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Comparative evaluation of cell types for endothelialization in cardiovascular tissue engineering

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ABSTRACT

Tissue engineering represents a promising approach to overcome the limitations of current vascular grafts by promoting endothelialization. However, the successful fabrication of small-diameter (<6 mm) tissue-engineered vascular grafts (TEVGs) that maintain the desired mechanical strength, biocompatibility, and long-term patency of native vessels remains a significant challenge. The development of an ideal TEVG depends largely on achieving complete endothelial coverage using appropriate autologous cells that can mimic the functional properties of native endothelium. Several studies have highlighted various autologous and stem cell sources with potential for graft endothelialization, including endothelial progenitor cells (EPCs), embryonic stem cells (ESCs), human umbilical vein endothelial cells (HUVECs), mesenchymal stem cells (MSCs), and induced pluripotent stem cells (iPSCs). Despite extensive preclinical progress, the most suitable cell source for generating stable and functional endothelium in small-diameter TEVGs remains unclear, representing a key gap in current knowledge. This review therefore evaluates the principal cell types investigated in cardiovascular tissue engineering, outlining their main advantages, limitations, and translational potential. Overall, a critical comparison of these cell sources highlights the need for further research to determine their long-term safety, durability, and suitability for clinical application in the construction of functional and durable TEVGs.

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Introduction

Cardiovascular disease (CVD) refers to a group of disorders affecting the heart and blood circulation and is recognized as one of the leading causes of morbidity and mortality worldwide (1). In the United Kingdom, CVD accounts for approximately 25% of all annual deaths, with an overall prevalence of around 4% of the total population (2). Among these diseases, coronary artery disease (CAD) contributes to the majority of CVD-related deaths and is characterized by the obstruction of the coronary arteries, leading to a reduction in blood flow to the myocardium. This reduction can cause myocardial perfusion deficits, ischemia, and eventually irreversible myocardial injury (3,4). It has been reported that approximately 2.3 million people in the UK are currently living with CAD, and nearly 63,000 individuals die each year as a result of this condition, making it one of the country's most common causes of death (2). Despite advances in both prevention and treatment strategies, the number of CAD cases continues to increase due to the ageing population and the growing prevalence of major risk factors such as hypertension, diabetes, and obesity (1, 2). The main pathological mechanism responsible for CAD development is atherosclerosis, a progressive process mainly driven by chronic inflammation and the accumulation of low-density lipoproteins (LDL) within the subendothelial intimal layer of the arterial wall (3, 4). This process leads to thickening of the arterial wall and narrowing of the vascular lumen, which reduces oxygen and nutrient delivery to cardiac tissues. As a result, the coronary arteries that supply the myocardium can become occluded, compromising myocardial perfusion and leading to ischemic injury and cardiac dysfunction (4). Importantly, these vascular alterations are rooted in endothelial dysfunction, which impairs nitric oxide (NO) bioavailability, enhances leukocyte adhesion, and promotes a pro-thrombotic luminal environment, mechanisms that directly influence graft compatibility and long-term patency (3, 4).

A major limitation in the management of CAD using autologous vessels, particularly saphenous vein grafts (SVGs), is vein graft failure (VGF), a serious complication defined as total graft occlusion that obstructs blood flow to

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the revascularized myocardial area. Graft failure can result in several adverse cardiac outcomes, including recurrent angina, myocardial infarction, and even death (5). The pathological mechanism underlying VGF is mainly driven by endothelial injury and poor endothelialization and is associated with several pathological processes, including early thrombosis, vascular inflammation, intimal hyperplasia, and late accelerated atherosclerosis (6, 7). These biological failures reflect instability of the endothelial phenotype and loss of antithrombotic signaling, which are central mechanistic challenges targeted by vascular tissue engineering (6, 8). Figure 1 summarizes the main mechanisms contributing to graft failure. Although multiple studies have explored the use of different cell types for constructing vascular grafts, the most suitable cell source for achieving complete and functional endothelialization remains unclear. Successful endothelialization requires selecting cells capable of producing nitric oxide, regulating vascular tone, exhibiting antithrombotic behavior, suppressing inflammatory pathways, and integrating mechanically with the graft matrix (9,10). Accordingly, identifying an optimal endothelial cell source remains a major unmet need in vascular biology and TEVG development (11, 12).

