Advances in Cellular Models of Atherosclerosis

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Abstract

Atherosclerosis is a disease with a complex pathogenesis, consisting of the interrelationships of many different elements. In light of the increasing spread of cardiovascular diseases, the precursor of which is atherosclerosis, the study of the intricacies of its pathogenesis remains an important research task. For its achievement, it is necessary to choose the right model. To date, the most common are models of small animals, in particular mice. However, extensive work is being carried out towards the development of cellular models that would allow moving away from the use of animals as model objects, as well as bypassing the problems of translating the results. In this review, we collected data on the current advances in the field of cellular models of atherosclerosis.

Keywords: Cellular models; In Vitro models; Atherosclerosis.

Significance | Atherosclerosis models, from 2D cultures to 3D tissue-engineered vessels, offer cost-effective avenues for drug screening and personalized medicine, advancing therapeutic discovery.

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Introduction

Atherosclerosis is a complex biological process that occurs due to low shear stress. It progresses due to impaired functioning of endothelial cells (ECs), leading to the accumulation of oxidized low-density lipoproteins (LDL) beneath the endothelium, inflammation, and the de-differentiation, migration, and growth of vascular smooth muscle cells (VSMCs). The development of atherosclerosis is mainly observed in the bends and branches of arteries where blood flow is weak or fluctuating, resulting in a low time-averaged wall shear stress (<10 dyn/cm2), in the presence of systemic risk factors such as genetic predisposition, smoking, hypertension, hyperlipidemia, and diabetes (Zhou et al., 2023; Botts et al., 2021).

Preclinical models play a clinical role in the study of the pathophysiology of atherosclerosis. They also help in the development of anti-atherosclerotic treatments. Experiments with animal models of atherosclerosis provide valuable mechanistic information, but they cannot reproduce the complex biology of blood vessels and the natural course of atherosclerosis as it occurs in humans (Zhang et al., 2021).

Moreover, animal experiments can be time-consuming and expensive. In contrast, in vitro atherosclerosis models on cell cultures are more affordable and time-saving. However, it should be noted that they lack the ability to reproduce cellular interactions and complex molecular pathways that occur in vivo. Currently, three types of cell culture methods are used in vascular biology. (1) Single-cell cultures of ECs, monocytes, or VSMCs on tissue culture plates. They are among the most popular and simple cultures, but they do not allow the study of complex interactions between different cell types and the extracellular matrix (Vedder et al., 2020).

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(2) Two-cell cultures in which two types of cells are either co-cultured on top of each other, separated by a porous membrane (indirect co-culture), or placed next to each other (direct co-culture). These cultures are more complex and advanced than single-cell cultures, but they still cannot fully demonstrate the complex multicellular interactions that occur in humans (Kuppusamy et al., 2020). (3) Three-cell co-cultures, which are more representative of human vascular biology. They also allow for complex communication between different cell types (direct co-cultures). However, they may be less reproducible and homogeneous, and their development typically requires more time, resources, and equipment. Further development of in vitro co-culture models that include a human-like vascular wall could provide economically efficient atherosclerosis research and support more targeted and significant research on animals and humans (Liu et al., 2023). A valuable tool for studying the pathophysiology of atherosclerosis and conducting drug research could be a highly reproducible and economically efficient three-cell co-culture model of atherosclerosis that allows for direct interaction between the major types of vascular cells (Figure 1), (Chen et al., 2022).

Cellular Models in the Search for Antiatherosclerosis Therapy

A biological model has proven to be a valuable tool for the development of new pharmaceutical and nutraceutical products. Unlike traditional safety and efficacy tests that must be conducted before clinical trials on humans, biological models can be quickly deployed and analyzed. These models allow for rapid assessment of potential drug activity, metabolism, and direct therapeutic effect. However, when simplifying the model, it is important to maintain similarity to the biological system. One application of this cellular model strategy is the search for natural anti-atherosclerotic agents (Mitra and Murthy, 2022).

To study this approach, two cellular models were used in one of the studies, in vitro and ex vivo. In the in vitro model, primary cultures of subendothelial cells were used. They were obtained from the thoracic aortas of deceased men and women aged 40 to 65. In the ex vivo model, the same cell cultures were used but in a different way. The in vitro model was developed to assess the ability of synthetic and plant compounds to reduce the atherogenicity of human serum and prevent the accumulation of cholesterol in the intima of blood vessels (Zakiev et al., 2017). To do this, live cells were extracted using collagenase from various sections of the aorta. Then, they were cultured for 7-10 days at 37°C in fresh media. The resulting heterogeneous cell population consisted mainly of pericyte-like cells and typical and modified smooth muscle cells. To obtain a "control" cellular model with properties different from the affected cellular model, cells were cultured from a healthy section of the aorta in a similar manner. By incubating cells with atherogenic serum from patients with atherosclerosis, abnormal lipid deposition in cells was induced (He et al., 2020). The tested products were added to both models. This allowed for the assessment of their ability to prevent lipid accumulation in relatively "control" cells and reduce cellular lipid deposits in affected cells. The capability to reduce the accumulation of lipids in cells was tested on cells that were already laden with lipids, and a decrease in intracellular cholesterol level that was statistically significant was considered as a positive antiatherosclerotic effect. The ability to prevent lipid buildup was measured on a second cellular model, which was cultured from a normal intimal aorta and exposed to atherogenic serum (Agarwal et al., 2020). If the cells showed resistance to lipid accumulation, it was considered a positive result. If both stages yielded positive results, the tested product proceeded to the next stages of development. The third (ex vivo) model was based on macrophages derived from monocytes obtained from the venous blood of healthy volunteers through 14-day culturing at 37°C. The tested product was given to volunteers with sufficient blood serum atherogenicity, meaning their serum had the ability to induce the pathological accumulation of cholesterol in the cellular model (Nikiforov et al., 2019). A series of blood collections were conducted, with the first sample taken before administering the product, and subsequent samples collected at 2, 4, 6, 8, 12, and 24 hours to assess short- and long-term effects. The serum from collected blood samples was added to the culture of aortic cells or monocytes-macrophages. Then, the assessment of serum atherogenic potential, intracellular lipid content, and cellular protein content was conducted. This model allowed the authors to assess the antiatherogenic effect of substances taking into account the metabolic processes in the human body. These two models eliminated ineffective botanicals and identified only a few non-pharmaceutical compounds. Three drugs were developed and further evaluated in clinical studies (Figure 2), (Bowen and Remaley, 2014).

In vitro 2D Models for Atherosclerosis Studies

For decades, 2D in vitro models have been crucial for studying the pathology of various diseases and evaluating drug efficacy. The most commonly used 2D in vitro models are single-cell culture systems that contain only one type of cell component found in atherosclerotic plaques, such as foam cells, macrophages, ECs, and SMCs. These single-cell cultures have played an important role in evaluating new therapeutic agents, such as microRNAs and exosomes, and in studying mechanisms related to atherosclerosis (Mohandas et al., 2023). Recently, they have also been widely used to assess the efficacy of drug delivery systems for treating atherosclerosis, as free drug administration is associated with its own problems. However, despite their widespread use, single-cell models may be unreliable in predicting therapeutic efficacy in

