

Review

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Endothelial dysfunction in atherosclerosis: from classical pathways to emerging mechanisms

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Abstract

Endothelial dysfunction is a pivotal factor in the pathogenesis of atherosclerosis, driving plaque formation, inflammation, and thrombosis. This review synthesizes classical mechanisms and emerging perspectives on endothelial dysfunction, emphasizing its role in atherogenesis. Traditional contributors, including lifestyle factors, lipid dysregulation, shear stress, and nitric oxide (NO) deficiency, are discussed alongside novel insights from single-cell RNA sequencing, metabolomics, and intercellular communication. scRNA-seq has unveiled endothelial cell (EC) heterogeneity and endothelial-to-mesenchymal transition (EndMT) as critical contributors to plaque instability. Metabolites such as trimethylamine N-oxide (TMAO) and homocysteine derivatives exacerbate endothelial injury, while gut microbiome interactions further modulate disease progression. Exosome-mediated



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cross-talk between ECs, immune cells, and vascular smooth muscle cells (VSMCs) highlights new pathways in vascular inflammation and remodeling. Current pharmacotherapies, such as lipid-lowering and anti-inflammatory drugs, improve endothelial function, and emerging strategies like nanotechnology and exosome-based therapies show promise as well. Integrating classical and novel approaches could enhance our understanding of endothelial biology and lead to targeted therapies, addressing atherosclerosis-related diseases.

Keywords: Endothelial dysfunction, atherosclerosis, nitric oxide, shear stress, single-cell RNA sequencing, exosomes, metabolites

INTRODUCTION

Atherosclerosis is a prevalent chronic inflammatory condition of arterial walls, significantly contributing to global cardiovascular morbidity and mortality^[1]. It impedes blood circulation, potentially leading to thrombus formation, which may result in heart attacks, ischemic strokes, or peripheral artery disease (PAD)^[2,3]. The prevalence of coronary artery disease among U.S. adults over 20 years old is 7.1%, with myocardial infarction at 3.2%^[4]. These conditions persist as primary global health threats. Atherosclerosis, initially influenced by lipoproteins, incites plaque development via intimal inflammation, necrosis, fibrosis, and calcification^[3]. Over time, such plaques can induce arterial stenosis, leading to ischemic conditions, particularly in coronary, carotid, or lower limb arteries. Additionally, plaques may precipitate acute thrombosis, often due to rupture in thin-cap fibroatheromas with substantial necrotic cores, or thrombus formation from plaque erosion in pathological intimal thickening or fibroatheromas^[3,5].

Endothelial dysfunction plays a critical role in the pathogenesis of atherosclerosis^[6], and its role extends to related cardiovascular conditions, including myocardial ischemia-reperfusion injury and impaired microvascular flow^[7,8]. Atherosclerosis originates from the subendothelial region^[1]. The association between endothelial injury and atherosclerosis was initially suggested by Ross and Glomset in 1976. They introduced the “response-to-injury” hypothesis, positing that endothelial injury initiates atherogenesis^[9]. This foundational theory continues to inform subsequent research^[1]. By the mid-1980s, endothelial dysfunction characterized by impaired vasodilation became linked to atherosclerosis^[10-12]. A significant breakthrough was the identification of endothelium-derived nitric oxide (NO), establishing the endothelium as a paracrine regulator of vasodilation and thromboprotection^[13].

The endothelium functions as an organ interacting with blood flow and pressure, subject to varying flow patterns throughout the cardiac cycle. A defining characteristic of atherosclerosis is its preferential plaque formation at sites of altered hemodynamics, such as arterial branch points, rather than a uniform distribution^[14]. This focal vulnerability correlates with endothelial responses to mechanical forces. The endothelium also regulates vascular homeostasis, affecting processes including vasodilation, inflammation, and coagulation. Endothelial dysfunction is characterized by impaired vascular tone and homeostasis, often indicated by reduced NO production, increased oxidative stress, and elevated inflammatory responses^[15]. Compromised endothelial integrity results in lipoprotein retention, leukocyte adhesion, and smooth muscle cell proliferation, all contributing to plaque formation^[16]. Endothelial inflammation promotes thrombosis, which is a key factor in adverse cardiovascular outcomes, while thrombosis, in turn, exacerbates endothelial inflammation^[17]. Furthermore, clinical studies have established endothelial vasodilator dysfunction as a critical marker of atherosclerotic coronary artery disease, corroborated by animal models^[18-20]. Moreover, endothelial dysfunction exacerbates myocardial ischemia-reperfusion injury by impairing microvascular perfusion and amplifying oxidative and inflammatory damage^[7,21].

In addition to traditional mechanisms, recent studies utilizing cutting-edge techniques have uncovered new insights, marking a new era in the study of endothelial dysfunction in atherosclerosis. Single-cell RNA sequencing (scRNA-seq) has revealed important details about the cellular transitions of endothelial cells and their distinct roles in atherogenesis^[22]. Metabolites, once considered terminal byproducts, have been found to play crucial roles in regulating cell functions, with many gut microbes contributing to this process^[23]. Moreover, intercellular signaling - beyond intracellular changes - has been shown to play a critical role, leading to numerous novel findings^[24]. These emerging perspectives open new avenues for understanding mechanisms and potential therapies.

In this review, we summarize both classical mechanisms and recent discoveries, with a focus on novel perspectives such as cellular heterogeneity, the roles of metabolites [Figure 1], and intercellular communication [Figure 2]. This review provides a comprehensive overview of endothelial dysfunction in atherosclerosis and offers insights into future research directions.

CAUSES OF ENDOTHELIAL DYSFUNCTION

Lifestyle and environmental risk factors

Lifestyle and environmental factors play a significant role in the development of endothelial dysfunction, a key contributor to atherogenesis. Modifiable risk factors, such as poor diet, physical inactivity, smoking, and exposure to environmental pollutants, can accelerate the progression of atherosclerosis. Understanding the impact of these factors is essential for developing effective strategies to prevent and treat cardiovascular disease.

Lifestyle determinants significantly contribute to the pathophysiology of endothelial dysfunction. Tobacco use represents a major modifiable risk factor that diminishes NO bioavailability while elevating the expression of adhesion molecules, consequently resulting in endothelial dysfunction and fostering a proinflammatory milieu^[25]. Diets characterized by high cholesterol and saturated fat content elevate low-density lipoprotein (LDL) cholesterol levels, which may compromise endothelial function through the reduction of NO bioavailability. Furthermore, excessive consumption of saturated fats is associated with increased levels of oxidized LDL (oxLDL), which directly inflict damage upon endothelial cells^[26,27]. The presence of trans fats further exacerbates the likelihood of thrombosis by facilitating endothelial dysfunction and inflammation^[28,29]. Conversely, foods abundant in antioxidants, such as fruits and vegetables, mitigate the risk of endothelial dysfunction by alleviating oxidative stress and augmenting NO production^[26,27]. Engagement in regular physical exercise has been demonstrated to enhance endothelial function by promoting NO production, reducing oxidative stress, and stabilizing atherosclerotic plaques^[30,31].

Certain additional environmental determinants also play a significant role in the manifestation of endothelial dysfunction. Atmospheric contamination induces oxidative stress and inflammatory responses, thereby aggravating endothelial dysfunction and facilitating atherogenic transformations^[32]. Moreover, psychosocial stressors^[33,34] and auditory pollution^[35,36] are correlated with heightened inflammatory activity and endothelial dysfunction, thereby contributing to the advancement of coronary artery disease.

