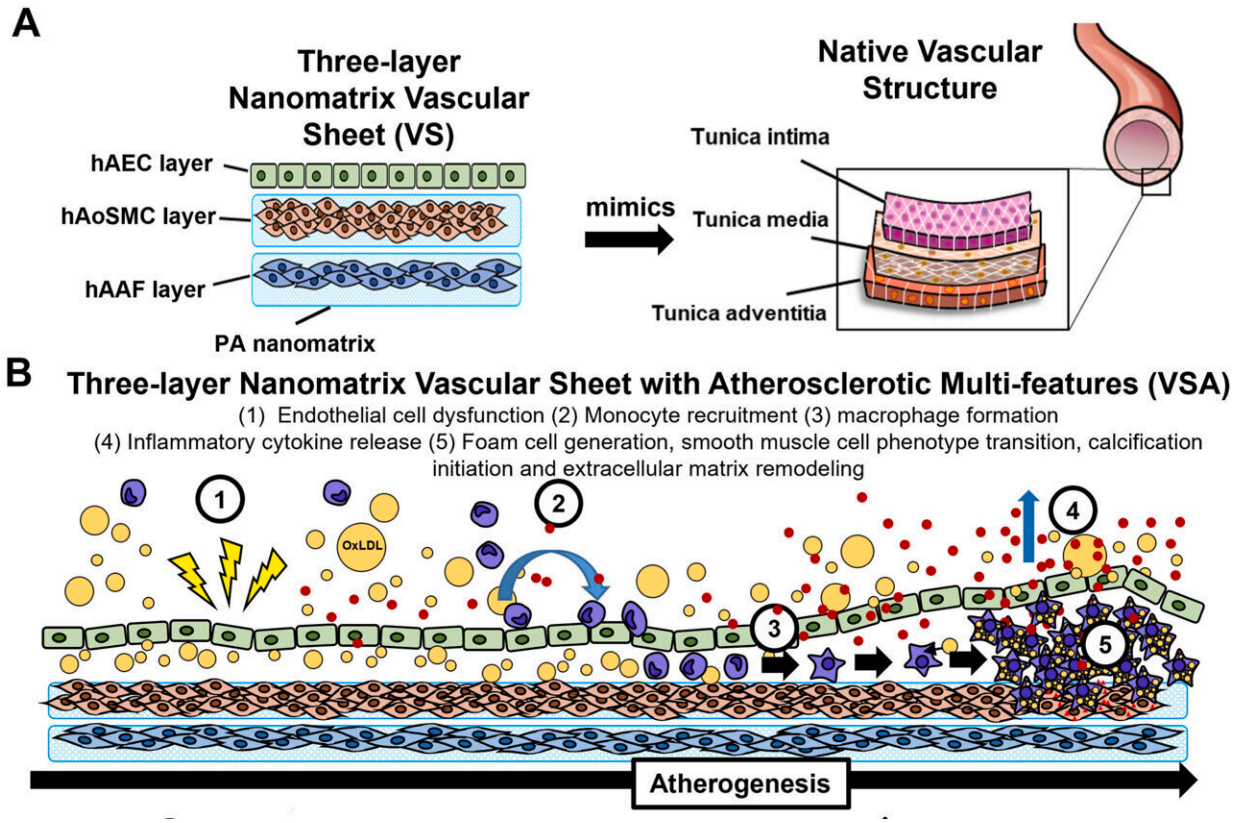

Nanomatrix vascular sheet (VS), nanomatrix vascular sheet with atherosclerosis (VSA), and VSA functional assays for therapeutic evaluation. Credit: *Biomaterials* (2023). DOI: 10.1016/j.biomaterials.2023.122450

A new 3D, three-layer nanomatrix vascular sheet that possesses multiple features of atherosclerosis has been applied for developing a high-

throughput functional assay of drug candidates to treat this disease, University of Alabama at Birmingham researchers report in the journal *Biomaterials* in a paper titled "Atherosclerotic three-layer nanomatrix vascular sheets for high-throughput therapeutic evaluation."

"Current in vitro atherosclerosis models have significant limitations, including the lack of three-layer vascular architecture and limited atherosclerotic features," said Ho-Wook Jun, Ph.D., a professor in the UAB Department of Biomedical Engineering and the corresponding author. "Moreover, no scalable 3D atherosclerosis model is available for the evaluation of potential therapeutics via high-throughput assays."

Cardiovascular disease—primarily caused by atherosclerosis—is the leading cause of death in the United States. In the development of effective therapies for atherosclerosis, in vitro models are commonly utilized to assess the efficacy and safety of novel therapeutics before proceeding to complex in vivo and clinical studies.