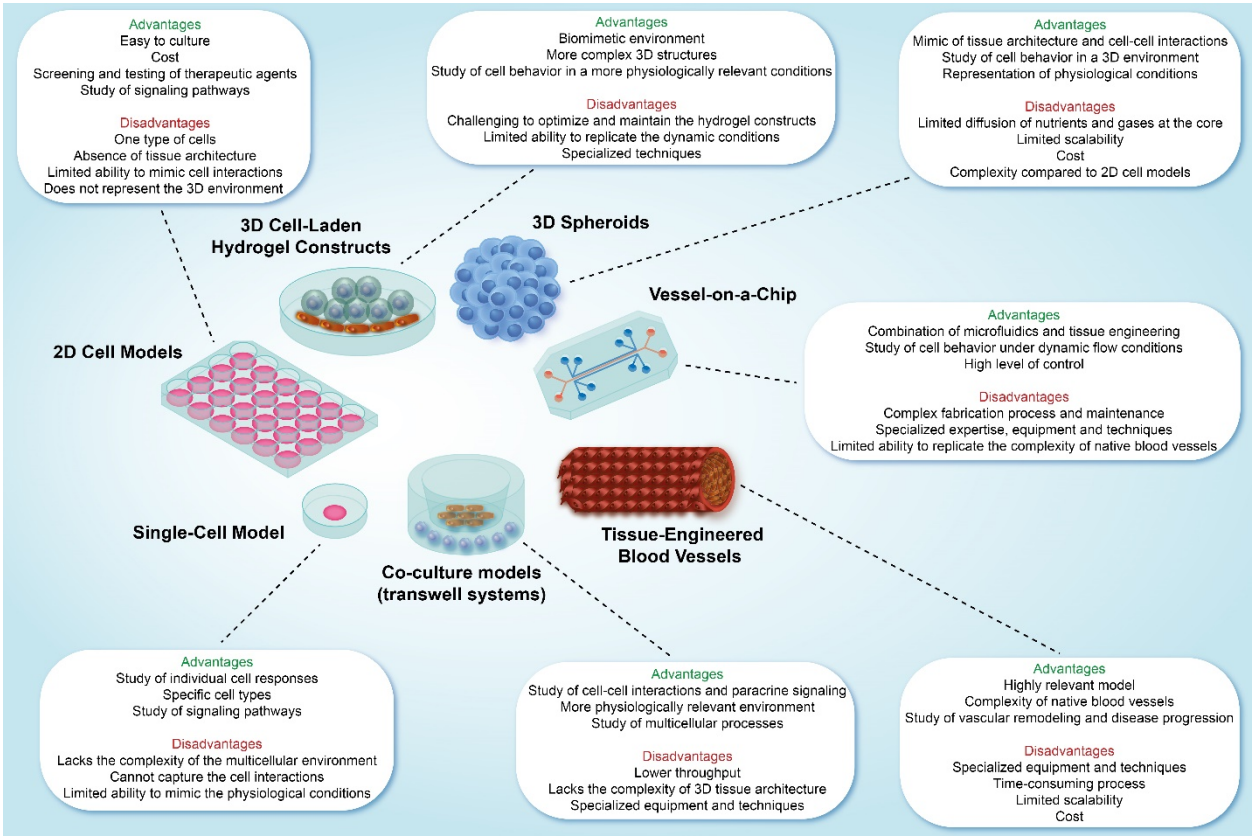

Figure 1. In Vitro Models in the Search for Antiatherosclerosis Therapy. In Vitro Models for Antiatherosclerosis Therapy: Overview of common in vitro models including 2D cell models, single-cell models, co-culture models, 3D spheroids, 3D cell-laden hydrogel constructs, tissue-engineered blood vessels, and vessel-on-a-chip. Emphasizes advantages and disadvantages of each model type.

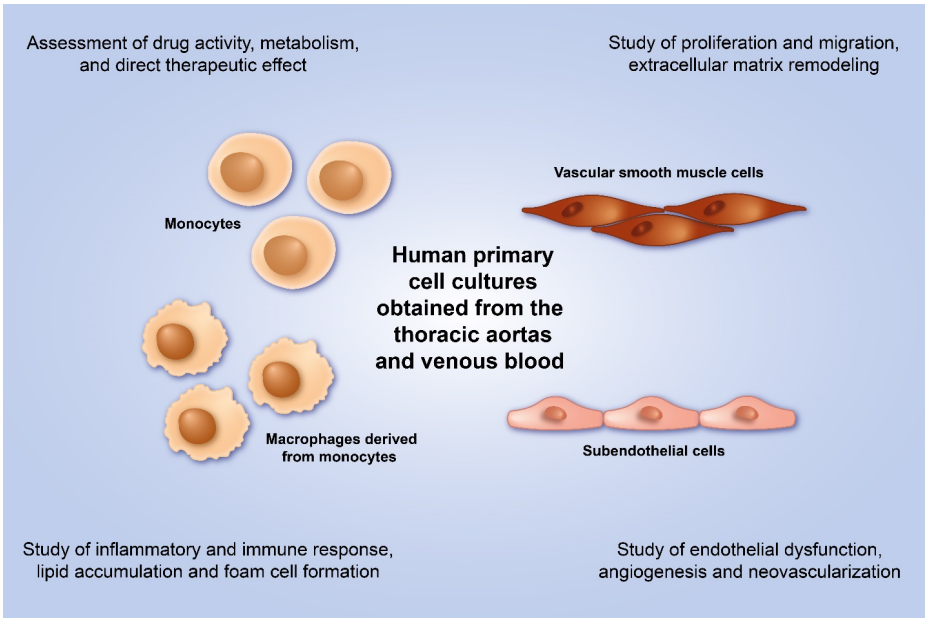

Figure 2. Human primary cell cultures used in the study of antiatherosclerosis. By utilizing human primary cell cultures, researchers can mimic the cellular interactions and responses that occur in the human body during atherosclerosis. These studies provide valuable insights into the molecular mechanisms underlying the disease and can aid in the development of novel therapeutic strategies. Furthermore, such cell models make it possible to evaluate the antiatherogenic effect of various substances, taking into account metabolic processes in the human body.

patients due to their inability to accurately mimic the structure of blood vessels and human plaques. Therefore, single-cell models are usually combined with in vivo models to obtain a significant understanding of the therapeutic efficacy and pathogenesis of atherosclerosis (Yang et al., 2022).

In addition to single-cell culture systems, research has also focused on developing co-culture models for atherosclerosis studies. These co-culture models can be direct, with cells in physical contact, or indirect, using transwell systems. As far back as 1986, various cell types involved in atherosclerosis pathology, including macrophages, ECs, and SMCs, were used in direct and indirect co-culture studies to create blood vessels and study inflammation in atherosclerosis. With advancements in engineering, significant efforts have been made to develop more sophisticated 2D in vitro systems, such as those using 2D scaffolds or microfluidic chips, to better mimic the physiological and pathological environment of atherosclerosis. This section summarizes these systems (Liu et al., 2023).

Single-Cell Model

Single-cell models have become widely used in assessing drug delivery systems due to their simplicity of production and easy availability. These models typically involve specific types of cells, such as macrophages, SMCs, ECs, or foam cells. One application of these models is the assessment of the cellular absorption of drug delivery systems. For instance, Schwendeman et al. developed a single-cell model using THP-1 monocytes that were differentiated into macrophages to evaluate the cellular uptake of synthetic high-density lipoproteins that were labeled with DiD dye (DiD-sHDL) (Owsiany et al., 2019). The results revealed that 99% of macrophages displayed positive staining for DiD after a 2-hour incubation with the particles. Other studies have utilized various cell types, such as HUVECs treated with TNF-α and vascular smooth muscle cells (VSMCs) from mice, to demonstrate efficient cellular uptake of β-cyclodextrin nanoparticles and biomimetic nanoparticles that were functionalized with macrophage membrane, respectively. Additionally, the Liu group reported using single-cell systems comprised of HUVECs, RAW cells, or foam cells to examine the cellular binding of platelet-mimicking nanoparticles to these cells. With these single-cell models, they discovered that platelet-mimicking nanoparticles strongly bound to foam cells, but not to other cell types (Ma et al., 2020).

The efficacy of drug delivery systems loaded with therapeutics to combat atherosclerosis depends on their ability to modulate critical biological processes associated with macrophages and macrophage-derived foam cells, such as foam cell formation, ROS generation, inflammation resolution, and cholesterol efflux. Macrophages have been extensively used to assess the impact of drug delivery systems on these processes. For instance, Ghosh et al. employed macrophages to evaluate the regulation of cholesterol efflux and influx mediated by drug delivery systems (Ghosh et al., 2022). They demonstrated that mannose-functionalized dendrimeric nanoparticles (mDNPs) co-delivering siRNA and LXR ligands could significantly reduce cholesterol content in macrophages. Macrophage systems have also been utilized to investigate the effect of drug delivery systems on efferocytosis and plaque stability, as macrophages in the plaque express Ca2+/calmodulin-dependent protein kinase (CaMKIIγ) and suppress efferocytosis receptors, leading to efferocytosis suppression and plaque necrosis (Chen, 2021). Shi and colleagues incubated bone marrow-derived macrophages with siRNA-loaded nanoparticles to evaluate the effect of siRNA nanoparticles on efferocytosis (Shi et al., 2022). They found that siRNA-loaded nanoparticles could significantly decrease CaMKIIγ expression, thus increasing macrophage efferocytosis and plaque stability. Furthermore, the ability of therapeutic-loaded drug delivery systems to induce inflammation resolution is also being investigated for treating atherosclerosis. The Scott group showed that anti-inflammatory nanocarriers, such as celastrol-loaded nanocarriers, could significantly reduce TNF-α production in macrophages compared to untreated cells (Vincent et al., 2022; Davis et al., 2020).

Single-cell models have been used not only to evaluate drug delivery systems loaded with therapeutics, but also to study the mechanisms associated with atherosclerosis pathogenesis. For example, ECs have been used to investigate the effects of various pathophysiological stimuli, including hypercholesterolemia, hypertension, hemodynamic factors, and pro-inflammatory cytokines, on inflammation, endothelial dysfunction, and cellular senescence in atherogenesis. Similarly, single-cell models containing SMCs or monocytes have been used to assess the impact of these stimuli on SMC phenotype changes, monocyte differentiation, and foam cell formation, which are critical processes in the development of atherosclerosis (Yang et al., 2020).

In vitro 3D models for Atherosclerosis Studies

Conventional 2D in vitro platforms for studying atherosclerosis have limitations, as they lack the physiological 3D structure and pathological compositions observed in vivo. Additionally, 2D cultures suffer from issues with substrate topography and stiffness, and may provide misleading information on the safety and efficacy of lead compounds. On the other hand, 3D culture has emerged as a promising approach, allowing for cell constructs that mimic the 3D structure of organs with more natural extracellular matrix (ECM), and enabling better cell-cell and ECM-cell interactions (Savoji et al., 2019). This approach can improve predictability of therapeutic toxicity and sensitivity, as drug responses in 3D models may differ from those in 2D models. Developing in vitro 3D models through bio-fabrication has become a growing interest in atherosclerosis modeling and drug testing. This approach generates organized structures with biological function using living cells, cell

aggregates, biomaterials, and bioactive molecules via bio-assembly or bio-printing, followed by tissue maturation (Brancato et al., 2020).