While both lifestyle and environmental determinants significantly influence the advancement of endothelial dysfunction, it is crucial to evaluate the possibility of reversibility associated with this condition. Endothelial dysfunction should not be regarded as an immutable condition, as it can be ameliorated through specific interventions and modifications in lifestyle. Adjustments in lifestyle, such as the cessation of tobacco use, alterations in dietary habits, and augmentation of physical activity, have been empirically demonstrated to enhance endothelial function and mitigate cardiovascular risk^[37].

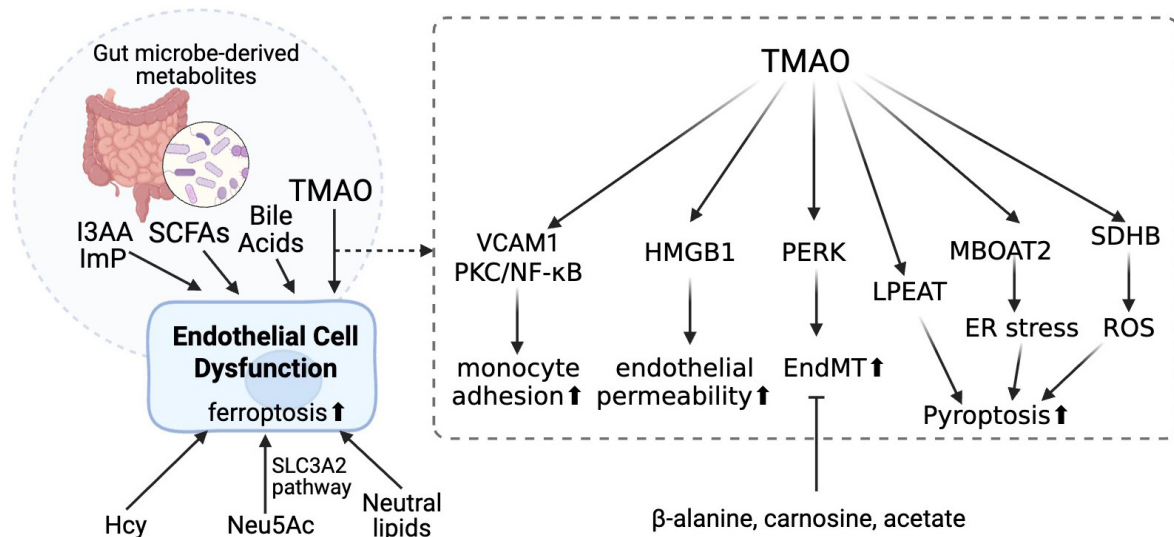


Figure 1. Gut microbiota-derived metabolites contribute to endothelial cell dysfunction through multiple pathways. Several metabolites produced by gut microbiota, including indole-3-acetic acid (I3AA), indolepropionic acid (Imp), short-chain fatty acids (SCFAs), bile acids, and trimethylamine N-oxide (TMAO), influence vascular endothelial function. These metabolites can induce endothelial cell dysfunction by promoting ferroptosis and altering cellular homeostasis. TMAO, in particular, activates various signaling pathways involving vascular cell adhesion molecule 1 (VCAM1), protein kinase C (PKC), nuclear factor kappa B (NF-κB), high mobility group box 1 (HMGB1), protein kinase RNA-like endoplasmic reticulum kinase (PERK), lysophosphatidylethanolamine acyltransferase (LPEAT), membrane-bound O-acyltransferase domain-containing 2 (MBOAT2), and succinate dehydrogenase complex iron sulfur subunit B (SDHB). These pathways contribute to increased monocyte adhesion, enhanced endothelial permeability, endothelial-to-mesenchymal transition (EndMT), endoplasmic reticulum (ER) stress, reactive oxygen species (ROS) production, and pyroptosis. Additionally, circulating factors such as homocysteine (Hcy), N-acetylneuraminic acid (Neu5Ac), and neutral lipids exacerbate endothelial dysfunction, partly through the solute carrier family 3 member 2 (SLC3A2) transport pathway. Conversely, protective metabolites including β-alanine, carnosine, and acetate may attenuate TMAO-induced endothelial injury. Created using <https://BioRender.com>.

Lipid products

Lipoproteins exhibit dual functions concerning the health of endothelial cells. Modified LDL and triglyceride-enriched lipoproteins induce morphological alterations in endothelial cells, manifesting as a diminished glycocalyx and heightened endoplasmic reticulum stress. These transformations, instigated by the interplay between modified lipoproteins and endothelial cells, compromise intercellular junction integrity and increase endothelial permeability^[38]. Arterial injury alters these lipoproteins, facilitating their infiltration into the subendothelial compartment^[1]. This accumulation incites monocyte recruitment and foam cell generation, thereby exacerbating endothelial dysfunction and the progression of atherosclerosis^[39].

Variations in palmitic acid concentrations also increase oxidative stress and inflammation in endothelial cells^[40]. This is evidenced by elevated levels of reactive oxygen species (ROS), primarily generated by mitochondria, Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase, endothelial Nitric Oxide Synthase (eNOS) uncoupling, and xanthine oxidase^[41]. Additionally, the upregulation of inflammatory genes and adhesion molecules, such as IL-6, MCP-1, ICAM, and VCAM, further impairs endothelial function^[40].

While lipid derivatives play a contributory role in endothelial dysfunction associated with atherosclerosis, high-density lipoprotein (HDL) exerts a protective influence by facilitating cholesterol efflux and mitigating oxidative stress under physiological conditions^[42]. Conversely, under pathological circumstances, HDL becomes dysfunctional, relinquishing its protective attributes and contributing to endothelial impairment^[43,44]. Dysfunctional HDL is ineffectual in inhibiting the formation of oxidized lipids, thereby