CVD continues to create a substantial clinical demand for vascular reconstruction, particularly in patients requiring bypass surgery or replacement of diseased small-diameter vessels (13). Although autologous vessels remain the preferred option for many procedures, they are not always available or suitable because of previous harvesting, poor vessel quality, advanced age, diabetes, diffuse vascular disease or donor-site morbidity (13). Conventional synthetic grafts can provide sufficient mechanical strength in large-diameter vessels, but their use in small-diameter applications remains limited by thrombosis, compliance

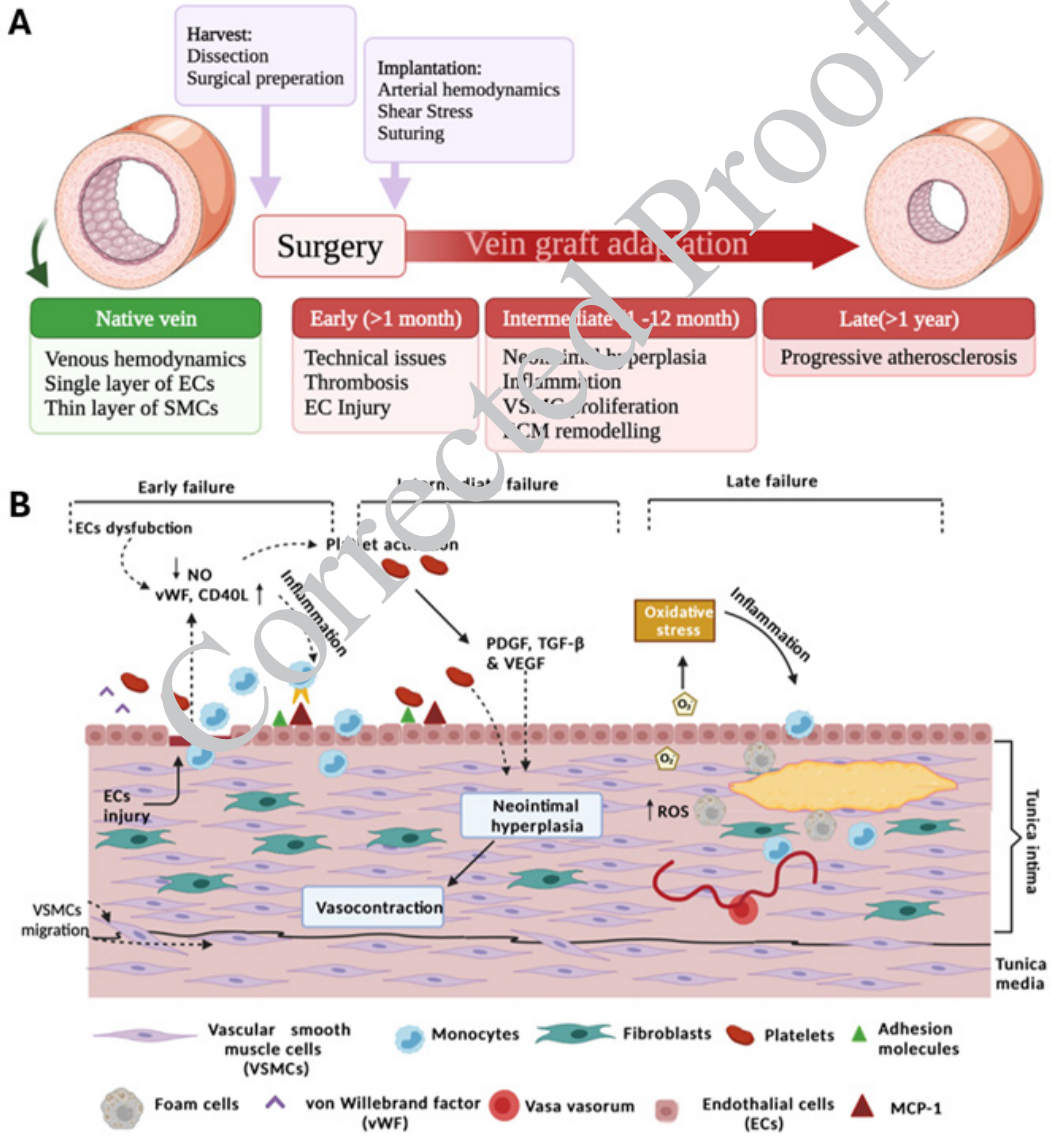

Figure 1. Stages and mechanisms of vein graft adaptation and failure
(A) Overview of saphenous vein graft adaptation after arterial implantation, showing the transition from normal venous structure to early injury, intermediate neointimal hyperplasia, and late atherosclerotic degeneration. (B) Key cellular and molecular events contributing to graft failure. Early failure involves endothelial injury, inflammation, and platelet activation. Intermediate failure is driven by VSMC proliferation and ECM deposition leading to neointimal hyperplasia. Late failure is characterized by oxidative stress, foam-cell formation, and atherosclerotic plaque development. Figure created with BioRender.com
ECs, endothelial cells; SMCs, smooth muscle cells; VSMCs, vascular smooth muscle cells; ECM, extracellular matrix; NO, nitric oxide; vWF, von Willebrand factor; CD40L, cluster of differentiation 40 ligand; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor beta; VEGF, vascular endothelial growth factor; ROS, reactive oxygen species; MCP-1, monocyte chemoattractant protein-1

mismatch, poor endothelial coverage and reduced long-term patency (13). These limitations have maintained interest in tissue-engineered vascular grafts (TEVGs) as biologically active alternatives that may overcome some of the shortcomings of both autologous and synthetic conduits (13-15). Recent reviews and experimental studies reinforce that TEVG development has shifted from simply producing mechanically competent conduits towards engineering living grafts capable of biological remodeling, host integration and functional endothelialization (13-16).

Compared with traditional vascular grafts, TEVGs offer the potential to act as dynamic vascular substitutes rather than inert tubes. Their proposed advantages include improved hemocompatibility, host-cell recruitment, extracellular matrix remodeling, potential growth capacity, and adaptation to local hemodynamic conditions (13). These features are particularly important in small-caliber grafts, where even modest thrombus formation or neointimal thickening can compromise luminal patency. Recent