In vitro 3D Spheroids

Three-dimensional spheroids are cell aggregates that resemble spheres and are increasingly used for therapeutic evaluation due to their better resemblance to natural tissue compared to 2D culture systems (Figure 3). The formation of spheroid cultures involves the use of extracellular matrix fibers with a ligand motif, such as tripeptide Arg-Gly-Asp, that bind with integrin membrane proteins on the cell surface, allowing the cells to aggregate and bind together with homophilic cadherins. This leads to solid adhesion and compaction of the cell mass, resulting in the formation of a spheroid (Białkowska et al., 2020). Over the past several decades, various fabrication methods have been used to develop spheroid cultures, including the hanging drop method, centrifugation, spinner flask, and culturing cells on a non-adherent substrate. It is important to note that spheroid cultures exhibit different gene expression, metabolism, and cellular motility, differentiation, and polarity compared to monolayer cultures in 2D. Spheroid models have been increasingly utilized for studying and modeling atherosclerosis due to their ability to more accurately mimic the structures of native tissue compared to traditional 2D monolayer cultures (Ryu et al., 2019).

Foam cells are macrophages that have absorbed lipids, which is the primary component of atherosclerotic plaque. In recent times, scientists have been exploring the development of a foam cell spheroid model to examine how specific compounds affect foam cell formation and associated inflammation (Poznyak et al., 2021). For example, Nguyen et al. demonstrated that using a 3D spheroid model of foam cells, dexamethasone (Dex) and fluocinolone acetonide (FA) could significantly decrease foam cell formation, but FA was more effective than Dex (Nguyen et al., 2018). Hydroxyl beta-cyclodextrin (HBCD) is a polysaccharide that increases cholesterol efflux and solubility. In another study, Kwan et al. utilized the foam cell spheroid model to assess the effectiveness of co-delivery of HBCD and sirolimus loaded poly-co-lactic-co-glycolic acid microparticles (mc-PLGA-MPs) on foam cell formation under ultrasound stimulation. These studies highlight the potential of the foam cell spheroid model for research on atherosclerosis (Su et al., 2021).

Vascular smooth muscle cells (VSMCs) are a critical element in the thickening of arterial walls, the formation of atherosclerotic plaques, and the regulation of plaque stability. In a study conducted by Chun et al., the effects of membrane-type 1 matrix metalloproteinase (MT1-MMP) on VSMC proliferation were examined using 3D spheroid models composed of silenced MT1-MMP gene VSMCs in mice (Chun et al., 2004). The results indicated that silencing the MT1-MMP gene significantly increased the proliferation of mouse VSMCs in this 3D model, whereas minimal effects on VSMC proliferation were observed in a 2D model. This study highlighted the importance of using 3D spheroid models for investigating biological processes associated with atherosclerosis, in addition to 2D models. Moreover, focal adhesion kinase (FAK) has been found to control VSMC proliferation through N-Cadherin and is significant for cell adhesion (Barnes et al., 2017). Vaidyanathan et al. developed a VSMC spheroid model to study the regulation of FAK and its downstream genes, Cdc42, Rac, and Rho, with the aim of identifying potential pathways for treating neointima formation (Vaidyanathan et al., 2021). By quantifying the expression of Rac and Rho in VSMC spheroids, the authors were able to establish that FAK-Rac-N-cadherin or FAK-Rho-N-cadherin are necessary for VSMC spheroid formation, which could become a future target for treating atherosclerosis-related neointima formation.

The mentioned models were focused on developing an early atherosclerosis model with one type of cellular component. It is worth noting that atherosclerosis is a chronic inflammatory disease encompassing various stages. As for an improved atherosclerosis model, Weber et al. conducted a study in which they first succeeded in creating a spheroid pseudo-atherosclerotic plaque in vitro consisting of a spheroidal core and a layer of surrounding myofibroblasts to simulate the late stage of human atherosclerotic lesion – fibroatheroma (Mallone et al., 2018). In addition to this, two types of pseudo-atherosclerotic plaques, t- and b-plaques, were developed using myeloid blood cells and THP-1 cells to manufacture the core. Spheroidal cores were also filled with dendritic cells and collagen, a lipid matrix with macrophages. It was found that the distribution of the cell population between t- and b-plaques was similar, including major components such as plasmacytoid dendritic cells, macrophages, monocytes, and activated dendritic cells. However, their components differed from human carotid plaques, which mainly consisted of activated dendritic cells and plasmacytoid dendritic cells. It is also worth mentioning that compared to pseudo-plaques, native sleep plaques demonstrated a significant reduction in the regulation of pro-inflammatory and remodeling genes. Although this is possibly the most reliable spheroid atherosclerosis model in vitro, age, gender, or genetic predisposition were not taken into account (Ilhan and Kalkanli, 2015).

In vitro 3D Cell-Laden Hydrogel Constructs

Cell-encapsulated hydrogel constructs consist of cells, hydrogels, and growth factors, creating a 3D in vitro environment that is useful for exploring atherosclerosis. In contrast to 2D cell sheets produced by seeding cells onto a 2D scaffold, cell-encapsulated hydrogels are usually made by embedding cells into 3D hydrogel matrices with growth factors, and then cultured statically or dynamically.

Notably, these constructs can be created in various geometries, sizes, and compositions, enabling cells to behave similarly to those in vivo. Regarding atherosclerosis research, cell-encapsulated hydrogel constructs offer a 3D in vivo-like model for observing cellular interactions and pathophysiology, with the added benefits of lower cost, improved controllability, and higher throughput when compared to animal models (Nicodemus and Bryant, 2008).

A hydrogel suitable for creating a cell-laden hydrogel system should possess certain characteristics such as biocompatibility, biodegradability, good porosity, and high-water content. It should also facilitate the growth, proliferation, and migration of cells by allowing nutrient diffusion throughout the scaffold. Collagen is the primary structural protein found in the extracellular matrix (ECM) and has been extensively used in creating cell-laden hydrogel constructs. For example, researchers have used collagen hydrogel constructs to investigate the impact of different factors on monocyte attachment (El-Sherbiny and Yacoub, 2013). Chiu et al. created a model using a collagen gel with embedded smooth muscle cells (SMCs) and seeded an endothelial cell (EC) monolayer over the SMC-laden hydrogel to study the influence of SMCs on inflammation and monocyte adhesion in atherosclerosis development (Chiu et al., 2022). In another study, SMC-laden collagen hydrogel constructs were used to examine the roles of SMCs and flow in leukocyte adhesion and transmigration. Moreover, the ability of the EC-seeded SMC-laden collagen-based hydrogel construct to mimic the intima-media structure of vessels has led to its use in modeling atherosclerosis (Liu et al., 2021). In a recent study, Su et al. induced EC dysfunction and SMC migration by treating the cell-hydrogel construct with IL-1β, TNFα, and Ox-LDL, allowing the quantification of SMC migration into the EC layer. This approach provides greater sensitivity than traditional transwell assays (Su et al., 2021). Furthermore, this system has potential as a tool for drug screening, as evidenced by the atheroprotective effects of vitamin D and metformin observed in experiments using this model. Nevertheless, a major limitation of this study is the lack of a monolayer EC layer, and its thickness being equivalent to that of the SMC layer, and the absence of a tunica adventitia (Su et al., 2021). In a separate study, Garcia-Sabate et al. utilized monocyte-laden collagen hydrogel constructs with low or high densities to mimic early or late-stage atherosclerotic tissues and examine the effects of the ECM (collagen) on macrophage behavior in these two environments (Garcia-Sabaté et al., 2020). The construct was created by first embedding THP-1 cells within the collagen hydrogel with Ox-LDL, then differentiating them into macrophages and activating them into pro- (M1) and anti-inflammatory (M2) phenotypes. Through the detection of inflammatory cytokines produced by the model, the authors discovered that M1 macrophages, M2 macrophages, and THP-1 monocytes displayed different responses in high and low tissue density hydrogel constructs (Zhang et al., 2018).

Dorweiler et al. also utilized a fibrin gel-based model to simulate the early stages of atherosclerosis. In this model, smooth muscle cells were initially encapsulated in the fibrin gel, followed by seeding of endothelial cells on top to form an endothelial-seeded smooth muscle cell-laden fibrin construct (Dorweiler et al., 2006). Lipoproteins and monocytes were then added to the culture to promote foam cell formation and atherosclerotic plaque development. The model was shown to be stable for up to six weeks and allowed for the study of autologous in vitro vascular models to investigate the progression of early atherosclerotic lesions. Similarly, Vogel et al. used a 3D engineered smooth muscle cell-fibrin construct with specific geometry to investigate the impact of hemodynamic forces on atherosclerosis development. They discovered that high shear stress could protect the newly formed extracellular matrix from degradation by metalloproteinases, whereas low shear stress would induce smooth muscle cell proteolytic activity and shift the balance towards increased collagen and reduced elastin, leading to a shift in smooth muscle cell phenotype. These findings highlight the potential of using cell hydrogel constructs to evaluate the effects of hemodynamic forces on cell behavior during atherosclerosis development (Hosseini et al., 2020).