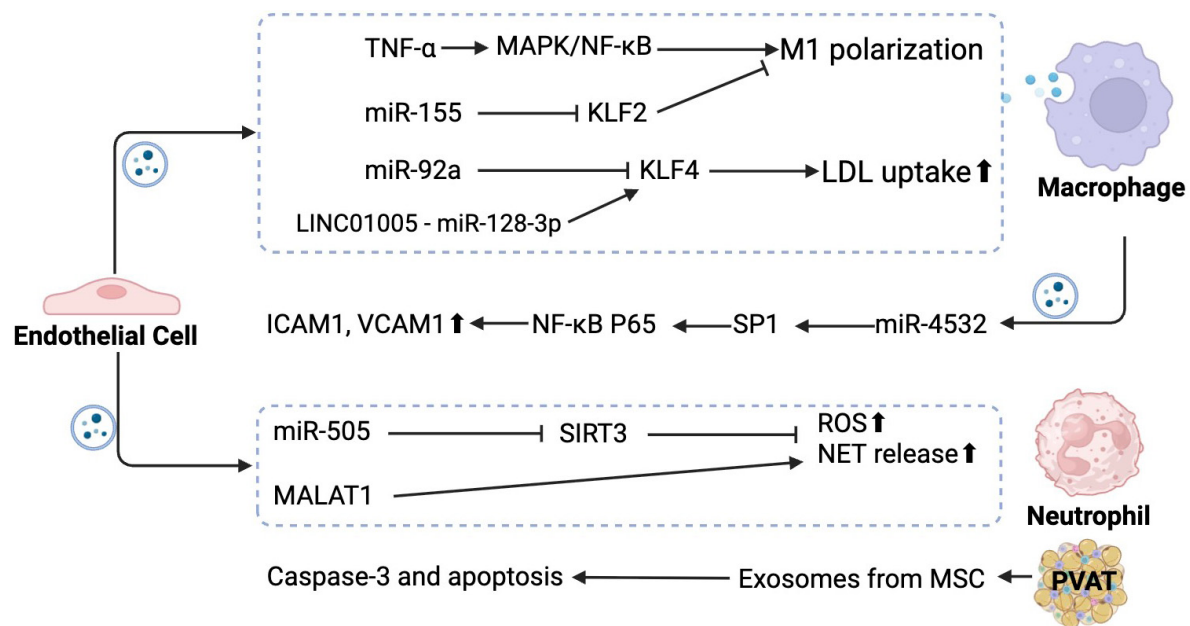


Figure 2. Endothelial-derived and incoming exosomes mediate vascular inflammation and immune cell activation in atherosclerosis. Exosomes released by endothelial cells influence the function of multiple immune and vascular cells. Endothelial cells secrete exosomes that activate the mitogen-activated protein kinase/nuclear factor kappa B (MAPK/NF- κ B) pathway, promoting proinflammatory M1 polarization in macrophages. Exosomal miR-155 inhibits Kruppel-like factor 2 (KLF2), and miR-92a suppresses Kruppel-like factor 4 (KLF4), enhancing low-density lipoprotein (LDL) uptake. The long non-coding RNA LINC01005 acts as a sponge for miR-128-3p, indirectly increasing KLF4 expression and contributing to atherosclerosis progression. Exosomes derived from endothelial cells also modulate neutrophil activation. miR-505 inhibits sirtuin 3 (SIRT3), leading to elevated reactive oxygen species (ROS) and enhanced neutrophil extracellular trap (NET) release. The long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), delivered by endothelial exosomes, also promotes NET formation. In the reverse direction, macrophage-derived exosomal miR-4532 targets specificity protein 1 (SP1) in endothelial cells, activating NF- κ B subunit p65 and upregulating intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1), thereby promoting leukocyte adhesion. Exosomes from neutrophils and perivascular adipose tissue (PVAT) exacerbate endothelial injury. In contrast, mesenchymal stem cell (MSC)-derived exosomes protect endothelial cells by inhibiting caspase-3-mediated apoptosis, reducing oxidative stress, and restoring redox balance. Created using <https://BioRender.com>.

exacerbating oxidative stress and endothelial dysfunction^[45].

Shear stress

Endothelial cells (ECs) are perpetually subjected to hemodynamic forces resultant from blood flow, which influences their functionality across various dimensions. Unidirectional, laminar shear stress is widely regarded as a protective factor against the onset of atherosclerosis^[46]. Conversely, the development of atherosclerosis is more prevalent in regions characterized by diminished wall shear stress or pulsatile disturbed shear stress, typically observed at arterial bifurcations and irregularities of the vessel wall. Such conditions facilitate the accumulation of LDL and the infiltration of inflammatory cells into the arterial wall^[47]. Furthermore, these conditions inhibit the expression of the anti-inflammatory, atheroprotective transcription factor KLF2 ^[48], which plays a crucial role in the development and progression of atherosclerotic plaques^[49].

Shear stress is detected by mechanosensors, including integrins (e.g., $\alpha 5$ -annexin A2), CD31/PECAM-1, VE-cadherin, and VEGFR2^[46,50], with PECAM-1 playing a pivotal role. The tension induced by flow on PECAM-1 initiates an upstream signaling cascade that connects PECAM-1 to the vimentin cytoskeleton, thereby transmitting force from myosin. This activation triggers the engagement of Src family kinases,

resulting in ligand-independent transactivation of VEGF receptors, activation of PI3K, stimulation of eNOS, and subsequent production of nitric oxide, ultimately resulting in vasodilation^[51-53].

Disturbed shear stress diminishes the expression of protective genes, such as Aff3ir-ORF2, which possesses anti-inflammatory and anti-atherosclerotic properties. The lack of Aff3ir-ORF2 exacerbates inflammatory responses governed by interferon regulatory factor 5 (IRF5), fostering endothelial activation and promoting atherosclerosis^[54]. Additionally, disturbed shear stress facilitates the degradation of MAPK6, an anti-atherosclerotic protein, via the ubiquitin ligase TRIM21. This degradation escalates endothelial inflammation through the EGR1/CXCL12 axis, thus contributing to plaque formation^[55]. Disturbed flow upregulates pro-atherosclerotic genes and proteins, whereas laminar flow and elevated shear stress generally confer a protective effect. Low shear stress induces pyroptosis in endothelial cells through the IKK ϵ /STAT1/NLRP3 signaling pathway, further exacerbating the progression of atherosclerosis^[56].

Nonetheless, certain exceptions are noted. Disturbed flow can also activate anti-inflammatory feedback mechanisms, exemplified by the endothelial adrenomedullin-calcitonin-like receptor (CALCRL), which transmits signals via cAMP to mitigate endothelial inflammation and lesion development^[57]. Although high shear stress is conventionally perceived as protective, it may also contribute to the formation of vulnerable plaques through mechanisms such as angiogenesis, thereby complicating the assertion that only low shear stress is detrimental^[58].

DYSREGULATION OF ENDOTHELIAL NITRIC OXIDE IN ATHEROGENESIS

In 1980, it was demonstrated that acetylcholine-induced vasodilation necessitated an intact endothelium and was mediated by a key humoral factor, later identified as endothelium-derived relaxing factor (EDRF). Due to the similarities in both pharmacological properties^[59,60] and biological effects^[61,62] between EDRF and NO, EDRF was ultimately acknowledged as NO. NO is a highly unstable molecule, with a half-life of only a few seconds in oxygenated physiological environments^[63]. It exerts its physiological effects by activating soluble guanylate cyclase (sGC), which elevates intracellular levels of cyclic guanosine monophosphate (cGMP) and triggers a protein kinase G (PKG) signaling cascade in vascular smooth muscle cells^[64].

Synthesis of NO in endothelial cells

Endothelial cells synthesize NO through the metabolic conversion of L-arginine mediated by the eNOS^[65]. The activation of eNOS transpires through a series of intricate steps. This activation is modulated by various transcriptional factors and sophisticated post-translational modification mechanisms^[66,67]. In its dormant conformation, eNOS is predominantly associated with caveolin within the caveolae, which represent invaginations of the cellular membrane^[68]. The binding of calcium ions (Ca²⁺) to calmodulin induces conformational alterations, facilitating the interaction of calmodulin with eNOS located in caveolae. This interaction results in the liberation and subsequent activation of eNOS^[69]. Once situated in the cytosolic compartment, the activated eNOS catalyzes the transformation of L-arginine into NO.

The production of NO can be augmented by receptor-mediated agonists, such as acetylcholine and bradykinin, as well as non-receptor-mediated agonists like calcium ionophores, and variations in blood flow dynamics^[66,70]. Moreover, hemodynamic forces exert a significant influence on the transcriptional regulation of the eNOS gene, with endothelial cells positioned in regions of stable laminar flow within arteries exhibiting enhanced NO synthesis^[71,72]. Additionally, NO has the propensity to react with superoxide to yield peroxynitrite anion, which subsequently results in its inactivation.

Physiological roles of NO in the vascular system

The basal production of NO in endothelial cells is crucial for regulating vascular tone and maintaining the nonthrombogenic properties of blood vessels. Once produced, NO rapidly permeates cell membranes and functions as a potent paracrine signaling molecule.

It influences adjacent vascular smooth muscle cells (VSMC) through several mechanisms. First, it diffuses into VSMC, activating sGC to promote cGMP release, leading to VSMC relaxation^[73]. Second, under hypoxic conditions, NO-induced sGC generates inosine 3',5'-cyclic monophosphate (cIMP) instead of cGMP^[74], which activates Rho-associated protein kinase (ROCK), inhibiting myosin light chain phosphatase (MLCP) and promoting VSMC contraction. Third, NO facilitates S-nitrosylation, resulting in the formation of nitrosothiols that mediate prolonged VSMC relaxation^[75].

