# Research Progress on the Role of Endothelial Cell Injury and Its Dysfunction in Atherosclerosis: Postprint

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## Abstract

Cardiovascular diseases are common disorders with high morbidity and mortality. Atherosclerosis (AS) constitutes the pathological basis of multiple cardiovascular diseases, characterized primarily by lipid accumulation and plaque formation in arterial walls, leading to ischemia or necrosis in surrounding tissues or organs. This article systematically and comprehensively discusses the role of endothelial cells (ECs) in AS, summarizing the mechanisms of their injury and dysfunction, as well as their interactions with macrophages and vascular smooth muscle cells in AS. This article demonstrates that ECs play a crucial role in AS, and that alleviating their injury and dysfunction helps to mitigate the onset and progression of AS, thereby offering new therapeutic strategies for AS.

## Full Text

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### Abstract

Cardiovascular disease is a prevalent condition characterized by high incidence and mortality rates. Atherosclerosis (AS) serves as the underlying pathological

mechanism for numerous cardiovascular diseases, primarily marked by lipid accumulation and plaque formation within arterial walls that can lead to tissue or organ ischemia and necrosis. This comprehensive review systematically examines the pivotal role of endothelial cells (ECs) in atherosclerosis, elucidating the mechanisms of EC injury and dysfunction as well as their interactions with macrophages and vascular smooth muscle cells (VSMCs) during disease progression. Our findings underscore the critical involvement of ECs in AS pathogenesis and demonstrate that mitigating EC damage and preserving endothelial function can potentially ameliorate the onset and progression of AS. This work aims to provide novel therapeutic avenues for AS treatment.

**Keywords:** Atherosclerosis; Endothelial cells; Dysfunction; Energy metabolism

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## 1. Literature Search Strategy

We conducted a computerized search of the PubMed database from inception to August 2024 using English search terms including "atherosclerosis," "human umbilical vein endothelial cell (HUVEC)," "endothelial cell," and "energy metabolism." Inclusion criteria comprised literature addressing the impact of vascular endothelial cells on AS, mechanisms of endothelial cell injury and dysfunction, and interaction mechanisms between endothelial cells and macrophages/vascular smooth muscle cells. Exclusion criteria eliminated studies unrelated to the topic, those of poor quality, or articles where full text was unavailable. Ultimately, 81 articles were included in this review.

## 2. EC Function and Energy Metabolism

Endothelial cells are specialized epithelial cells that synthesize and secrete various bioactive substances essential for maintaining vascular health and normal function, playing a crucial physiological role in preserving metabolic homeostasis and vascular integrity. During tissue injury and inflammatory stress, activated ECs present adhesion factors on their surface and release chemokines such as vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and E-selectin to facilitate leukocyte recruitment, adhesion, and transendothelial migration. Recent research has revealed that EC function is regulated not only by vascular endothelial growth factor (VEGF) and other signaling pathways but also by endothelial energy metabolism.

ECs primarily rely on glycolysis rather than oxidative phosphorylation (OXPHOS) for energy production, a mechanism known as aerobic glycolysis or the Warburg effect. When glucose is abundant, glycolysis can synthesize ATP more rapidly while generating less reactive oxygen species (ROS), maximizing oxygen transfer to perivascular cells and adapting to hypoxic environments. Additionally, various glycolytic regulators such as Krüppel-like factor 2 (KLF2) participate in the functional regulation of ECs.

Fatty acid (FA) metabolism is also crucial for ECs, as lipid absorption in almost all tissues requires transport across the endothelial barrier. Fatty acid synthase (FASN), a key enzyme in FA synthesis, can modulate endothelial nitric oxide synthase (eNOS) bioavailability to produce nitric oxide (NO). ECs deficient in adipose triglyceride lipase (ATGL) exhibit neutral lipid accumulation in blood vessels, reduced vascular tone, and decreased NO synthesis, leading to endothelial dysfunction. In AS, ECs induce inflammatory responses through activation of the nuclear factor B (NF- B) pathway, while trimetazidine attenuates inflammation by inhibiting fatty acid oxidation (FAO) and reducing NOD-like receptor thermal protein domain associated protein 3 (NLRP3) activation.

Beyond glucose and FA metabolism, amino acid metabolism also holds significant importance for ECs. In ECs, eNOS converts arginine to citrulline and NO through multiple cofactors to maintain normal vascular homeostasis. However, in AS, arginine becomes rate-limiting. When the availability of arginine and its cofactor tetrahydrobiopterin (BH4) decreases, eNOS generates ROS instead of NO and citrulline—a process termed eNOS uncoupling that represents a key factor in AS pathogenesis. Reduced eNOS activity and increased uncoupling disrupt the balance between NO and superoxide, triggering AS development. Therefore, given the role of cellular metabolism in regulating EC function, deepening our understanding of EC energy metabolism mechanisms and their alterations during AS initiation and progression may open new avenues for developing preventive therapeutics.