work has shown that endothelialization may be promoted not only by pre-seeding endothelial cells, but also through host-cell-mediated mechanisms and scaffold-driven recruitment of circulating vascular or immune cell populations (16-18). For example, circulating monocytes have been shown to contribute to endothelialization of arterial grafts under appropriate biochemical and biomechanical cues, while peptide-functionalized grafts and bioactive scaffold designs have been developed to enhance endothelial colony-forming cell capture, angiogenic activity and vascular tissue reconstruction (16-20). These recent findings support the view that TEVG success depends on the coordinated interaction between scaffold properties, host response and endothelial cell source.

Endothelialization is central to TEVG function because the endothelial layer regulates the blood–graft interface. A stable and functional endothelium provides an antithrombotic surface, supports nitric oxide-mediated vascular regulation, reduces platelet adhesion, limits inflammatory cell recruitment and suppresses pathological vascular smooth muscle cell migration (15, 16). Therefore, the cell type used to establish endothelial coverage is a major determinant of graft performance. Candidate cells differ substantially in endothelial maturity, proliferative capacity, shear-stress resistance, immunogenicity, thrombogenicity, scalability and translational safety (13, 14, 21). Recent studies have highlighted several approaches, including human induced pluripotent stem cell-derived endothelial cells, endothelial colony-forming cell capture, scaffold functionalization, flow-based endothelial conditioning and multicellular vascular models incorporating vascular smooth muscle cells (14, 17, 21, 22). However, much of this evidence remains preclinical, and no single cell source has yet resolved all requirements for durable, safe and clinically scalable TEVG endothelialization (13-15). This review therefore evaluates the principal cell types investigated for TEVG endothelialization, comparing their biological advantages, limitations and translational potential.

While several reviews have examined isolated aspects of vascular graft engineering, few have directly compared current cell types across endothelial functional performance, mechanistic properties, and translational feasibility. Therefore, this review aims to evaluate the current cell types investigated in cardiovascular tissue engineering and to discuss their advantages, limitations, and potential clinical applications. First, the review outlines limitations of existing grafting approaches, then evaluates autologous and stem-cell-derived endothelial sources, and finally highlights translational considerations for future TEVG development.