Beyond its impact on smooth muscle cells, NO also affects circulating blood platelets and leukocytes, both of which play essential roles in maintaining vascular homeostasis. Upon entering the vessel lumen, NO inhibits the activation of $\alpha\text{IIb}\beta_3$ integrins in platelets, suppresses platelet aggregation^[76,77], and obstructs the adhesion of inflammatory cells to the vessel endothelium^[78]. These effects are largely mediated by sGC activation and the subsequent conversion of guanosine triphosphate (GTP) to cGMP.

Roles of NO-cGMP deficiency during the process of atherogenesis

In vivo investigations have demonstrated that mice lacking eNOS display heightened interactions between endothelial cells and leukocytes, increased platelet aggregation, and a propensity for thrombosis, as previously documented^[79,80]. Additionally, Apolipoprotein E/endothelial nitric oxide synthase double-knockout mice manifest an accelerated progression of atherosclerosis^[81]. Various animal models of atherosclerosis, encompassing species such as mice, rabbits, and primates, have similarly revealed that hypercholesterolemia induces significant modifications in endothelium-dependent vasodilation^[19,20,82].

In human studies, coronary arteries obtained from patients undergoing cardiac transplantation, who are afflicted with coronary atherosclerosis, exhibited diminished cGMP synthesis in contrast to those without atherosclerotic conditions^[19]. Furthermore, peripheral manifestations indicative of endothelial dysfunction have been recognized as independent prognosticators of atherosclerotic cardiovascular disease (ACVD) events^[83]. Collectively, these observations imply that a deficiency in endothelial nitric oxide production or its bioavailability-evidenced in both human subjects and animal models-may precede the onset of clinically relevant atherosclerotic lesions.

Mechanisms of NO dysregulation

NO deficiency can occur through mechanisms such as eNOS uncoupling, abnormal phosphorylation, inhibition by endogenous methylarginines, and increased NO degradation due to ROS. These mechanisms are detailed in prior literature^[84,85].

The regulation of eNOS is intricate, involving transcription, translation, protein maturation, and cofactor assembly. While eNOS activity is primarily regulated through phosphorylation, other modifications like S-nitrosylation, acetylation, and glutathionylation also affect its function^[85]. A recent study revealed that Ser 615 undergoes O-GlcNAc modification, which diminishes eNOS activity in glucose dysregulation contexts, such as type-2 diabetes^[86].

Furthermore, microRNAs serve as posttranscriptional regulators of nitric oxide release from endothelial cells by influencing eNOS^[87]. In endothelial cells, miR-92a modulates eNOS mRNA levels by targeting Klf2^[88]. Additionally, miR-221 and miR-222 expression increases during early atherosclerosis, negatively

correlating with eNOS signaling^[89]. Moreover, miR-195 and miR-532, potential biomarkers for thrombosis, inversely relate to eNOS expression^[87].

ENDOTHELIAL CELLULAR STATE TRANSITION DURING ATHEROSCLEROSIS

With the advancement of research, several novel perspectives have emerged. While classical mechanisms involved in endothelial dysfunction, such as oxidative stress, inflammation, hemodynamic disturbances, apoptosis, and cell death, have been briefly discussed above and extensively explored in previous reviews^[6,16], the focus here will be on newer mechanisms.

Endothelial-to-mesenchymal transition

Endothelial-to-mesenchymal transition (EndMT) is a major feature of atherosclerotic plaque development and was reported to drive the development and progression of atherosclerosis^[90]. EndMT is marked by reduced endothelial markers (e.g., CD31, NOS3) and increased mesenchymal markers [e.g., fibroblast activation protein (FAP), alpha-actin 2 (ACTA)]^[91]. Lineage-tracing experiments in *ApoE*^{-/-} mice on a high-fat diet (HFD) revealed that EndMT is common in atherosclerotic lesions, as about 45% of FAP+ fibroblast-like cells originated from ECs after 30 weeks on HFD^[91]. This process destabilizes plaques by promoting collagen degradation and reducing collagen synthesis^[91]. Single-cell RNA sequencing (scRNA-seq) of ECs from *LDLR*^{-/-} mice fed a diabetogenic HFD identified three EC clusters expressing mesenchymal markers, showing reduced fatty acid oxidation, increased inflammation, and altered extracellular matrix organization^[92]. Inflammation, shear stress, and oxidative stress in the form of oxLDL are among the factors known to contribute to atherosclerosis and promote EndMT^[93].

Various molecular pathways and factors have been identified as key regulators of EndMT. *In vitro* studies have demonstrated that EndMT is induced by TGF- β signaling, oxidative stress, and hypoxia^[91,94]. Similar to TGF- β signaling, fibroblast growth factor (FGF) pathways are activated by inflammation and shear stress, but endothelial FGF receptor (FGFR) signaling offers protection against atherosclerosis^[90]. In *ApoE*^{-/-} mice, the absence of FGFR substrate 2a leads to enhanced plaque burden and neointima formation, driven by excessive EndMT and fibronectin accumulation^[90]. The USF1/USP14/NLRC5 axis has been identified as a promoter of EndMT in atherosclerosis, triggering the activation of the Smad2/3 signaling cascade, which is essential for EndMT progression. Silencing USF1 can block this pathway, thus reducing EndMT and atherosclerosis development *in vitro* and *in vivo*^[95]. Furthermore, a study using scRNA-seq identified ENO1, regulated by disturbed flow, as a key player in EC inflammation and EndMT in a shear flow model^[96]. ENO1 knockdown in human aortic endothelial cells (HAECs) upregulated EC markers (CD31 and CDH5) while downregulating mesenchymal markers induced by disturbed flow^[96].

Epigenetic regulation, including histone modification and DNA methylation, plays a crucial role in controlling the expression levels of endothelial-specific genes and upstream modulators that regulate EndMT. The molecular mechanisms reported have been comprehensively summarized in the review by Singh^[97]. Recent studies demonstrated the role of epigenetics in EndMT in atherosclerotic mouse models. Histone lactylation, particularly at H3K18la, promotes EndMT and atherosclerosis. This modification is regulated by the ASF1A/P300 complex, which enhances SNAI1 transcription. Inhibiting glycolysis and ASF1A reduces H3K18la levels, decreasing EndMT and offering a potential therapeutic approach for atherosclerosis^[98]. Lecce *et al.* found that the knockout of HDAC9 in endothelial cells prevented EndMT and maintained an endothelial-like phenotype^[99]. *In vivo*, mice with endothelial-specific HDAC9 knockout showed reduced EndMT and smaller plaque areas^[99]. In line with this, another study found that valproic acid (VPA), an HDAC inhibitor, induced TGF β -mediated EndMT in endothelial cells^[100]. Additionally, DNA methyltransferases (DNMTs) have been recognized as crucial molecular mediators of EndMT.

5-aza-2'-deoxycytidine (5-Aza), an inhibitor of DNMTs, suppressed EndMT in both cultured cells and mice^[101]. Furthermore, Endothelial-specific knockout of Dnmt1 protected the vasculature from remodeling induced by disturbed flow and atherosclerosis^[101]. Together, these findings highlight the potential of targeting epigenetic regulators as a therapeutic strategy for atherosclerosis.