In vitro 3D Vessel Based Systems

Tissue-Engineered Blood Vessels (TEBVs)

In recent years, the fields of tissue engineering, regenerative medicine, biomaterials, and cell biology have undergone remarkable progress, leading to the development of tissue-engineered blood vessels (TEBVs) as potential vascular grafts for treating atherosclerosis. As a result, TEBVs have become essential tools for studying atherogenesis and have been transformed into in vitro atherosclerosis models that can simulate the critical aspects of this disease. These models offer an alternative to traditional 2D cell cultures and animal models, providing a valuable platform for research on atherosclerosis and therapies for this condition (Song et al., 2018).

Advancements in tissue engineering, regenerative medicine, biomaterials, and cell biology have allowed for the development of tissue-engineered blood vessels (TEBVs) as a means of treating atherosclerosis. TEBVs are created by seeding vascular cells onto biodegradable polymeric scaffolds, such as poly-(l-lactide-co-ε-caprolactone) (PLCL), and maturing the tissue in a perfusion system (Truskey, 2016). For example, single-layered TEBVs was fabricated by seeding mouse smooth muscle cells on a PLCL scaffold coated with fibronectin and gelatin. The TEBVs achieved mechanical properties similar to those of native arteries. In addition to the seeding strategy, cell sheet technology has been used to create TEBVs. The Germain group developed single-layered TEBVs by

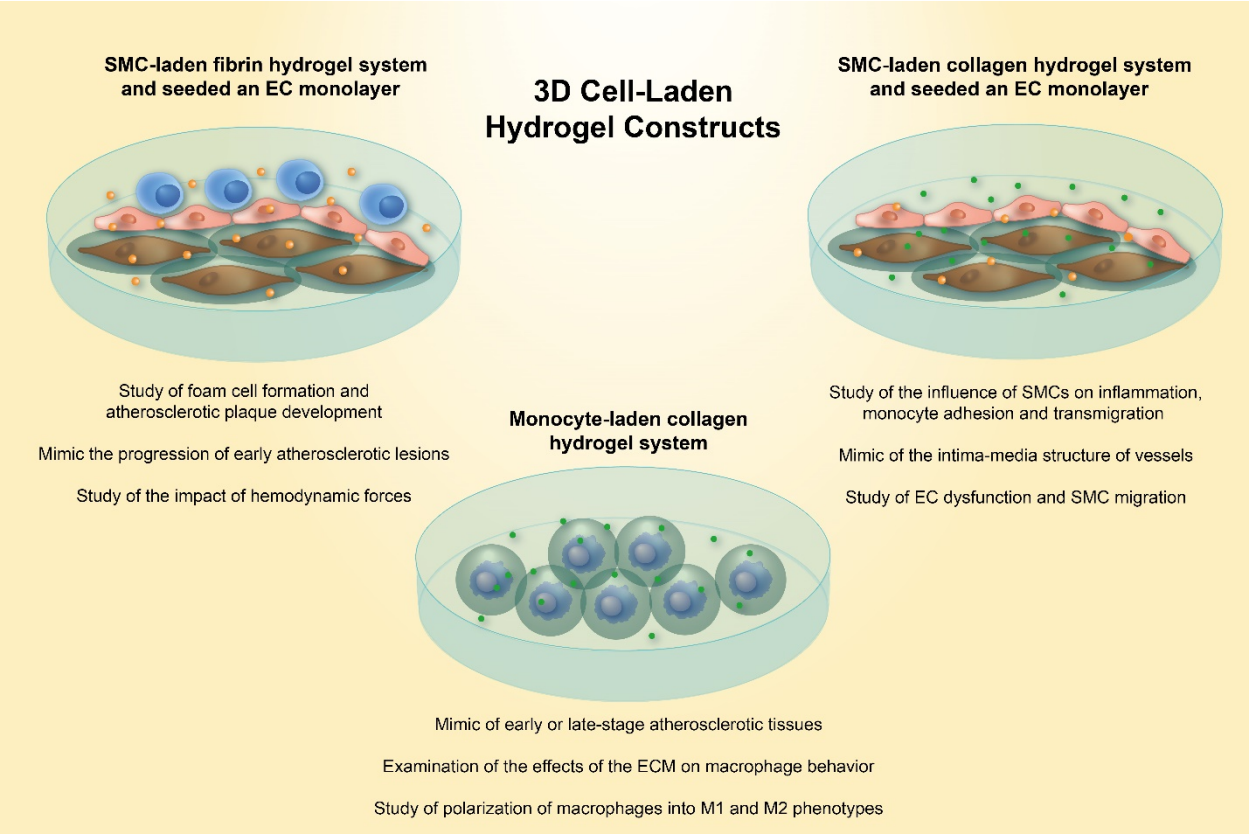

Figure 3. In vitro 3D Cell-Laden Hydrogel Constructs. Using in vitro 3D cell-laden hydrogel constructs in atherosclerosis research provides a more physiologically relevant and biomimetic platform for studying the disease. This approach offers opportunities for investigating cell-cell interactions, recapitulating tissue architecture, and evaluating therapeutic interventions in a controlled environment.

Table 1. Atherosclerosis models and their limitations.

Model	Limitation	Consequence	Recommended Approach
2D Cell Models	Inability to accurately mimic the complex structure of blood vessels and human plaques.	Single-cell models may not reliably predict therapeutic efficacy in patients.	Combine 2D single-cell models with in vivo models for comprehensive understanding.
3D Spheroids	Limited scalability and standardization of fabrication methods.	Difficulty in comparing results across studies due to variability in spheroid formation.	Establish standardized protocols for reproducible 3D spheroid generation.
3D Cell-Laden Hydrogel Constructs	Challenges in modeling the complete structure of blood vessels, including all layers.	Lack of tunica adventitia and accurate vessel architecture representation.	Enhance models to incorporate complete vessel layers for better mimicry.
Tissue-Engineered Blood Vessels (TEBVs)	Difficulty in replicating all aspects of native atherosclerotic lesions in vitro.	Incomplete representation of complex disease progression.	Further refine TEBV models to better mimic advanced disease stages.
Vessel-on-a-Chip Models	Current focus on non-diseased models with limited diseased vessel-on-a-chip systems.	Limited application of vessel-on-a-chip models in studying atherosclerosis disease mechanisms.	Develop more disease-relevant vessel-on-a-chip models to advance atherosclerosis research.

rolling fibroblast cell sheets into a vessel-like structure, while a multi-layered TEBV was developed by decellularizing a single-layered TEBV, seeding SMCs and ECs in the decellularized TEBV, and maturing the TEBV in a bioreactor (Laterreur et al., 2014). Rolle et al. recently developed spatially controlled TEBVs by fusing SMC ring units into a vessel structure with heterogeneous compositions similar to those observed in intimal hyperplasia or atherosclerosis (Strobel et al., 2018). The TEBV was created by self-assembling human aortic SMCs (hAoSMC) ring units, threading them onto a mandrel, and maturing them in a dynamic environment using a bioreactor. Although some TEBVs have been generated by seeding cells on 2D scaffolds followed by rolling and maturing of the vessel structure, we still discuss them in the 3D section as those TEBVs provide a 3D vessel shape. These advances in TEBV fabrication have provided valuable models for studying atherogenesis and have offered alternative in vitro platforms for atherosclerosis and associated therapy studies, supplementing the traditional 2D and animal models (Pan et al., 2022).

In the field of atherosclerosis research, 2-layered tissue-engineered blood vessels (TEBVs) have been utilized for investigating the roles of various factors in atherosclerosis development. The Truskey group, for instance, used a 2-layered TEBV to investigate the impact of PCSK9, an enzyme linked to high cholesterol levels, on atherogenesis, independent of LDL. In addition, the group examined the effect of oxidative stress on vascular inflammation and senescence using a 2-layered TEBV consisting of endothelial and fibroblast layers (Yurtseven et al., 2020). The results indicated that oxidative stress could promote atherosclerosis by increasing vascular inflammation. Similarly, Chen et al. developed a 2-layered TEBV containing both endothelial and smooth muscle cells, and employed it in an imaging chamber to visualize the real-time process of leukocyte recruitment and penetration through the endothelium into the intima, offering a novel approach for studying the pathogenesis of atherosclerosis and for testing the efficacy of potential drugs in a micro-physiological system (Chen et al., 2018).