Management of CAD

The treatment strategies for CAD mainly depend on the severity of arterial obstruction and the overall condition of the patient. The initial management often includes optimal medical therapy together with percutaneous coronary intervention (PCI), which involves angioplasty and stent implantation. These approaches are generally preferred in patients without total occlusion of the left anterior descending artery (LAD) or in the absence of other complicating vessel blockages (5). In contrast, when complete occlusion is present, coronary artery bypass grafting (CABG) becomes necessary to relieve anginal symptoms and restore myocardial function and viability (5). Overall, CABG is considered the standard revascularization method for patients with small-artery blockage and multivessel disease because of its long-term clinical benefits. It has been reported that approximately 400,000 CABG surgeries are performed annually in the United States in patients with advanced CAD, highlighting the widespread use of this procedure (5). However, while clinically important, these treatment decisions do not address the underlying vascular biological limitations, particularly endothelial dysfunction and loss of antithrombotic signaling, that directly motivate the development of bioengineered grafts (3, 6).

Autologous vessels are the most commonly used conduits in coronary bypass surgery. These include the saphenous vein graft (SVG) and the mammary arteries, such as the right internal mammary artery (RIMA) and the left internal mammary artery (LIMA). The RIMA and LIMA are typically anastomosed to the right and left anterior descending arteries, respectively (Figure 2). In addition to these, other autologous grafts such as the radial artery have been utilized and were found to provide superior long-term patency rates ranging between 5 and 15 years compared with SVG (Table 1)(6). The choice of graft is usually determined by factors such as the patient's surgical history, comorbid conditions, and the specific site of obstruction. Moreover, the selection of arterial grafts often depends on the surgeon's experience and preference, as the implantation of multiple arterial grafts can be technically demanding and more time consuming compared with venous grafts (6). These conduit-related considerations further highlight the continuing reliance on native vessels and underscore the clinical need for alternative grafts capable of providing durable endothelial stability and improved biological compatibility (5, 11).

Despite their advantages, autologous grafts, particularly SVGs, are associated with a high risk of VGF, a major postoperative complication that can lead to total graft occlusion and subsequent impairment of blood flow to the revascularized region. VGF can result in severe cardiac outcomes, including recurrent angina, myocardial infarction, and even death (5). The underlying pathological mechanisms of graft failure are multifactorial and primarily related to endothelial injury and insufficient endothelialization. These alterations trigger a series of pathological events that include acute thrombosis, vascular inflammation, neointimal hyperplasia, and late-stage