EC heterogeneity revealed by scRNA-seq

In addition to the well-known EndMT state of ECs, scRNA-seq has significantly advanced the understanding of EC heterogeneity in atherosclerosis by providing detailed insights into the diverse cellular phenotypes and functions within atherosclerotic lesions^[22,102]. A comprehensive scRNA-seq study identified more than 78 distinct EC clusters across 11 murine tissues^[103]. Kalluri *et al.* demonstrated that two EC subsets have specialized functions in lipoprotein handling, angiogenesis, and extracellular matrix production^[104]. He *et al.* identified three primary EC populations in the murine aorta: CD34^{high} ECs, THY1^{high} ECs, and “activated ECs”^[105]. While CD34^{high} and THY1^{high} ECs are primarily associated with endothelial proliferation and vascular development, activated ECs are enriched in pathways related to vessel dilation and blood pressure regulation^[105]. Another study highlighted the functional heterogeneity of ECs in atherosclerosis, showing that SULF1⁺ (sulfatase-1) endothelial cells contribute to plaque vulnerability and cerebrovascular events through proinflammatory activities^[106].

METABOLIC SIGNALING PATHWAYS

Effects of key metabolites

Recent studies have highlighted the significant role of metabolites in influencing endothelial function and atherogenesis [Figure 1], with homocysteine metabolites being particularly impactful^[107]. Elevated levels of homocysteine (Hcy) and its metabolites, such as Hcy-thiolactone and N-Hcy-protein, are strongly associated with endothelial dysfunction and cardiovascular diseases (CVD)^[107]. Hcy-thiolactone modifies lysine residues on proteins, resulting in the formation of N-Hcy-proteins, which are cytotoxic, proinflammatory, and proatherogenic^[108]. These metabolites trigger pro-atherogenic changes in gene expression, activate mTOR signaling, and inhibit autophagy through epigenetic mechanisms, all of which contribute to endothelial dysfunction^[109,110]. Even small physiological increases in plasma total homocysteine (tHcy), induced by low-dose methionine or dietary animal protein, have been shown to impair vascular endothelial function. This suggests that diet-induced elevations in plasma tHcy may contribute to the progression of atherosclerosis by compromising vascular function^[111].

Another metabolite, Neu5Ac, has been shown to promote endothelial ferroptosis via the SLC3A2 pathway, contributing to endothelial cell injury and accelerating atherosclerotic plaque progression in Apoe^{-/-} mice^[112]. Additionally, supplementation with functional metabolites such as β -alanine, carnosine, and acetyl-CoA (acetate) has been found to reverse EndMT, a key process in atherogenesis. This effect is likely mediated through the inhibition of Smad2/3 phosphorylation^[101]. Furthermore, the accumulation of neutral lipids resulting from the deletion of adipose triglyceride lipase (ATGL) in the endothelium leads to impaired endothelial-dependent vascular tone and reduced nitric oxide synthesis, exacerbating endothelial dysfunction. Loss of ATGL also induces endoplasmic reticulum stress, which triggers inflammation in the endothelium and increases lesion size in atherosclerosis models^[113].

Gut microbiome metabolism

A variety of metabolites derived from the gut microbiota play a crucial role in the progression of atherosclerosis, including short-chain fatty acids (SCFAs), trimethylamine N-oxide (TMAO), secondary bile acids (BAs), and other molecules^[17,114-116].

TMAO induces endothelial dysfunction through multiple mechanisms^[117]. Querio *et al.* systematically investigated how it induces endothelial dysfunction^[118]. Using bovine aortic endothelial cell line (BAE-1 cells) as a model, the results showed that TMAO altered the purinergic response, which means it affected intracellular ATP-induced calcium increase, nitric oxide release, and phosphorylation of eNOS.

The expression of adhesion molecules is also regulated by TMAO. It increases monocyte adhesion by upregulating vascular cell adhesion molecule 1 (VCAM-1) and activates the protein kinase C (PKC)/nuclear factor kappa B (NF- κ B) pathway, thereby promoting atherogenesis^[119]. Additionally, TMAO increases endothelial permeability and dysfunction by disrupting cell junctions through the action of high-mobility group box protein 1 (HMGB1)^[120]. Furthermore, TMAO induces EndMT and apoptosis through protein kinase RNA-like endoplasmic reticulum kinase (PERK) signaling, worsening plaque formation and cardiac function^[121]. Finally, TMAO promotes vascular inflammation by activating mitogen-activated protein kinase (MAPK) and NF- κ B pathways, enhancing inflammatory gene expression and leukocyte recruitment, which increases the risk of atherosclerosis^[122].

Studies have also shown that TMAO affects pyroptosis, a form of programmed cell death associated with inflammation. This process involves the lysophosphatidylethanolamine acyltransferase (LPEAT) and mitophagy pathways, leading to excessive cell death and promoting the progression of atherosclerosis^[23]. In ApoE^{-/-} mice, TMAO accelerates atherosclerosis by triggering pyroptosis through membrane-bound O-acyltransferase domain-containing 2 (MBOAT2)-mediated endoplasmic reticulum stress^[123]. Additionally, TMAO promotes pyroptosis via the succinate dehydrogenase complex iron sulfur subunit B (SDHB)/ROS pathway, further damaging endothelial cells and contributing to atherosclerosis progression^[124].

In addition to TMAO, certain SCFAs produced by specific microbiota species from indigestible carbohydrates also contribute to endothelial inflammation^[17]. The mechanisms by which SCFAs are involved in atherosclerosis have been extensively reviewed^[114-116]. Recent studies have focused more on the therapeutic effects of microbiome-associated SCFAs. For instance, Zhu *et al.* found that *E. faecium* NCIMB11508 positively impacted gut microbiota composition by increasing SCFA levels, which, in turn, reduced inflammation and protected the intestine^[125]. Yang *et al.* demonstrated that dietary methionine restriction (MR) promoted SCFA production and bile acid metabolism, reduced cell adhesion molecules, and prevented foam cell formation in the aorta, thereby helping to prevent atherosclerosis^[126].

Additionally, other gut microbiome-derived metabolites are involved in atherogenesis. Indole-3-acrylic acid (I3AA), a metabolite derived from microbes, plays a key role in alleviating atherosclerosis (AS) by activating the aryl hydrocarbon receptor (AhR) and inhibiting the TGF- β /Smad pathway. This may help mitigate endothelial-to-mesenchymal transitions in atherosclerotic mice^[127]. In ApoE^{-/-} mice, imidazole propionate (ImP) was found to increase atherosclerotic lesion size. Mechanistically, ImP disrupts insulin receptor signaling by inhibiting the PI3K/AKT pathway, leading to prolonged activation of the FOXO1 (forkhead box protein O1) transcription factor^[128].

INTERCELLULAR COMMUNICATION AND EXOSOME-MEDIATED CROSSTALK

Endothelial-derived exosomes as key mediators of vascular crosstalk

Exosomes, which can carry small molecules, serve as mediators in communication among various cell types and play a role in endothelial dysfunction and atherosclerosis [Figure 2]. Endothelial-derived exosomes can activate macrophages, a crucial cell type in atherosclerotic plaques^[129]. When endothelial cells are exposed to inflammatory cytokines like TNF- α , they release exosomes that promote a proinflammatory M1 phenotype

in macrophages through the MAPK/NF- κ B signaling pathway. This polarization exacerbates lipid accumulation and triggers macrophage apoptosis, contributing to plaque instability^[130]. Several studies have identified specific molecules within exosomes derived from endothelial cells. MiR-155, for example, was found to be highly expressed in exosomes from both oxLDL-treated HUVECs and VSMCs, driving proinflammatory M1 polarization in THP-1 monocytes^[131]. This effect occurs through the downregulation of KLF2, a repressor of miR-155, resulting in elevated levels of miR-155 in both cells and exosomes. KLF2 is known for its anti-inflammatory role in endothelial cells during atherogenesis. MiR-92a, contained in exosomes derived from endothelial cells, downregulated the expression of its target gene KLF4 in macrophages. This led to enhanced proinflammatory responses, increased LDL uptake, and impaired macrophage migration. The study further demonstrated that these harmful effects were alleviated in both miR-92a knockdown and KLF4 overexpression models. Observations in atherosclerotic Apoe^{-/-} mice revealed an inverse correlation between intravesicular miR-92a levels in blood serum and KLF4 expression in lesions, suggesting a potential role for extracellular miR-92a in the formation of atherosclerotic lesions^[132].