Despite early efforts to design TEBVs without curvature, recent research has increasingly focused on developing branched TEBVs to study disease. This is due to the fact that different vascular geometries can lead to varying flow patterns that may have a significant impact on atherogenesis (Lee et al., 2021). For example, the Leong research team constructed a branched TEBV to investigate the impact of flow patterns on atherogenesis (Chen et al., 2018). Their findings revealed that the athero-prone region (branched side outlets) had greater monocyte adhesion than other regions. In another study, Chavez et al. developed an angulated TEBV that mimicked the bent human vessel to study the effects of stents on atherogenesis in athero-prone regions (Chavez et al., 2019). The TEBV was constructed by seeding HUVECs on an expanded polytetrafluoroethylene tubular scaffold with bent geometry. The authors discovered that endothelialization was significantly reduced on the stented TEBV compared to the un-stented control. These studies demonstrate that a controllable in vitro TEBV platform with specific geometries can simulate the athero-prone conditions of the artery, making it an ideal candidate for future intravascular device evaluations.

Diseased TEBVs, which exhibit features of atherosclerosis, have garnered significant attention in the field due to their potential to model this disease. However, the complexity of the atherosclerotic plaque has made it challenging to develop TEBVs with relevant features (Cai et al., 2021). One notable study by Hoerstrup et al. involved the creation of a 2-layered diseased TEBV by seeding vascular cells on a biodegradable tubular scaffold and adding LDL and inflammatory cells under high and low shear stress (Hoerstrup et al., 2002). The diseased TEBV model exhibited early atherosclerotic features such as monocyte attachment and LDL accumulation. In another study, Truskey et al. attempted to develop a 3-layered TEBV comprising endothelial cells, smooth muscle cells, and dermal fibroblasts (Salmon et al., 2020). Early atherosclerosis was induced in this model using LDL with or without TNF-α, and the TEBV displayed the maintenance of vascular cell phenotype and early atherosclerosis symptoms such as endothelial activation, vasoactivity, monocyte accumulation, foam cell formation, and macrophage polarization. Furthermore, this model was utilized to examine the effects of various substances on disease progression, including LDL, lovastatin, and P2Y11 inhibitor (NF157). Lovastatin was found to prevent the altered vasoactivity and NO production induced by eLDL and TNF-α in this model (Zhang et al., 2020).

As a result, utilizing diseased TEBVs can provide an effective means of studying specific vascular functions that are difficult to assess in vivo. In another study, Cho et al. employed cell printing technology to fabricate three-layered vascular constructs with atherosclerotic features, including tunable geometries such as stenosis and tortuous structures, a monolayer of confluent endothelium, and condensed smooth muscle cell (SMC) layers. The resulting turbulent flow in the TEBVs with stenosis and tortuous structures, along with the co-culture of SMCs and endothelial cells (ECs), led to greater endothelial dysfunction, LDL accumulation, foam cell formation, and THP-1 cell recruitment - all of which are early hallmarks of atherosclerosis. These findings underscored the importance of athero-prone vascular structures and the co-existence of multiple vascular cell types to generate atherogenesis in the model (Greco et al., 2022). Notably, the authors also demonstrated the significance of TEBVs in drug testing for atherosclerosis. They showed that atorvastatin was effective in reducing atherosclerotic symptoms in the TEBV model, including endothelial dysfunction, monocyte recruitment, LDL oxidation and uptake, and improvement of free cholesterol efflux. Therefore, this study provided further evidence

that TEBVs are a promising tool for biomedical applications, including pathological research and novel drug identification and evaluation (Demir et al., 2018).

Vessel-on-a-Chip

Vessel-on-a-chip is a type of organ-on-a-chip (OOC) system that has become increasingly popular among atherosclerosis researchers. OOC systems are biomimetic microfluidic platforms that combine microfluidic technology, biomaterial science, tissue engineering, and cell biology to mimic the micro-physiological environment of functional human organs. Typically, an OOC system consists of an engineered architecture built in a micron-sized electron fluidic chip chamber that recreates the functional architectures and micro-physiological environment of a human organ (Paloschi et al., 2021). This chamber can be connected to a pump with controlled flow rates and shear stresses. Alternatively, certain critical features of human diseases in a specific tissue can be induced in the OOC to model the disease. In comparison to traditional static 2D cultures, OOC systems provide a more dynamic environment that more accurately represents vascular physiology, morphology, and response. Furthermore, the high-throughput capacity of OOC systems makes them more cost-effective than animal models, which is an important consideration for reducing R&D costs. As a result, OOC systems, particularly vessel-on-a-chip, have become promising platforms for studying biochemical and metabolic processes, investigating cellular responses during atherogenesis, and evaluating the therapeutic efficacy and safety of treatments for atherosclerosis. In the following section, we will review recent advances in this field (Ronaldson-Bouchard and Vunjak-Novakovic, 2018).

A vessel-on-a-chip system consists of a vessel-like structure on a microchip, either with or without disease features. These systems have been created by seeding cells onto a fiber scaffold. To address issues commonly encountered in vessels grown under static conditions, such as shedding of endothelial cells and irregular orientation, the Li research group developed a chip by seeding endothelial cells (ECs) onto a highly oriented electrospun scaffold made of poly(ε-caprolactone) fibers. The ECs cultured on this chip showed enhanced endothelialization when perfused with nutrients, and were able to align themselves in a manner similar to natural blood vessels (Hasan et al., 2015). Aside from seeding cells directly onto a scaffold, vessel-on-a-chip systems have also been fabricated by creating cell-laden constructs and then seeding cells onto the hybrid constructs. For example, a gelatin methacryloyl gel was developed that contained fibroblast and smooth muscle cells. They subsequently seeded ECs onto the construct through perfusion, which resulted in the formation of a confluent endothelium layer in just 3 days (Klotz et al., 2016). This vessel-on-a-chip system is useful for drug safety screening because of its three-layered structure and perfusion system. Li et al. conducted a study on their blood-vessel-on-chip system that enables the creation of controllable vessel structures (straight, wavy, or helical) to mimic different physiological environments of blood vessels. Firstly, the researchers created a hollow microfiber with a desired structure by adjusting the flow rate, injection device radius, and fluid composition of each channel. Then, the inner surface of collagen and alginate-coated hollow microfibers were seeded with HUVECs to achieve a fully covered endothelium layer. Finally, a proof-of-concept test was conducted to show that this cell-laden microfiber could be developed into a vessel-on-a-chip (Delannoy et al., 2022).

Despite some headway in the development of vessel-on-a-chip systems, the majority of research has been focused on creating non-diseased vessel-on-a-chip models with limited practical use, and only one study has demonstrated the creation of atherosclerotic vessel-on-a-chip. This groundbreaking research was conducted by Hoerstrup et al., who used tissue-engineered arteries (hiTEV) made from cells derived from human-induced pluripotent stem cells (hiPSCs) to induce atherosclerosis on a chip (Hoerstrup et al., 2002). The researchers successfully induced SMC, EC, and macrophage-derived cells from hiPSCs, and showed that the hiPSC-derived SMCs and ECs could express SMC contractile phenotype markers and endothelial phenotype markers. By seeding the hiPSC-derived SMCs in the biodegradable tubular-shaped conduits carrying fibrin scaffold and cultivating, followed by seeding the hiPSC-derived ECs in the inner lumen of the conduits on a microfluidic chip, the researchers were able to fabricate two-layered hiTEV. The matured hiTEV was composed of endothelium and SMC layers that displayed positive expressions of EC and SMC phenotype markers, respectively. Additionally, the hiTEV underwent plaque formation when treated with hiPSC-derived macrophages and LDL, resulting in plaque-like structures (Stephenson et al., 2019). The authors noted that the tissue-engineered plaque contained dendritic cells, ECs, and macrophages that closely resembled those found in native plaque. Furthermore, the expression of ECM assembly and remodeling genes in the tissue-engineered plaques was similar to that of native plaques. Despite the close emulation of native plaque, the study had limitations and drawbacks. One issue was that the hiPSC-derived cells had a fetal-like cell phenotype and did not exhibit the aged phenotype seen in atherosclerosis patients of different ages. Additionally, while T and B cells play a critical role in atherosclerosis development, they were not included in the model. Finally, the study did not demonstrate any specific applications of this system (Dudley andGriffioen, 2023).

Vessel-on-a-chip systems have been widely employed for studying the biological mechanisms of atherosclerosis and evaluating the safety of different compounds. Joore et al. designed tubular vessels-on-a-chip to investigate the adhesion of monocytes to endothelial cells (Poussin et al., 2020). The tubular vessels were generated by

seeding human coronary artery endothelial cells into a collagen gel and culturing them with perfused flow. The authors found that after treatment with TNF-α, the vessels expressed high levels of ICAM-1 proteins and recruited significant numbers of monocytes. They also used this model to demonstrate that aerosol extract was less toxic to the endothelium than cigarette extract, as shown by the lower expression of ICAM-1 and monocyte adhesion induced by the aerosol extract.