## 3. Impact of EC Dysfunction on AS

The development of AS can be divided into several stages: lipid deposition, inflammatory response, foam cell formation, VSMC proliferation, and fibrous cap formation. In AS pathogenesis, ECs are considered the first cells affected by various factors (oxidative stress, mechanical stress, aging, etc.) that alter their structure and function.

### 3.1 Effects of Oxidative Stress and Inflammatory Response on ECs

Oxidative stress refers to an imbalance between oxidation and antioxidant capacity leading to excessive free radical accumulation. EC oxidative stress and inflammatory response represent critical risk factors in AS initiation and progression. During AS development, stimulated ECs release inflammatory factors and reactive oxygen species that react with proteins, lipids, and DNA in ECs, causing cellular injury and dysfunction that drive AS pathogenesis. Consequently, alleviating EC oxidative stress and inflammation has become a major research focus. For example, 3' -sialyllactose inhibits LPS-induced endothelial hyperpermeability by suppressing superoxide activity, while the SIRT3-SOD2-mtROS pathway and Nrf2 pathway improve mitochondrial dysfunction and lipid peroxidation to suppress inflammatory responses. PCSK9 and Nogo-B modulate EC oxidative stress and inflammation through the NF- B pathway, and simvastatin and Bio-LN/SPMs (a novel targeted nanomedicine) can resolve inflammation in

AS. In AS, elevated ROS levels in ECs originate not only from eNOS uncoupling but also from NADPH oxidase (NOX enzymes), which serve as an important ROS source. NOX-derived ROS also function as signaling molecules affecting redox-sensitive key signaling factors such as NF- B, hypoxia-inducible factor 1$\alpha$, and p53, thereby regulating adhesion factor release and vascular permeability. Pro-inflammatory cytokines, growth factors, hypoxia, high glucose, high FA levels, and shear stress can all induce NOX enzyme expression. Currently, NOX1/NOX2 antagonists have been developed for cardiovascular diseases; for instance, Rab27a reduces EC oxidative stress by inhibiting NOX2 and caspase-3 expression, while ginsenosides reduce oxidative stress and inflammation by disrupting NOX2 complex assembly. These findings demonstrate that mitigating EC oxidative stress and inflammation is crucial for AS treatment.

**3.2.1 EC Apoptosis** Apoptosis is a form of programmed cell death characterized by energy-dependent biochemical changes and distinct morphological alterations, regulated by the intrinsic mitochondrial-dependent pathway controlled by the BCL2 protein family or by external factors activating death receptors. EC apoptosis represents a fundamental aspect of AS pathophysiology. Current research has identified that the CD137 signaling pathway promotes EC apoptosis through pro-oxidative and pro-inflammatory mechanisms mediated by Nrf2 and NF- B pathways, while insulin receptor substrate 1 and transmembrane protein 215 (TMEM215) regulate EC apoptosis through mitochondrial oxidative stress and endoplasmic reticulum stress. Recently, microRNAs (miRNAs —single-stranded non-coding RNAs that reduce protein expression by regulating mRNA) have emerged as novel apoptosis regulators. Studies show that miR-106a-5p downregulation alleviates ox-LDL-induced EC injury by targeting STAT3, while miR-122 promotes EC apoptosis by targeting XIAP (an endogenous mammalian caspase inhibitor), thereby exacerbating AS. These findings illustrate that EC apoptosis in AS arises through multiple pathways, and reducing EC apoptosis holds significant therapeutic importance.

**3.2.2 EC Pyroptosis** Pyroptosis is a newly recognized form of programmed cell death characterized by cellular swelling, plasma membrane bubbling, and intense release of pro-inflammatory cytokines induced by multiple factors. This process involves several biological mechanisms and can be classified into canonical and non-canonical pathways based on caspase-1 activation requirement. In AS, EC pyroptosis reduces plaque stability and triggers cardiovascular events, with the ROS (or mtROS)-NLRP3-caspase-1 pathway potentially playing an important role. Recent studies have identified novel pyroptosis regulatory pathways beyond caspase-1, such as caspase-3 and caspase-8 mediating apoptosis by cleaving gasdermin family proteins (GSDME and GSDMD). Circ-USP9X interacts with EIF4A3 to promote EC pyroptosis by regulating GSDMD stability in AS, while IQ motif-containing GTPase-activating protein 1 (IQGAP1) induces EC pyroptosis through the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway, leading to AS. Conversely, Rnd3 suppresses

EC pyroptosis in AS by regulating ubiquitination of TRAF6. miRNAs also regulate EC pyroptosis, including miR-635, miR-302-3p, and miR-455-5p. Current evidence suggests that further investigation is needed into the non-canonical pyroptosis pathway in AS.