Exosomes released from ECs play a crucial role in regulating the phenotypic changes, proliferation, and migration of VSMCs, which are pivotal in the development of atherosclerosis^[133,134]. The long non-coding RNA LINC01005, highly expressed in oxLDL-treated HUVECs, is also abundant in exosomes derived from these cells^[135]. LINC01005 promotes the phenotypic shift, proliferation, and migration of VSMCs. Mechanistically, LINC01005 functions as a sponge for miR-128-3p, leading to the upregulation of its target gene KLF4. These findings indicate that LINC01005-mediated modulation of the miR-128-3p/KLF4 axis in exosomes from oxLDL-treated HUVECs reduces contractile markers while increasing the synthetic smooth muscle cell marker osteopontin (OPN), thereby contributing to the progression of atherosclerosis^[135].

Exosomes derived from endothelial cells can be transferred to immune cells, where they influence immune cell function^[136]. For instance, exosomes containing interleukin-6 (IL-6) can promote the differentiation of Th17 cells, leading to increased production of inflammatory cytokines and contributing to the development of atherosclerosis^[137]. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) has also been identified as a factor that activates neutrophil extracellular traps (NETs). It is delivered to neutrophils via exosomes released by HUVECs treated with oxLDL. In atherosclerotic Apoe^{-/-} mice, treatment with exosomes from oxLDL-stimulated HUVECs triggered an inflammatory response and promoted NET release, thereby exacerbating atherosclerosis^[138]. Additionally, the upregulation of miR-505, which is encapsulated within exosomes from HUVECs, inhibits Sirtuin 3 (SIRT3) in neutrophils. This inhibition increases ROS production and further enhances NET formation by neutrophils^[139].

Reverse communication: neighboring cells to endothelium

Exosomes derived from various cell types and organs, including macrophages, mesenchymal stem cells, dendritic cells, and perivascular adipose tissue (PVAT), can exacerbate endothelial dysfunction in atherosclerosis^[136].

For instance, macrophage-derived exosomal miR-4532 has been shown to promote endothelial cell injury by targeting SP1 and activating NF- κ B p65 signaling, leading to increased expression of adhesion molecules such as ICAM-1 and VCAM-1, which facilitate leukocyte adhesion and transmigration^[140]. MicroRNAs involved in the inflammatory response of monocytes, miR-155 and miR-233, are significantly altered within these exosomes. The target of these exosomes is primarily endothelial cells, which were shown to effectively internalize these bioactive vesicles, subsequently causing an increase in the expression of NF- κ B^[141].

MSC-derived exosomes were internalized by oxLDL-treated ECs, where they inhibited caspase-3 activation and cell apoptosis, promoted EC proliferation, and reduced proinflammatory cytokines and oxidative stress levels. Additionally, they increased antioxidant levels (SOD and GSH-PX), offering protection against oxLDL-induced EC dysfunction^[142]. Furthermore, exosomes derived from adipose-derived mesenchymal stem cells (ADMSCs) were shown to protect ECs from atherosclerosis. In a H₂O₂-treated HUVEC lesion model, miR-324-5p expression was decreased, and miR-324-5p inhibited the expression of Protein Phosphatase 1 Regulatory Subunit 12B (PPP1R12B), highlighting its protective role^[143].

Endothelial cells can also internalize platelet-derived exosomes. One example is miR-223, which inhibits ICAM-1 expression and protects atherosclerotic ApoE^{-/-} mice from endothelial inflammation^[144].

Myoendothelial junction

Myoendothelial junctions (MEJs) are specialized structures where endothelial cells directly interface with vascular smooth muscle cells, enabling intercellular signaling that regulates vascular tone and blood pressure^[145]. MEJs enable the transfer of ions and small molecules, which are essential for coordinating vasodilation and vasoconstriction^[146]. Dysfunction of MEJs impairs vasodilation, increases vascular resistance, and contributes to the progression of atherosclerosis.

Endothelial cells, sensitive to hemodynamic changes, activate signaling pathways involving ion channels and NO production to respond to changes in blood flow and wall tension^[147]. MEJs provide a direct signaling pathway between endothelial cells and VSMCs, particularly for transporting small molecules such as Ca²⁺ and IP₃^[148,149]. This signaling forms the “myoendothelial feedback” (MEF) loop, where endothelial NO release and Ca²⁺-activated K⁺ channels help inhibit excessive VSMC contraction, thereby reducing vascular tension^[147]. The localization of signaling proteins within MEJs further emphasizes their critical role in vascular regulation.

Continuous mechanical strain can induce phenotypic changes and functional remodeling of VSMCs, altering the structure of the vascular wall and its ability to adapt to stimuli^[150,151]. This mechanical regulation is essential for maintaining vascular function and ensuring stable blood flow.

PHARMACOTHERAPIES TARGETING ENDOTHELIAL DYSFUNCTION IN ATHEROSCLEROSIS

Accumulating evidence suggests that pharmacological agents-ranging from approved therapeutics to investigational compounds, with diverse chemical structures and mechanisms of action-can modulate endothelial function. This section provides an overview of classical pharmacological agents and summarizes some novel drugs with promising potential and related clinical trials [Tables 1 and 2].

Lipid-lowering drug

Lipid-lowering agents, especially statins, are highly effective in improving endothelial dysfunction. Their primary mechanisms involve increasing NO bioavailability and reducing oxidative stress, thereby preserving NO function and maintaining normal vasodilation^[6,37]. Furthermore, statins markedly diminish the generation of ROS, thus mitigating oxidative stress that contributes to endothelial injury^[152]. Additionally, statins exhibit notable anti-inflammatory properties by substantially lowering inflammatory markers such as C-reactive protein (CRP), thereby enhancing vascular stability^[153].

Numerous clinical investigations have demonstrated that statin therapy markedly improves endothelial biomarkers, particularly in individuals with metabolic syndrome and dyslipidemia. Prolonged