In another study, Li et al. created a carotid artery model by combining EC and gelatin. This model was designed to mimic the common carotid artery (CCA), internal carotid artery (ICA), external carotid artery (ECA), and carotid sinus (CS) using a tuning fork-shaped artery composed of four distinct parts (Chen et al., 2019). To generate a flow, a perfusion loop consisting of a medium and a pump was used. Interestingly, the laminar flow in the CCA region changed to disturbed flow with lower wall shear stress in the CS regions due to its curvature structure when the flow was applied. However, the flow pattern changed when it reached the ICA and ECA regions, creating a laminar flow with higher WSS. The chip's distinctive design led to various flow conditions in different areas, allowing the authors to study the hemodynamic effects, including wall shear stress (WSS), on endothelial cells (ECs). The authors discovered that the ECs in the chip responded differently to the changes in flow. In the ECA region, the ECs stretched and aligned with the flow direction, while in the CS region, they had a round shape and were more disorganized (Sui et al., 2008). Furthermore, an endothelialized monolayer formed in the ECA region, while the expression of ICAM-1 and VCAM-1 was more significant in the CS region than in the ECA region. Over time, WSS was found to increase nitric oxide production in the system, but decreased expression of ZO-1, the primary EC tight junction marker, was observed in the CS regions compared to the ECA region. This study showcases an example of how a vessel-on-a-chip with unique features can be created and utilized to study the effects of hemodynamic force on critical cellular components associated with atherosclerosis, which is not achievable in most other static in vitro systems (Chen et al., 2019). In Table 1, we summarized the limitations and their consequences of the range of models.

Conclusion

Despite advances in bioengineering in cell model development, murine models remain the most popular choice among atherosclerosis researchers. Many different models have been developed in recent years, from a classic 2D single-cell culture system on TCP to advanced 3D TEBVs and vessel-on-a-chip models. The main advantages of using such models are their low cost, the speed of cultivation, as well as the relative ease in evaluating the result compared to animals. Despite this, the available models cannot fully model human atherosclerosis, but they show significant potential. 3D models seem to be especially promising. All of them have their own benefits and limitations, but the choice should be made individually according to the purpose of an exact experiment. Research using cellular models in atherosclerosis holds promising translational potential and offers a glimpse into future advancements. These models play a pivotal role in drug screening and development, facilitating the discovery of novel therapeutics. They also open doors to personalized medicine by utilizing patient-derived cells to tailor treatments based on individual genetic and environmental factors.

Furthermore, cellular models aid in biomarker discovery, identifying crucial markers for early detection and monitoring disease progression. By delving deeper into the molecular and cellular mechanisms of atherosclerosis, these models pave the way for targeted interventions.

Looking ahead, future directions in atherosclerosis research involve integrating multicellular models to mimic complex cell interactions within blood vessels. By incorporating bioengineering approaches, researchers can recreate dynamic microenvironments, including flow patterns and extracellular matrix composition. Advanced imaging technologies will provide real-time visualization of cellular responses, enhancing research outcomes.

Validation studies with animal models and clinical data will ensure the relevance of findings from cellular models. Additionally, the development of high-throughput screening platforms will expedite drug discovery processes by quickly screening compounds and potential therapeutic targets.

By embracing these future directions and leveraging the translational potential of cellular models, researchers can advance our understanding of atherosclerosis, identify new therapeutic targets, and ultimately improve patient outcomes.

Author contribution

A.V.P. wrote, drafted; V.N.S., V.A.K., V.Y.G., I.A.S., A.N.O. wrote, reviewed, edited, prepared the graph of the article. All authors have read and agreed to the published version of the manuscript.

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Competing financial interests

The authors have no conflict of interest.

References

Agarwal, P., Combes, T. W., Shojaee-Moradie, F., Fielding, B., Gordon, S., Mizrahi, V., & Martinez, F. O. (2020). Foam Cells Control Mycobacterium tuberculosis Infection. Frontiers in microbiology, 11, 1394. https://doi.org/10.3389/fmicb.2020.01394

Barnes, R. H., 2nd, Akama, T., Öhman, M. K., Woo, M. S., Bahr, J., Weiss, S. J., Eitzman, D. T., & Chun, T. H. (2017). Membrane-Tethered Metalloproteinase Expressed by Vascular Smooth Muscle Cells Limits the Progression of Proliferative Atherosclerotic Lesions. Journal of the American Heart Association, 6(7), e003693. https://doi.org/10.1161/JAHA.116.003693

Białkowska, K., Komorowski, P., Bryszewska, M., & Miłowska, K. (2020). Spheroids as a Type of Three-Dimensional Cell Cultures-Examples of Methods of Preparation and the Most Important Application. International journal of molecular sciences, 21(17), 6225. https://doi.org/10.3390/ijms21176225

Bowen, R. A., & Remaley, A. T. (2014). Interferences from blood collection tube components on clinical chemistry assays. Biochemia medica, 24(1), 31–44. https://doi.org/10.11613/BM.2014.006

Brancato, V., Oliveira, J. M., Correlo, V. M., Reis, R. L., & Kundu, S. C. (2020). Could 3D models of cancer enhance drug screening?. Biomaterials, 232, 119744. https://doi.org/10.1016/j.biomaterials.2019.119744

Cai, Q., Liao, W., Xue, F., Wang, X., Zhou, W., Li, Y., & Zeng, W. (2021). Selection of different endothelialization modes and different seed cells for tissue-engineered vascular graft. Bioactive materials, 6(8), 2557–2568. https://doi.org/10.1016/j.bioactmat.2020.12.021

Chavez, R. D., Walls, S. L., & Cardinal, K. O. (2019). Tissue-engineered blood vessel mimics in complex geometries for intravascular device testing. PloS one, 14(6), e0217709. https://doi.org/10.1371/journal.pone.0217709

Chen M. F. (2021). The role of calmodulin and calmodulin-dependent protein kinases in the pathogenesis of atherosclerosis. Tzu chi medical journal, 34(2), 160–168. https://doi.org/10.4103/tcmj.tcmj_119_21

Chen, J., Zhang, X., Millican, R., Lynd, T., Gangasani, M., Malhotra, S., Sherwood, J., Hwang, P. T., Cho, Y., Brott, B. C., Qin, G., Jo, H., Yoon, Y. S., & Jun, H. W. (2022). Recent Progress in in vitro Models for Atherosclerosis Studies. Frontiers in cardiovascular medicine, 8, 790529. https://doi.org/10.3389/fcvm.2021.790529

Chen, R., Wang, B., Liu, Y., He, J., Lin, R., & Li, D. (2019). Gelatin-based perfusable, endothelial carotid artery model for the study of atherosclerosis. Biomedical engineering online, 18(1), 87. https://doi.org/10.1186/s12938-019-0706-6

Chen, R., Wang, B., Liu, Y., He, J., Lin, R., & Li, D. (2019). Gelatin-based perfusable, endothelial carotid artery model for the study of atherosclerosis. Biomedical engineering online, 18(1), 87. https://doi.org/10.1186/s12938-019-0706-6

Chen, Z., Tang, M., Huang, D., Jiang, W., Li, M., Ji, H., Park, J., Xu, B., Atchison, L. J., Truskey, G. A., & Leong, K. W. (2018). Real-time observation of leukocyte-endothelium interactions in tissue-engineered blood vessel. Lab on a chip, 18(14), 2047–2054. https://doi.org/10.1039/c8lc00202a

Chen, Z., Tang, M., Huang, D., Jiang, W., Li, M., Ji, H., Park, J., Xu, B., Atchison, L. J., Truskey, G. A., & Leong, K. W. (2018). Real-time observation of leukocyte-endothelium interactions in tissue-engineered blood vessel. Lab on a chip, 18(14), 2047–2054. https://doi.org/10.1039/c8lc00202a

Chiu, C. W., Hsieh, C. Y., Yang, C. H., Tsai, J. H., Huang, S. Y., & Sheu, J. R. (2022). Yohimbine, an α2-Adrenoceptor Antagonist, Suppresses PDGF-BB-Stimulated Vascular Smooth Muscle Cell Proliferation by Downregulating the PLCγ1 Signaling Pathway. International journal of molecular sciences, 23(14), 8049. https://doi.org/10.3390/ijms23148049

Chun, T. H., Sabeh, F., Ota, I., Murphy, H., McDonagh, K. T., Holmbeck, K., Birkedal-Hansen, H., Allen, E. D., & Weiss, S. J. (2004). MT1-MMP-dependent neovessel formation within the confines of the three-dimensional extracellular matrix. The Journal of cell biology, 167(4), 757–767. https://doi.org/10.1083/jcb.200405001

Davis, J. L., Zhang, Y., Yi, S., Du, F., Song, K. H., Scott, E. A., Sun, C., & Zhang, H. F. (2020). Super-Resolution Imaging of Self-Assembled Nanocarriers Using Quantitative Spectroscopic Analysis for Cluster Extraction. Langmuir : the ACS journal of surfaces and colloids, 36(9), 2291–2299. https://doi.org/10.1021/acs.langmuir.9b03149

Delannoy, E., Tellier, G., Cholet, J., Leroy, A. M., Treizebré, A., & Soncin, F. (2022). Multi-Layered Human Blood Vessels-on-Chip Design Using Double Viscous Finger Patterning. Biomedicines, 10(4), 797. https://doi.org/10.3390/biomedicines10040797

Demir, V., Doğru, M. T., Ede, H., Yilmaz, S., Alp, C., Celik, Y., & Yildirim, N. (2018). The effects of treatment with atorvastatin versus rosuvastatin on endothelial dysfunction in patients with hyperlipidaemia. Cardiovascular journal of Africa, 29(3), 162–166. https://doi.org/10.5830/CVJA-2018-008

Dorweiler, B., Torzewski, M., Dahm, M., Ochsenhirt, V., Lehr, H. A., Lackner, K. J., & Vahl, C. F. (2006). A novel in vitro model for the study of plaque development in atherosclerosis. Thrombosis and haemostasis, 95(1), 182–189.