Recently, the United States Food and Drug Agency Modernization Act 2.0 has permitted the use of alternative models other than animals for drug testing before progressing to human trials. This transformative change in regulations serves as a driving force, inspiring the pursuit of advanced in vitro models, such as cell-based assays and organoid- or artificial intelligence-based models that are capable of replacing or reducing animal use in assessing drug efficacy and safety.

The goal is to expedite progression from preclinical research to human clinical trials via a more efficient and cost-effective drug development process.

The novel in vitro 3D, three-layer nanomatrix vascular sheet with critical atherosclerosis multi-features, or VSA, includes endothelial cell dysfunction, monocyte recruitment, presence of macrophages,

extracellular matrix remodeling, smooth muscle cell phenotype transition, inflammatory cytokine secretion, foam cells and calcification initiation. The VSA thus provides a human atherosclerosis-mimicking environment for drug evaluation.

The three layers of the robust vascular sheet, or VS, structure are composed of: 1) human aortic endothelial cells, 2) human aortic smooth muscle cells and 3) human aortic adventitial fibroblasts layers. These mimic the layered structure of the native vascular wall, which, from inside out, is composed of the tunica intima, tunica media and tunica adventitia tissues.

The researchers created the critical atherosclerosis multi-features by adding monocytes and various pro-atherosclerotic cytokines, colony-stimulating factors and oxidized low-density lipoprotein to stimulate atherogenesis on the 3D, layered nanomatrix vascular sheet.

Co-first author Jun Chen, Ph.D., and colleagues used this VSA system to create high-throughput functional assays by fabricating multiple VSAs in 48-well plates. The VSAs were subjected to drug treatments and then were comprehensively characterized, with a focus on evaluating foam cells, inflammation and atherosclerosis-associated genes.

High-throughput functional assays were validated using two classic atherosclerosis drugs, rosuvastatin and sirolimus, and were used to evaluate two drug candidates, curcumin and colchicine, and a potential gene therapy candidate, microRNA-146a-loaded liposomes, for treating atherosclerosis. The researchers found that the VSAs replicated a number of results seen by others in in vivo tests of these treatments.

"The high efficiency and scalability of the VSA-evaluated functional assays should facilitate drug discovery and development for atherosclerosis," Chen said.

"Our study focuses on demonstrating the use of VSAs as a cost-effective and efficient way to investigate therapeutic effectiveness," Jun said.

"The VSAs offer a high-throughput methodology and allow for a relatively large number of biological replicates, also making them ideal for mechanistic research. For instance, VSAs can be customized to induce atherosclerosis on single-, double- or three-layer structures, which provides insights into the effect of discrete layers on atherogenesis, particularly the fibroblast layer. Furthermore, the vascular sheets can be scaled up to develop high-throughput assays for drug safety testing, helping determine pharmacological and toxicological parameters for use in animal models."

The Birmingham-based Endomimetics, LLC, has licensed the new 3D, three-layer nanomatrix vascular sheet atherosclerosis model technology through UAB's Bill L. Harbert Institute for Innovation and Entrepreneurship, which manages university intellectual property.

"The atherosclerosis drug market is a large and growing segment of the pharmaceutical industry," said Joseph Garner, Ph.D., CEO of Endomimetics.

"This market is experiencing significant growth due to the rising prevalence of cardiovascular diseases, advancements in pharmaceutical research and the development of innovative atherosclerosis treatments. By 2032, this market is projected to reach $26.9 billion with a 2.8 percent compound annual growth rate from 2023 to 2032. North America currently holds the dominant market share at more than 41 percent."

Brigitta Brott, M.D., a co-author and an interventional cardiologist and professor in the UAB Department of Medicine Division of Cardiovascular Disease, said, "Endomimetics and UAB will collaborate

with pharmaceutical companies to evaluate potential candidates for atherosclerosis treatment, utilizing our VSA-based efficacy and safety assays."

"This approach can also be extended to assess other drugs for conditions such as diabetes, obesity and liver-related diseases, where atherosclerosis is prevalent among many patients."

Endomimetics anticipates providing this innovative atherosclerosis model this spring for evaluating various types of potential therapeutics.

"Our atherosclerosis assays will pave the way for therapeutic candidates directly targeting human plaque and the atherosclerotic artery wall, and they will generate extensive predictive data on their responses, which is crucial for defining the therapeutic window of these candidates and providing essential groundwork for future studies," Jun said.

More information: Jun Chen et al, Atherosclerotic three-layer nanomatrix vascular sheets for high-throughput therapeutic evaluation, *Biomaterials* (2023). DOI: 10.1016/j.biomaterials.2023.122450

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