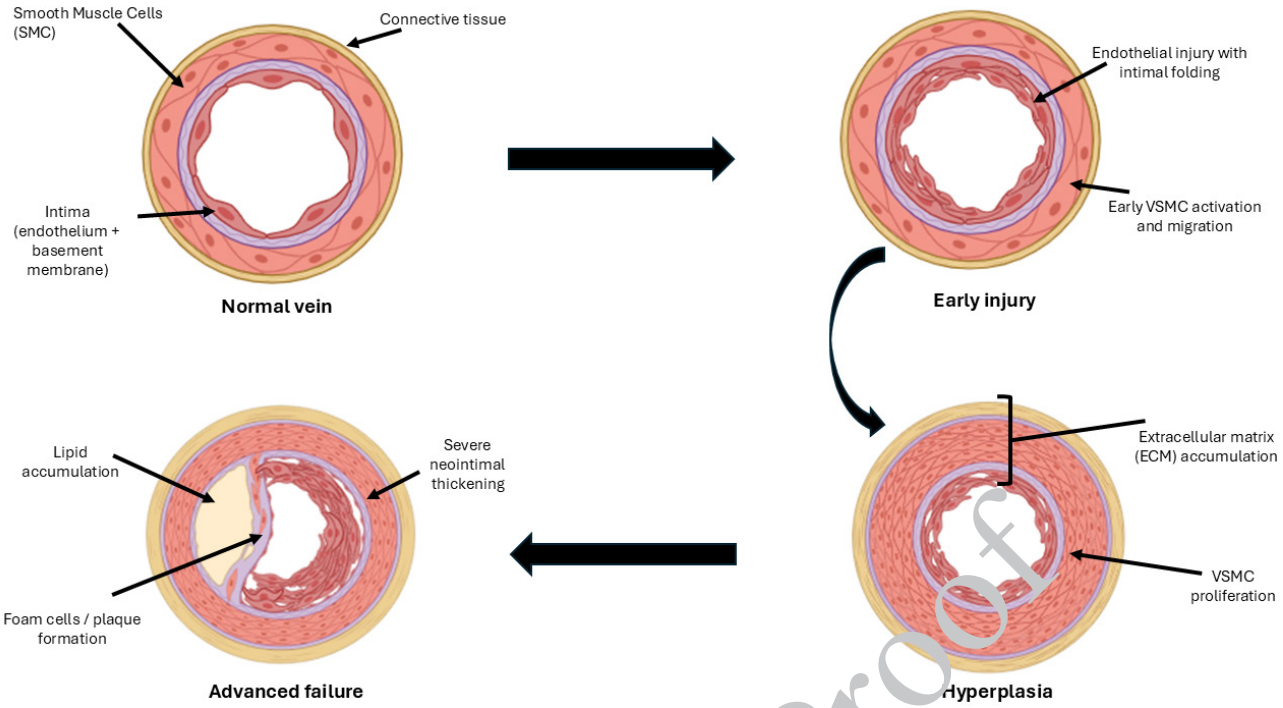

Figure 2. Stages of vein graft adaptation and failure following arterial implantation
The diagram illustrates the key structural and cellular changes occurring in saphenous vein grafts after exposure to arterial pressure and shear stress. Normal vein: The venous wall consists of an intact endothelial monolayer supported by the basement membrane, a media layer composed of smooth muscle cells (SMCs), and an outer connective tissue adventitia. Early injury: Arterialization leads to endothelial injury with characteristic intimal folding, accompanied by early SMC activation and migration toward the intima. Hyperplasia: Progressive neointimal thickening occurs due to SMC proliferation and extracellular matrix (ECM) accumulation, resulting in luminal narrowing. Advanced failure: Chronic inflammation, lipid infiltration, and foam cell accumulation contribute to plaque formation and severe neointimal thickening, ultimately leading to graft stenosis and failure. Figure created with BioRender.com
SMC, smooth muscle cell; VSMC, vascular smooth muscle cell; ECM, extracellular matrix

Table 1. Summary of the main advantages and disadvantages of cell types used for endothelialization of TEVGs and their current clinical findings when applicable

Cell type	Clinical studies	Advantages	Disadvantages
EPCs	EPC-based therapies are safe and beneficial for organ recovery in human subjects e.g. vascular injury and neurological disease EPCs-derived TEVGs have not been clinically studied	Noninvasive harvesting Capability to differentiate into ECs Angiogenesis and vascular homeostasis properties	Few sources of EPCs Difficult to isolate The lack of standard detection and cultivation methods
MSCs	Approximately 470 Clinical studies in different phases including 70 trails in CHD area have shown that MSCs are safe However, MSCs-derived TEVGs have not been investigated in clinical trials	Anti-inflammatory and immunosuppressive ability Secret cardio-protection factors Can be obtained from countless types of tissue Multipotent differentiation potential Thromboresistant	Restricted multipotency Senescence leads to fast reduction of differentiation potency on *in vitro* environment
ESCs	ESCs seeded grafts have not been clinically investigated yet	Multipotent differentiation into various cell types including ECs and VSMCs	Ethical concerns The risk of tumorigenesis.
HUVECs	HUVECs are frequently in various areas of tissue engineering including bone, muscles and vascular tissue engineering However, HUVECs-derived TEVGs have not been clinically studied due to more safety issues	Noninvasively obtained Rabidly and easily isolated Widely studied in tissue engineering field Morphology and extracellular matrix similar to saphenous vein cells Express range of EC markers	Limited capability to durably adhere to grafts Risk of cells washout and subsequent thrombosis
iPSCs	iPSCs-derived TEVGs have not been clinically studied due to more safety issues	Can differentiate into several cell types High proliferation capacity Anti-inflammatory properties and low risk of immune repossess Obtained noninvasively Wide range of cell sources	Risk of tumorigenesis epigenetic alteration because of reprograming process High cost and time consuming

TEVGs, tissue-engineered vascular grafts; ECs, endothelial cells; VSMCs, vascular smooth muscle cells; EPCs, endothelial progenitor cells; MSCs, mesenchymal stem cells; ESCs, embryonic stem cells; HUVECs, human umbilical vein endothelial cells; iPSCs, induced pluripotent stem cells; CHD, coronary heart disease

atherosclerosis, all of which contribute to graft occlusion (7). These biological failures, loss of endothelial integrity, impaired nitric oxide signaling, and pro-thrombotic remodeling, directly define the functional criteria that engineered endothelial cell sources must meet to support durable TEVG performance (6, 23).