Table 1. Pharmacotherapies targeting endothelial dysfunction

Drug class	Agent	Mechanisms influencing endothelial function
Lipid-lowering drugs	Statins (e.g., Rosuvastatin, Simvastatin)	↑ NO, ↓ ROS, ↓ CRP, improved FMD
	PCSK9 inhibitors (e.g., Evolocumab)	↓ Oxidative stress & inflammation, improved endothelial function
Antihypertensive drugs	ARBs (e.g., Losartan)	↓ Endothelin-1, ↓ inflammation
	ACEIs (e.g., Enalapril)	Improved FMD in multiple vascular beds
	Direct renin inhibitor (Aliskiren)	↓ EPCs, no FMD improvement
	Calcium channel blockers (e.g., Amlodipine)	Restore endothelium-dependent vasodilation
	β-blockers (Nebivolol vs. Atenolol)	Nebivolol: beneficial; Atenolol: negative
Anti-diabetic drugs	GLP-1 receptor agonists	↑ NO, ↓ inflammation, improved FMD
	SGLT-2 inhibitors	Improved endothelial markers, mechanism unclear
	Metformin	↑ insulin sensitivity, ↓ ROS
	TZDs (e.g., Rosiglitazone)	PPAR-γ activation, anti-inflammatory
	Insulin analogs	Mechanisms unclear
Anti-inflammatory drugs	Aspirin	Improved endothelium-dependent vasodilation
	IL-35	↓ Vascular inflammation
	Flavonoids, antioxidant mimetics	↓ Oxidative stress, improved vascular function (animal)
	Colchicine	NLRP3 inhibition, improved endothelial markers
	MCC950	NLRP3 inhibition (preclinical)
	Resolvin D1 (RvD1)	↓ NF-κB, ↓ adhesion molecules
eNOS enhancers/antioxidants	BH4 + antioxidants	Restore eNOS coupling, ↑ NO
	Resveratrol	↑ eNOS via Nrf2, ↓ ROS
	Vitamin C & E	Inhibit NAD(P)H oxidase, ↑ FMD
	AVE3085	↑ eNOS transcription, ↓ oxidative stress
	N-acetylcysteine (NAC)	Antioxidant effect (preclinical)
	Genistein	↑ eNOS, ↑ calmodulin-1, ↓ ROS
Novel strategies	β2 receptor agonists	↑ eNOS, ↑ NO, ↓ ROS
	Molsidomine	Direct NO donor, no effect in angina
	KCa channel activators	↑ NO, ↓ oxidative stress
	AMPK activators	↓ lipid inflammation, ↑ NO
	Exosomal circ_0001785	↓ EC injury via miR-513a-5p/TGFB3

NO: Nitric oxide; ROS: reactive oxygen species; CRP: C-reactive protein; FMD: flow-mediated dilation; PCSK9: proprotein convertase subtilisin/kexin type 9; ARBs: angiotensin II receptor blockers; ACEIs: angiotensin-converting enzyme inhibitors; EPCs: endothelial progenitor cells; GLP-1: glucagon-like peptide-1; SGLT-2: sodium-glucose cotransporter 2; TZDs: thiazolidinediones; PPAR-γ: peroxisome proliferator-activated receptor gamma; IL-35: interleukin-35; NLRP3: NOD-, LRR- and pyrin domain-containing protein 3; NF-κB: nuclear factor kappa B; eNOS: endothelial nitric oxide synthase; BH4: tetrahydrobiopterin; Nrf2: nuclear factor erythroid 2-related factor 2; AMPK: AMP-activated protein kinase; KCa: calcium-activated potassium channel; circ_0001785: circular RNA_0001785; miR-513a-5p: microRNA-513a-5p; TGFB3: transforming growth factor beta receptor 3; NAC: N-acetylcysteine; RvD1: Resolvin D1; EC injury: endothelial cell injury.

administration of statins, such as rosuvastatin, leads to sustained enhancement of endothelial function and reduced lipid peroxidation, which may promote the regression of atherosclerotic lesions. High-dose rosuvastatin treatment improves endothelium-dependent flow-mediated dilation (FMD) of the brachial artery in patients with ST-segment elevation myocardial infarction (STEMI)^[154]. Additionally, aggressive lowering of low-density lipoprotein cholesterol (LDL-C) with rosuvastatin significantly improved the reactive hyperemia peripheral arterial tonometry (RH-PAT) index, suggesting that it may improve endothelial function in patients with coronary artery disease^[155]. Previous studies have suggested that simvastatin may help preserve endothelial function in prediabetic individuals with normal or mildly to moderately elevated cholesterol levels^[156]. Continued inquiry into the molecular underpinnings of endothelial dysfunction, alongside the multifaceted effects of lipid-lowering treatments such as statins, presents promising prospects for developing innovative therapeutic strategies to improve endothelial

Table 2. Clinical studies on endothelial function and atherosclerosis

Study (PMID)	Drug	Target	Disease	Sample size	Results
21349594	Rosuvastatin	HMG-CoA reductase	STEMI	87	Positive
29209775	Rosuvastatin	HMG-CoA reductase	CAD	50	Positive
20199786	Simvastatin	HMG-CoA reductase	PreDM	50	Positive
37468450	Evolocumab	PCSK9	ACS	46	Positive
23615219	Aliskiren	renin	EA	22	No effect
30683457	Colchicine	NLRP3	CAD	28	Positive in part
25875387	Molsidomine	NO	SAP	180	No effect

STEMI: ST-elevation myocardial infarction; CAD: coronary artery disease; PreDM: prediabetes; ACS: acute coronary syndrome; EA: endothelial activation; SAP: stable angina pectoris; NO: nitric oxide; PCSK9: proprotein convertase subtilisin/kexin type 9; HMG-CoA reductase: 3-hydroxy-3-methylglutaryl-coenzyme A reductase.

health^[6].

PCSK9 inhibitors (PCSK9i), an emergent lipid-lowering modality, attenuate endothelial dysfunction and platelet activation in patients with acute coronary syndrome^[157]. In the context of atherosclerosis, PCSK9i enhance endothelial function by abating oxidative stress and inflammation, thus counteracting vascular aging and dysfunction^[158].

Antihypertensive drugs

Angiotensin II receptor blockers (ARBs) and angiotensin-converting enzyme inhibitors (ACEIs) have demonstrated positive effects on endothelial function. Drugs such as losartan can reduce levels of endothelin-1 and other serum inflammatory markers, thereby alleviating endothelial dysfunction. ACEIs have also been shown to improve endothelial function in multiple vascular regions^[159]. A meta-analysis of 38 clinical trials found that, compared to other antihypertensive drugs, ACE inhibitors and ARBs are more effective in improving endothelial function, as measured by FMD, although conflicting data exist among these studies^[160]. Additionally, a four-month treatment with the direct renin inhibitor aliskiren was shown to decrease circulating endothelial progenitor cells (EPCs) without affecting endothelial function^[161]. Calcium channel blockers, particularly the dihydropyridine class, can also significantly reverse endothelium-dependent vasodilation impairment. In contrast, traditional β -blockers such as atenolol may have negative effects on endothelial function, whereas newer β -blockers like nebivolol show beneficial effects^[162].

Anti-diabetic drugs

Various anti-diabetic drugs not only exert their effects through glycemic control but also show independent benefits in improving endothelial function. For instance, GLP-1 receptor agonists, SGLT-2 inhibitors, and thiazolidinediones (TZDs) can enhance NO production, inhibit inflammatory cytokines, and reduce oxidative stress, significantly improving endothelial function indicators such as flow-mediated dilation^[163]. Metformin reduces endothelial injury by increasing insulin sensitivity and scavenging free radicals^[164], while TZDs such as rosiglitazone activate the PPAR- γ pathway, exert anti-inflammatory effects, and improve vascular responsiveness^[165]. These mechanisms help restore endothelial cell physiological function, potentially slow the progression of atherosclerosis, and reduce cardiovascular risk^[166]. However, the mechanisms of action for certain agents, such as insulin analogs and some SGLT-2 inhibitors, remain incompletely understood, and further clinical studies are needed to verify their long-term efficacy and cardiovascular outcomes. Future research should focus on comprehensively evaluating the multifaceted vascular effects of anti-diabetic drugs to support more personalized and targeted therapeutic strategies.

Anti-inflammatory drugs

Given the central role of inflammation in endothelial dysfunction and the development and progression of atherosclerosis, anti-inflammatory drugs have garnered significant attention in recent years. Widely used anti-inflammatory agents, such as aspirin, have been shown to improve endothelium-dependent vasodilation in high-risk individuals and patients with established atherosclerosis^[167]. Emerging anti-inflammatory cytokines, like interleukin-35 (IL-35), have also been identified as potential therapeutic targets, offering new avenues for managing vascular inflammation in CVD^[168]. Additionally, natural compounds with both antioxidant and anti-inflammatory properties-such as flavonoids and antioxidant enzyme mimetics-have demonstrated promise in reducing oxidative stress and improving vascular function in animal models^[169].