Dudley, A. C., & Griffioen, A. W. (2023). Pathological angiogenesis: mechanisms and therapeutic strategies. Angiogenesis, 26(3), 313–347. https://doi.org/10.1007/s10456-023-09876-7

El-Sherbiny, I. M., & Yacoub, M. H. (2013). Hydrogel scaffolds for tissue engineering: Progress and challenges. Global cardiology science & practice, 2013(3), 316–342. https://doi.org/10.5339/gcsp.2013.38

Garcia-Sabaté, A., Mohamed, W. K. E., Sapudom, J., Alatoom, A., Al Safadi, L., & Teo, J. C. M. (2020). Biomimetic 3D Models for Investigating the Role of Monocytes and Macrophages in Atherosclerosis. Bioengineering (Basel, Switzerland), 7(3), 113. https://doi.org/10.3390/bioengineering7030113

Ghosh, N., Das, A., Biswas, N., Mahajan, S. P., Madeshiya, A. K., Khanna, S., Sen, C. K., & Roy, S. (2022). Myo-Inositol in Fermented Sugar Matrix Improves Human Macrophage Function. Molecular nutrition & food research, 66(8), e2100852. https://doi.org/10.1002/mnfr.202100852

Greco, M. F., Rizzuto, A. S., Zarà, M., Cafora, M., Favero, C., Solazzo, G., Giusti, I., Adorni, M. P., Zimetti, F., Dolo, V., Banfi, C., Ferri, N., Sirtori, C. R., Corsini, A., Barbieri, S. S., Pistocchi, A., Bollati, V., Macchi, C., & Ruscica, M. (2022). PCSK9 Confers Inflammatory Properties to Extracellular Vesicles Released by Vascular Smooth Muscle Cells. International journal of molecular sciences, 23(21), 13065. https://doi.org/10.3390/ijms232113065

Hasan, A., Paul, A., Memic, A., & Khademhosseini, A. (2015). A multilayered microfluidic blood vessel-like structure. Biomedical microdevices, 17(5), 88. https://doi.org/10.1007/s10544-015-9993-2

He, D., Mao, A., Zheng, C. B., Kan, H., Zhang, K., Zhang, Z., Feng, L., & Ma, X. (2020). Aortic heterogeneity across segments and under high fat/salt/glucose conditions at the single-cell level. National science review, 7(5), 881–896. https://doi.org/10.1093/nsr/nwaa038

Hoerstrup, S. P., Kadner, A., Melnitchouk, S., Trojan, A., Eid, K., Tracy, J., Sodian, R., Visjager, J. F., Kolb, S. A., Grunenfelder, J., Zund, G., & Turina, M. I. (2002). Tissue engineering of functional trileaflet heart valves from human marrow stromal cells. Circulation, 106(12 Suppl 1), I143–I150.

Hosseini, V., Mallone, A., Mirkhani, N., Noir, J., Salek, M., Pasqualini, F. S., Schuerle, S., Khademhosseini, A., Hoerstrup, S. P., & Vogel, V. (2020). A Pulsatile Flow System to Engineer Aneurysm and Atherosclerosis Mimetic Extracellular Matrix. Advanced science (Weinheim, Baden-Wurttemberg, Germany), 7(12), 2000173. https://doi.org/10.1002/advs.202000173

Ilhan, F., & Kalkanli, S. T. (2015). Atherosclerosis and the role of immune cells. World journal of clinical cases, 3(4), 345–352. https://doi.org/10.12998/wjcc.v3.i4.345

Klotz, B. J., Gawlitta, D., Rosenberg, A. J. W. P., Malda, J., & Melchels, F. P. W. (2016). Gelatin-Methacryloyl Hydrogels: Towards Biofabrication-Based Tissue Repair. Trends in biotechnology, 34(5), 394–407. https://doi.org/10.1016/j.tibtech.2016.01.002

Kuppusamy, P., Kim, D., Soundharrajan, I., Hwang, I., & Choi, K. C. (2020). Adipose and Muscle Cell Co-Culture System: A Novel In Vitro Tool to Mimic the In Vivo Cellular Environment. Biology, 10(1), 6. https://doi.org/10.3390/biology10010006

Laterreur, V., Ruel, J., Auger, F. A., Vallières, K., Tremblay, C., Lacroix, D., Tondreau, M., Bourget, J. M., & Germain, L. (2014). Comparison of the direct burst pressure and the ring tensile test methods for mechanical characterization of tissue-engineered vascular substitutes. Journal of the mechanical behavior of biomedical materials, 34, 253–263. https://doi.org/10.1016/j.jmbbm.2014.02.017.

Lee, J. H., Chen, Z., He, S., Zhou, J., Tsai, A., Truskey, G., & Leong, K. W. (2021). Emulating Early Atherosclerosis in a Vascular Microphysiological System Using Branched Tissue-Engineered Blood Vessels. Advanced biology, 5(4), e2000428. https://doi.org/10.1002/adbi.202000428

Liu, M., Samant, S., Vasa, C. H., Pedrigi, R. M., Oguz, U. M., Ryu, S., Wei, T., Anderson, D. R., Agrawal, D. K., & Chatzizisis, Y. S. (2023). Co-culture models of endothelial cells, macrophages, and vascular smooth muscle cells for the study of the natural history of atherosclerosis. PloS one, 18(1), e0280385. https://doi.org/10.1371/journal.pone.0280385

Liu, M., Samant, S., Vasa, C. H., Pedrigi, R. M., Oguz, U. M., Ryu, S., Wei, T., Anderson, D. R., Agrawal, D. K., & Chatzizisis, Y. S. (2023). Co-culture models of endothelial cells, macrophages, and vascular smooth muscle cells for the study of the natural history of atherosclerosis. PloS one, 18(1), e0280385. https://doi.org/10.1371/journal.pone.0280385

Liu, M., Wang, D., Gu, S., Tian, B., Liang, J., Suo, Q., Zhang, Z., Yang, G., Zhou, Y., & Li, S. (2021). Micro/nano materials regulate cell morphology and intercellular communication by extracellular vesicles. Acta biomaterialia, 124, 130–138. https://doi.org/10.1016/j.actbio.2021.02.003

Ma, C., Xia, R., Yang, S., Liu, L., Zhang, J., Feng, K., Shang, Y., Qu, J., Li, L., Chen, N., Xu, S., Zhang, W., Mao, J., Han, J., Chen, Y., Yang, X., Duan, Y., & Fan, G. (2020). Formononetin attenuates atherosclerosis via regulating interaction between KLF4 and SRA in apoE-/- mice. Theranostics, 10(3), 1090–1106. https://doi.org/10.7150/thno.38115

Mallone, A., Stenger, C., Von Eckardstein, A., Hoerstrup, S. P., & Weber, B. (2018). Biofabricating atherosclerotic plaques: In vitro engineering of a three-dimensional human fibroatheroma model. Biomaterials, 150, 49–59. https://doi.org/10.1016/j.biomaterials.2017.09.034

Méndez-Barbero, N., Gutiérrez-Muñoz, C., & Blanco-Colio, L. M. (2021). Cellular Crosstalk between Endothelial and Smooth Muscle Cells in Vascular Wall Remodeling. International journal of molecular sciences, 22(14), 7284. https://doi.org/10.3390/ijms22147284

Mitra, S., Murthy, G.S. Bioreactor control systems in the biopharmaceutical industry: a critical perspective. Syst Microbiol and Biomanuf 2, 91–112 (2022). https://doi.org/10.1007/s43393-021-00048-6

Mohandas, S., Gayatri, V., Kumaran, K., Gopinath, V., Paulmurugan, R., & Ramkumar, K. M. (2023). New Frontiers in Three-Dimensional Culture Platforms to Improve Diabetes Research. Pharmaceutics, 15(3), 725. https://doi.org/10.3390/pharmaceutics15030725

Nguyen, L. T. H., Muktabar, A., Tang, J., Wong, Y. S., Thaxton, C. S., Venkatraman, S. S., & Ng, K. W. (2018). The Potential of Fluocinolone Acetonide to Mitigate Inflammation and Lipid Accumulation in 2D and 3D Foam Cell Cultures. BioMed research international, 2018, 3739251. https://doi.org/10.1155/2018/3739251

Nicodemus, G. D., & Bryant, S. J. (2008). Cell encapsulation in biodegradable hydrogels for tissue engineering applications. Tissue engineering. Part B, Reviews, 14(2), 149–165. https://doi.org/10.1089/ten.teb.2007.0332

Nikiforov, N. G., Wetzker, R., Kubekina, M. V., Petukhova, A. V., Kirichenko, T. V., & Orekhov, A. N. (2019). Trained Circulating Monocytes in Atherosclerosis: Ex Vivo Model Approach. Frontiers in pharmacology, 10, 725. https://doi.org/10.3389/fphar.2019.00725