Tissue-engineered grafts (TEGs)

In current clinical practice, synthetic grafts such as Dacron and polytetrafluoroethylene are frequently used for large-diameter vascular replacements (>6 mm) because they provide excellent mechanical strength and satisfactory long-term patency (24). However, their use in small-diameter applications (<6 mm), including coronary artery bypass grafting, is limited, as these grafts are associated with poor biocompatibility and high thrombogenicity leading to early graft failure (24). Abbott *et al.* (25) reported that, after five years, large-diameter synthetic grafts demonstrated a patency rate of around 90%, whereas small-diameter grafts achieved only about 30%. Similar observations were made in animal studies, where patency rates ranging between 0 and 25% were reported within weeks or months following implantation of small-diameter synthetic grafts (26, 27). Therefore, synthetic grafts are not recommended for coronary artery bypass procedures and cannot be routinely considered as reliable alternatives to autologous conduits. These findings underscore the fundamental biological limitation of synthetic materials, the absence of a functional endothelium capable of regulating thrombosis, inflammation, and vascular tone, which is critical for small-caliber graft patency (8, 11).

To overcome the limitations associated with both autologous vessels and synthetic prostheses, extensive research has focused on the development of TEVGs (11). The main goal of vascular tissue engineering is to recreate a graft that closely mimics the native vessel in structure and function by integrating appropriate biomaterials, mechanical cues, and biological signals (11). TEVGs are generally fabricated by seeding biodegradable scaffolds with autologous or stem-cell-derived populations, such as vascular cells, somatic cells, embryonic stem cells (ESCs), or endothelial progenitor cells (EPCs)(8, 24). A critical advantage of TEVGs is their capacity to support *in situ* endothelialization, enabling the establishment of a stable, antithrombotic endothelial lining capable of producing nitric oxide and modulating vascular remodeling, features essential for long-term patency (8, 11). These bioengineered constructs have shown promising results in preclinical studies, offering improved endothelialization and reduced thrombogenicity compared with conventional synthetic grafts. Nevertheless, their long-term success remains highly dependent on the selection of an optimal endothelial cell source, making the evaluation of available cellular candidates a key focus for advancing TEVG design and clinical translation (8, 12).

Cells used for endothelialization of TEVGs

To prevent thrombosis and neointimal hyperplasia following graft implantation, establishing a continuous luminal layer of endothelial cells (ECs) is crucial. This endothelial monolayer mimics the physiological function of native blood vessels by providing an antithrombogenic surface, reducing platelet aggregation, and inhibiting VSMC proliferation and migration into the vessel lumen, which would otherwise result in vascular stenosis (8). Beyond these protective functions, the endothelium also regulates nitric oxide (NO) bioavailability, vascular tone, and inflammatory signaling, all essential properties that TEVG-seeded cells must replicate to achieve long-term patency (9, 10). Several studies have demonstrated that re-endothelialized TEVGs exhibit significantly higher patency rates compared with non-endothelialized grafts, confirming that complete endothelial coverage is essential for long-term graft success. The primary cellular sources utilized for TEVG endothelialization include EPCs, ESCs, human umbilical vein endothelial cells (HUVECs), induced pluripotent stem cells (iPSCs), and mesenchymal stem cells (MSCs)(24). However, these cell types differ markedly in their ability to maintain endothelial phenotype stability, antithrombotic behavior, and mechanical integration, core criteria that determine their suitability for TEVG applications (11, 12). Table 1 summarizes the main advantages and disadvantages of each cell type and presents relevant preclinical and clinical findings when available.

Endothelial progenitor cells (EPCs)

EPCs are commonly described as bone marrow-derived circulating progenitor cells with the potential to contribute to endothelial repair and neovascularization. EPCs can also be obtained non-invasively from peripheral blood or umbilical cord blood, reducing the need to harvest mature vascular tissue from the patient (31, 32). Circulating EPCs are often implicated in angiogenesis, vascular homeostasis and endothelial repair, although EPC identity remains heterogeneous and no single marker uniquely defines this population (33, 34). EPCs and related endothelial progenitor populations share several endothelial features with mature ECs, including the expression of cluster of differentiation 31 (CD31), von Willebrand factor (vWF), vascular endothelial cadherin (VE-cadherin) and vascular endothelial growth factor receptor 2 (VEGFR-2), which are associated with endothelial identity, vascular integrity and neovascularization (32, 33, 35). Two broad EPC subtypes are commonly described: early-growth EPCs and late-growth EPCs. Early-growth EPCs primarily support angiogenesis through paracrine secretion of pro-angiogenic cytokines, whereas late-growth EPCs, also referred to as endothelial colony-forming cells, exhibit higher proliferative potential and greater capacity to form endothelial-like monolayers, making them particularly relevant for re-endothelialization strategies. The use of EPCs in vascular tissue engineering has been supported by *in vivo* investigations. Kaushal *et al.* (36) reported that small-diameter grafts seeded with peripheral blood-derived EPCs maintained patency for approximately 130 days after implantation in sheep, compared with only 15 days for non-seeded grafts. The EPC-seeded grafts also exhibited contractility and nitric oxide (NO)-mediated vasodilation similar to native carotid arteries, suggesting that EPC seeding can improve graft patency and support endothelial-like functional behavior in preclinical TEVG models.