**3.2.3 EC Ferroptosis and Cuproptosis** Recent research has identified ferroptosis and cuproptosis as novel cell death modalities. Iron and copper are essential trace elements in cells, but their elevated levels cause ROS accumulation and trigger programmed cell death. Ferroptosis is characterized by lipid peroxidation and DNA fragmentation, while cuproptosis occurs through oxidative stress responses, lysyl oxidase construction, and regulation of EC injury via vascular endothelial growth factor binding. Growing evidence demonstrates that EC ferroptosis causes direct EC damage and impairs normal EC function during AS development. For instance, adrenomedullin alleviates AS by inhibiting AMPK-mediated EC ferroptosis, while GLXB and melatonin suppress AS by activating the Nrf2 pathway to inhibit ferroptosis. High-dose ionizing radiation exacerbates AS by regulating p38/nuclear receptor coactivator 4 (NCOA4)-mediated EC ferroptosis. N-acetylneuraminic acid triggers solute carrier family 3 member 2 (SLC3A2) degradation, increasing EC ferroptosis and aggravating AS. Ferroptosis and cuproptosis are closely linked to other programmed death pathways, and understanding the underlying mechanisms connecting these cell death modalities may enable development of new drugs that simultaneously target multiple pathways for effective AS treatment.

**3.3 Effects of Senescence on ECs** Senescent cells can alter the cellular microenvironment and generate chronic inflammatory responses that cause tissue damage. Senescent ECs characterized by flattened morphology, increased size, polyploidy, reduced NO bioavailability, and secretion of numerous pro-inflammatory cytokines play a critical role in cardiovascular disease. SIRT1, SIRT6, angiotensin, insulin-like growth factor-binding protein (IGFBP), mTOR, and p53 are important in age-related vascular remodeling. For example, Compound Danshen Dripping Pill reduces EC senescence through SIRT1 activation, PM2.5 induces premature EC senescence via the SIRT1/peroxisome proliferator-activated receptor $\gamma$ coactivator $1\alpha$ (PGC-$1\alpha$)/SIRT3 signaling pathway, YPEL2 regulates EC senescence and AS through the p53/p21 pathway, and sodium-glucose cotransporter 2 (SGLT2) inhibitors and cilostazol can delay vascular aging by alleviating EC inflammation and mitochondrial dysfunction to regulate EC senescence. Since ECs are in direct contact with blood, EC senescence can also be triggered by mechanical stimuli; for instance, disturbed blood flow causes DNA damage, telomere dysfunction, and excessive ROS production in ECs. These findings highlight the critical importance of alleviating vascular aging to reduce AS incidence.

**3.4 Effects of Shear Stress on ECs** At arterial branch points and curvatures, blood flow transitions from stable laminar flow to oscillatory disturbed

flow, generating small but continuous shear stress on ECs. Similar to how flow patterns regulate EC function through MerTK-mediated efferocytosis, plaques on vascular walls increase shear stress on the intima, stimulating ECs to trigger intracellular signaling changes that increase vascular permeability. This facilitates the infiltration of cholesterol-rich lipoprotein particles into the intima, forming arterial wall lipid deposition, while leukocytes in blood are recruited and adhere to ECs in low-flow regions, further exacerbating AS progression. Under different shear stress conditions, altered function of EC mechanosensory receptors disrupts EC Ca2+ homeostasis, causing rapid Ca2+ influx into the cytoplasm and activating eNOS, which induces NO production and causes endothelial dysfunction and AS. The magnitude and duration of shear stress have distinct effects on vascular endothelial regulation: laminar flow induces KLF2/4 expression in ECs exposed to interferon-$\gamma$ (IFN-$\gamma$), inhibiting EC proliferation and protecting against AS, whereas disturbed flow induces ROS production, stimulates EC proliferation, and creates a pro-atherosclerotic environment. Although EC injury from shear stress is well recognized, strategies to mitigate this damage require further investigation.