As research continues to highlight the importance of NLRP3 (NOD-like receptor protein 3) as a key driver of atherosclerosis, drugs targeting this pathway are gaining attention. According to a recent review, the NLRP3 inflammasome plays a crucial role in endothelial dysfunction, with its activation triggered by factors such as ROS, ischemia, and high glucose, leading to inflammation and endothelial injury^[170]. The NLRP3 inflammasome inhibitor MCC950 has shown promising results in preclinical studies; however, its clinical utility still needs to be confirmed^[171]. Colchicine, another inhibitor of the NLRP3 inflammasome, has been tested in clinical trials and shown to improve endothelial function in patients with leukocyte activation, although it had no effect on endothelial function in the overall patient population^[172]. NLRP3 can be activated by oxLDL, mitochondrial ROS, and TMAO^[173]. Upon activation, NLRP3 assembles the inflammasome, activates caspase-1, and promotes the release of interleukin-1 β (IL-1 β) and interleukin-18 (IL-18)^[174,175]. These cytokines upregulate endothelial adhesion molecules and enhance leukocyte adhesion. NLRP3 inflammasome can also be regulated by gut microbiota, which warrants further investigation^[176].

Endogenous agents are also becoming better understood and show considerable promise. For example, specialized pro-resolving mediators derived from DHA and EPA, such as resolvin D1 (RvD1), have demonstrated promising effects in suppressing NF- κ B activation and reducing adhesion molecule expression, effectively mitigating endothelial inflammation induced by stimuli like cholesterol crystals^[177,178].

eNOS enhancers and antioxidant agents

Various pharmacological agents and natural substances enhance vascular endothelial function by mitigating oxidative stress, restoring eNOS coupling, and augmenting NO bioavailability. Antioxidants contribute to this process by scavenging free radicals and supporting the availability of essential enzyme cofactors, thus preserving eNOS activity and promoting NO production^[179].

Both BH4 and its combinations with antioxidants have been validated in research to enhance eNOS performance^[180]. Moreover, interventions targeting eNOS regulation-such as inhibiting VE-PTP or utilizing resveratrol-loaded nanoparticles-have shown promise in augmenting NO production and minimizing oxidative stress^[181]. Resveratrol enhances eNOS expression and activity via Nrf2 activation, BH4 elevation, and ROS inhibition^[182]. Vitamin C facilitates the conversion of oxidized BH4 back to its active state and supports sustained NO release^[180]. In healthy subjects, vitamins C and E have been demonstrated to enhance FMD by inhibiting NAD(P)H oxidase, leading to improved endothelial function^[183]. The compound AVE3085 has proven effective in ameliorating hypertension and endothelial dysfunction by promoting eNOS transcription and coupling, inhibiting oxidative stress, and reducing arginase activity^[184-186]. Furthermore, N-acetylcysteine (NAC) has shown promising effects on endothelial function in preclinical investigations^[187]. Genistein supports endothelial integrity by enhancing eNOS activity, increasing calmodulin-1 expression, and scavenging ROS^[188].

Novel potential therapeutic strategies

Beta2 (B2) receptor agonists, recently discovered drugs, show promise in treating endothelial dysfunction, particularly in cardiovascular diseases. They improve endothelial function by enhancing NO production, stimulating eNOS activity, reducing oxidative stress, preventing hypoxia/reoxygenation injury, and improving vascular permeability^[189]. However, a clinical study found that add-on treatment with Molsidomine, a direct NO donor, did not improve endothelial function in patients with stable angina pectoris^[190].

Enhancing the activity of endothelial Ca²⁺-activated K⁺ (KCa) channels^[191] and activating AMP-activated protein kinase (AMPK)^[192] are also promising strategies to restore endothelial function. These approaches aim to reduce oxidative stress, improve NO bioavailability, and counteract the proinflammatory effects of lipid products.

As summarized in the previous section on exosomes, certain exosomal components have demonstrated protective effects in atherosclerotic endothelial dysfunction. For instance, exosome-derived circ_0001785 has been shown to reduce endothelial cell injury and delay atherogenesis through the miR-513a-5p/TGFB3 ceRNA network, highlighting a potential therapeutic strategy for atherosclerosis^[193].

FUTURE DIRECTIONS

Endothelial cell heterogeneity and spatial omics

Emerging single-cell and spatial transcriptomic technologies have revealed substantial heterogeneity among ECs across different vascular beds, with distinct subpopulations associated with plaque rupture susceptibility, lipid transcytosis, and leukocyte adhesion^[22,102]. Future research should focus on deepening our understanding of the functions of each EC subtype. Furthermore, integrating spatial transcriptomics and/or spatial proteomics to map EC subtypes within human atherosclerotic lesions-especially in high-risk areas like plaque shoulders and necrotic cores-will be crucial. Combining these approaches with *in vivo* shear stress modeling could provide insights into how oscillatory flow drives proinflammatory EC phenotypes via mechanosensitive pathways. Additionally, lineage-tracing models could offer further evidence in these studies.

Nanotechnology for targeted therapeutics

Nanoparticle-based strategies offer unprecedented precision for modulating endothelial dysfunction. A huge advantage of nanoparticle delivery is that it can target certain cell types, avoiding unwanted effects. For example, dysregulation of YAP/TAZ contributes to plaque development, making them potential therapeutic targets. However, systemic modulation of YAP/TAZ poses risks of off-target effects. To overcome this, a study developed monocyte membrane-coated nanoparticles (MoNP) for targeted delivery to activated, inflamed endothelium on plaque surfaces, effectively reducing plaque development in mice without causing significant histopathological changes in major organs^[194]. Another study developed a biomimetic ROS-responsive hyaluronic acid-based nanomaterial, which demonstrated ROS responsiveness, evaded macrophage phagocytosis, and showed high uptake rates by inflamed endothelial cells^[195]. More nanoparticles are expected to be developed to enhance effectiveness and minimize side effects in targeting endothelial dysfunction and atherosclerosis.

Clinical translation and trials

As more mechanisms underlying endothelial dysfunction in atherogenesis are uncovered, specific therapies warrant further investigation in clinical trials to assess their potential for clinical application. For instance, microbiome-modulating interventions, such as prebiotics that promote butyrate production, may enhance endothelial barrier function. Long-term follow-up studies are crucial to determine whether short-term

endothelial repair leads to a reduction in major adverse cardiovascular events (MACE).

CONCLUSION

The endothelium plays a crucial role as both a sensor of hemodynamic stress and a regulator of vascular homeostasis, making it central to the pathogenesis of atherosclerosis. While traditional approaches targeting LDL-C and blood pressure remain essential, cutting-edge strategies could further reveal the mechanisms of endothelial heterogeneity, mechanotransduction, and metabolic reprogramming. Advances in gene editing, nanomedicine, and systems biology provide new tools to develop spatially resolved, cell-type-specific therapies. However, significant gaps remain in improving endothelial function and preventing adverse events due to a limited understanding of critical mechanisms such as redox-inflammation coupling and endothelial pyroptosis. These complex pathological processes highlight the need for multi-target therapies and more precise treatments. By prioritizing translational studies that connect endothelial biology with clinical outcomes, the field can move beyond plaque stabilization and aim for true vascular repair, offering hope for reversing atherosclerosis in its early stages.

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Authors' contributions

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All authors reviewed and approved the final manuscript.

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Consent for publication

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