Owsiany, K. M., Alencar, G. F., & Owens, G. K. (2019). Revealing the Origins of Foam Cells in Atherosclerotic Lesions. Arteriosclerosis, thrombosis, and vascular biology, 39(5), 836–838. https://doi.org/10.1161/ATVBAHA.119.312557

Paloschi, V., Sabater-Lleal, M., Middelkamp, H., Vivas, A., Johansson, S., van der Meer, A., Tenje, M., & Maegdefessel, L. (2021). Organ-on-a-chip technology: a novel approach to investigate cardiovascular diseases. Cardiovascular research, 117(14), 2742–2754. https://doi.org/10.1093/cvr/cvab088

Pan, C., Gao, Q., Kim, B. S., Han, Y., & Gao, G. (2022). The Biofabrication of Diseased Artery In Vitro Models. Micromachines, 13(2), 326. https://doi.org/10.3390/mi13020326

Poussin, C., Kramer, B., Lanz, H. L., Van den Heuvel, A., Laurent, A., Olivier, T., Vermeer, M., Peric, D., Baumer, K., Dulize, R., Guedj, E., Ivanov, N. V., Peitsch, M. C., Hoeng, J., & Joore, J. (2020). 3D human microvessel-on-a-chip model for studying monocyte-to-endothelium adhesion under flow - application in systems toxicology. ALTEX, 37(1), 47–63. https://doi.org/10.14573/altex.1811301

Poznyak, A. V., Nikiforov, N. G., Starodubova, A. V., Popkova, T. V., & Orekhov, A. N. (2021). Macrophages and Foam Cells: Brief Overview of Their Role, Linkage, and Targeting Potential in Atherosclerosis. Biomedicines, 9(9), 1221. https://doi.org/10.3390/biomedicines9091221

Ronaldson-Bouchard, K., & Vunjak-Novakovic, G. (2018). Organs-on-a-Chip: A Fast Track for Engineered Human Tissues in Drug Development. Cell stem cell, 22(3), 310–324. https://doi.org/10.1016/j.stem.2018.02.011

Ryu, N. E., Lee, S. H., & Park, H. (2019). Spheroid Culture System Methods and Applications for Mesenchymal Stem Cells. Cells, 8(12), 1620. https://doi.org/10.3390/cells8121620

S. R., Fish, J. E., & Howe, K. L. (2021). Dysfunctional Vascular Endothelium as a Driver of Atherosclerosis: Emerging Insights Into Pathogenesis and Treatment. Frontiers in pharmacology, 12, 787541. https://doi.org/10.3389/fphar.2021.787541

Salmon, E. E., Breithaupt, J. J., & Truskey, G. A. (2020). Application of Oxidative Stress to a Tissue-Engineered Vascular Aging Model Induces Endothelial Cell Senescence and Activation. Cells, 9(5), 1292. https://doi.org/10.3390/cells9051292

Savoji, H., Mohammadi, M. H., Rafatian, N., Toroghi, M. K., Wang, E. Y., Zhao, Y., Korolj, A., Ahadian, S., & Radisic, M. (2019). Cardiovascular disease models: A game changing paradigm in drug discovery and screening. Biomaterials, 198, 3–26. https://doi.org/10.1016/j.biomaterials.2018.09.036

Shi, J., Zhou, T., & Chen, Q. (2022). Exploring the expanding universe of small RNAs. Nature cell biology, 24(4), 415–423. https://doi.org/10.1038/s41556-022-00880-5

Song, H. G., Rumma, R. T., Ozaki, C. K., Edelman, E. R., & Chen, C. S. (2018). Vascular Tissue Engineering: Progress, Challenges, and Clinical Promise. Cell stem cell, 22(3), 340–354. https://doi.org/10.1016/j.stem.2018.02.009

Stephenson, M., Reich, D. H., & Boheler, K. R. (2019). Induced pluripotent stem cell-derived vascular smooth muscle cells. Vascular biology (Bristol, England), 2(1), R1–R15. https://doi.org/10.1530/VB-19-0028

Strobel, H. A., Hookway, T. A., Piola, M., Fiore, G. B., Soncini, M., Alsberg, E., & Rolle, M. W. (2018). Assembly of Tissue-Engineered Blood Vessels with Spatially Controlled Heterogeneities. Tissue engineering. Part A, 24(19-20), 1492–1503. https://doi.org/10.1089/ten.TEA.2017.0492

Su, C., Menon, N. V., Xu, X., Teo, Y. R., Cao, H., Dalan, R., Tay, C. Y., & Hou, H. W. (2021). A novel human arterial wall-on-a-chip to study endothelial inflammation and vascular smooth muscle cell migration in early atherosclerosis. Lab on a chip, 21(12), 2359–2371. https://doi.org/10.1039/d1lc00131k

Su, X., Rakshit, M., Das, P., Gupta, I., Das, D., Pramanik, M., Ng, K. W., & Kwan, J. (2021). Ultrasonic Implantation and Imaging of Sound-Sensitive Theranostic Agents for the Treatment of Arterial Inflammation. ACS applied materials & interfaces, 13(21), 24422–24430. https://doi.org/10.1021/acsami.1c01161

Sui, B., Gao, P., Lin, Y., Gao, B., Liu, L., & An, J. (2008). Blood flow pattern and wall shear stress in the internal carotid arteries of healthy subjects. Acta radiologica (Stockholm, Sweden : 1987), 49(7), 806–814. https://doi.org/10.1080/02841850802068624.

Truskey G. A. (2016). Advancing cardiovascular tissue engineering. F1000Research, 5, F1000 Faculty Rev-1045. https://doi.org/10.12688/f1000research.8237.1

Vaidyanathan, K., Wang, C., Krajnik, A., Yu, Y., Choi, M., Lin, B., Jang, J., Heo, S. J., Kolega, J., Lee, K., & Bae, Y. (2021). A machine learning pipeline revealing heterogeneous responses to drug perturbations on vascular smooth muscle cell spheroid morphology and formation. Scientific reports, 11(1), 23285. https://doi.org/10.1038/s41598-021-02683-4

Vedder, V. L., Aherrahrou, Z., & Erdmann, J. (2020). Dare to Compare. Development of Atherosclerotic Lesions in Human, Mouse, and Zebrafish. Frontiers in cardiovascular medicine, 7, 109. https://doi.org/10.3389/fcvm.2020.00109

Vincent, M. P., Navidzadeh, J. O., Bobbala, S., & Scott, E. A. (2022). Leveraging self-assembled nanobiomaterials for improved cancer immunotherapy. Cancer cell, 40(3), 255–276. https://doi.org/10.1016/j.ccell.2022.01.006

Yang, F., Xue, J., Wang, G., & Diao, Q. (2022). Nanoparticle-based drug delivery systems for the treatment of cardiovascular diseases. Frontiers in pharmacology, 13, 999404. https://doi.org/10.3389/fphar.2022.999404

Yang, L., Zang, G., Li, J., Li, X., Li, Y., & Zhao, Y. (2020). Cell-derived biomimetic nanoparticles as a novel drug delivery system for atherosclerosis: predecessors and perspectives. Regenerative biomaterials, 7(4), 349–358. https://doi.org/10.1093/rb/rbaa019

Yurtseven, E., Ural, D., Baysal, K., & Tokgözoğlu, L. (2020). An Update on the Role of PCSK9 in Atherosclerosis. Journal of atherosclerosis and thrombosis, 27(9), 909–918. https://doi.org/10.5551/jat.55400

Zakiev, E. R., Nikiforov, N. G., & Orekhov, A. N. (2017). Cell-Based Models for Development of Antiatherosclerotic Therapies. BioMed research international, 2017, 5198723. https://doi.org/10.1155/2017/5198723

Zhang, X., Bishawi, M., Zhang, G., Prasad, V., Salmon, E., Breithaupt, J. J., Zhang, Q., & Truskey, G. A. (2020). Modeling early stage atherosclerosis in a primary human vascular microphysiological system. Nature communications, 11(1), 5426. https://doi.org/10.1038/s41467-020-19197-8

Zhang, X., Huang, F., Li, W., Dang, J. L., Yuan, J., Wang, J., Zeng, D. L., Sun, C. X., Liu, Y. Y., Ao, Q., Tan, H., Su, W., Qian, X., Olsen, N., & Zheng, S. G. (2018). Human Gingiva-Derived Mesenchymal Stem Cells Modulate Monocytes/Macrophages and Alleviate Atherosclerosis. Frontiers in immunology, 9, 878. https://doi.org/10.3389/fimmu.2018.00878

Zhang, Y., Fatima, M., Hou, S., Bai, L., Zhao, S., & Liu, E. (2021). Research methods for animal models of atherosclerosis (Review). Molecular medicine reports, 24(6), 871. https://doi.org/10.3892/mmr.2021.12511

Zhou, M., Yu, Y., Chen, R., Liu, X., Hu, Y., Ma, Z., Gao, L., Jian, W., & Wang, L. (2023). Wall shear stress and its role in atherosclerosis. Frontiers in cardiovascular medicine, 10, 1083547. https://doi.org/10.3389/fcvm.2023.1083547