## 4. ECs Influence AS by Regulating Macrophages and Smooth Muscle Cells

The pathogenesis of AS involves complex interactions among multiple cell types and inflammatory mediators, including ECs, monocyte-macrophages, VSMCs, and other immune cells, with ECs playing a crucial role. In early AS, when vascular walls are stimulated, ECs secrete various growth factors and cytokines that increase EC permeability and alter subendothelial extracellular matrix composition, causing monocyte-macrophage infiltration and aggregation that promote AS development. For example, trimethylamine N-oxide (TMAO) and EC GATA-binding factor 6 (GATA6) trigger EC recruitment of leukocytes through endoplasmic reticulum/mitochondrial stress and cytidine/uridine monophosphate kinase 2 (CMPK2)-NLRP3 pathways, leading to AS, while metformin alleviates AS by interrupting macrophage infiltration and reducing pro-inflammatory cytokine production. Conversely, monocyte-macrophages that aggregate and infiltrate diseased vascular walls engulf lipid particles via scavenger receptors to form foam cells, secreting various growth factors and cytokines such as tumor necrosis factor-$\alpha$ (TNF-$\alpha$) and interleukin-1$\beta$ (IL-1$\beta$) that further aggravate EC injury and dysfunction, creating a vicious cycle. For instance, TNF-$\alpha$-stimulated ECs release exosomes that polarize macrophages toward the M1 phenotype, while histone deacetylase 3 (HDAC3) regulates HUVEC inflammatory responses by modulating inflammatory cytokine expression and monocyte adhesion. Therefore, understanding EC-monocyte-macrophage interactions is crucial for elucidating AS pathogenesis and developing therapeutic strategies, as demonstrated by amitriptyline and capmatinib inhibiting inflammation by blocking EC-monocyte interactions.

**4.2 ECs Influence AS Development by Regulating VSMC Proliferation and Migration** During AS pathogenesis, EC-VSMC interactions promote plaque formation and progression. When ECs are injured, they synthesize and secrete various bioactive substances such as growth factors, inflammatory factors, and adhesion molecules that attract leukocytes and VSMCs to migrate into the intima and promote plaque formation, exacerbating AS. For example, EC-derived Dickkopf-1 (DKK-1) promotes VSMC foam cell formation and AS development through CYP4A11/sterol regulatory element-binding protein 2 (SREBP2)/ATP-binding cassette transporter A1 (ABCA1) and ubiquitin-specific peptidase 53 (USP53)-mediated SR-A deubiquitination. Simultaneously, VSMCs can release various growth factors and cytokines that affect EC survival and function, further promoting AS development.

Targeting EC-VSMC interactions offers therapeutic strategies to inhibit AS progression. First, reducing VSMC proliferation and migration by inhibiting EC inflammatory responses and growth factor release represents one approach: EC CD137 signaling activation attenuates EC exosome release, inhibiting VSMC phenotypic switching and neointima formation, while Compound Danshen Dripping Pill modulates EC-VSMC interactions through the DKK-1/low-density lipoprotein receptor-related protein 6 (LRP6)/$\beta$-catenin signaling pathway to reduce vascular calcification. Second, inhibiting VSMC abnormal proliferation and functional abnormalities to improve EC function and survival may also represent an effective therapeutic strategy for preventing and treating AS.

## 5. Summary and Outlook

As essential components of the vascular system, ECs serve a critical barrier function. EC injury and dysfunction persist throughout AS pathogenesis through interconnected and mutually influencing mechanisms. With advancing scientific technology and deepening investigation into EC mechanisms, our understanding of the crucial role of ECs in AS initiation and progression has expanded considerably. To delay or reverse AS progression, researchers can explore lifestyle modifications and pharmacological interventions to reduce EC injury. First, correcting unhealthy habits such as smoking, alcohol consumption, hypertension, and hypercholesterolemia can protect ECs. Second, although some drugs for protecting and improving EC function—such as antioxidants and anti-inflammatory agents—are under development, more precise targeting strategies with fewer side effects should be pursued. In conclusion, understanding the pathogenic mechanisms underlying EC activation and endothelial dysfunction to further unravel the mysteries of EC biology will provide additional insights and approaches for AS treatment and offer critical information for identifying therapeutic targets in cardiovascular disease.

**Figure 1** [Figure 1: see original paper] Endothelial cells interact with monocyte-macrophages and vascular smooth muscle cells

**Author Contributions:** GAO Haijun was responsible for conceptualization,

literature collection, and manuscript writing; REN Jiayu, WANG Ruolin, and ZHOU Huiya edited and organized the tables; QU Peng revised the manuscript, provided quality control and final approval, and supervised the overall project.

**Conflict of Interest:** The authors declare no conflicts of interest.

*Note: Figure translations are in progress. See original paper for figures.*