Interestingly, EPCs derived from cord blood appear to outperform those from peripheral blood. A study (37) reported that cord blood EPC-seeded grafts displayed improved stability and sustained physiological function, remaining viable for nearly four months, whereas

peripheral blood EPC grafts were stable for less than three weeks following implantation in mice. Additionally, cord blood EPCs formed a dense and stable vascular network lasting over 77 days, while peripheral EPCs produced sparse vessel formation. These findings highlight the superior regenerative potential of cord blood-derived EPCs for vascular applications. Despite their promising properties, several challenges limit the clinical translation of EPC-based therapies. The major drawbacks include the lack of specific markers for EPC identification, their limited numbers in circulation and bone marrow, and the complex isolation and culture procedures that hinder the establishment of standardized protocols for their detection and expansion (38, 39). These limitations present significant barriers to reproducibility and scalability, reinforcing the need to compare EPCs against alternative endothelial cell sources that may offer greater stability, availability, or translational feasibility within TEVG development (11, 12).

Mesenchymal stem cells (MSCs)

MSCs, also referred to as stromal cells, are multipotent progenitor cells that can be isolated from bone marrow and a wide range of other tissues (40). Additional sources of MSCs include amniotic fluid, the amniotic membrane, and the umbilical cord, all of which provide accessible and ethically acceptable material for isolation. MSCs are thrombo-resistant and possess the capacity to self-renew and differentiate into multiple cell lineages, including VSMCs and endothelial cells (ECs)(41, 42). Extensive research has shown that MSCs secrete several biologically active molecules contributing to cardioprotection, such as angiogenic, mitogenic, and homing factors, as well as anti-apoptotic mediators (43). Their anti-inflammatory and immunomodulatory effects are mainly attributed to the release of soluble factors like hepatocyte growth factor (HGF) and transforming growth factor beta (TGF-β), both of which play an essential role in regulating VSMC proliferation, migration, and extracellular matrix (ECM) deposition (23). Furthermore, MSCs can modulate the immune response by suppressing the activation of natural killer (NK) cells through the expression of surface receptors and signaling molecules, including Toll-like receptors (TLRs) and programmed death ligand-1 (PD-L1)(44, 45). Consequently, MSCs may help limit inflammation, immune activation, and thrombosis, processes that collectively contribute to graft failure. Over the past three decades, MSCs have been intensively investigated in vascular tissue engineering, where they have been induced to differentiate into VSMCs capable of synthesising vascular tissue resembling the tunica media of native blood vessels (46, 47). The engineered TEVGs produced from MSCs were reported to be both histologically and molecularly comparable to natural vessels. A study (48) demonstrated in an ovine model that mesenchymal-derived grafts implanted into carotid arteries remained patent, antithrombogenic, and mechanically stable for up to five months, whereas non-seeded grafts became occluded within two weeks post-implantation. Similarly, researchers (49) evaluated human thymus-derived MSCs (hT-MSCs) in a piglet pulmonary artery model and found that the grafts remained patent for more than three months without evidence of stenosis, thrombosis, or degeneration. Macroscopic assessment revealed that hT-MSC-seeded grafts developed both a VSMC layer and an endothelial lining comparable to those observed in native arteries. Notably, this study was the first to demonstrate that hT-MSCs represent a feasible and effective cell source for TEVG construction. Despite these strengths, MSC-derived endothelial-like cells often exhibit limited long-term phenotypic stability and reduced NO-producing capacity compared with mature ECs, factors that may compromise their performance in TEVG endothelialization (9, 10).

Despite the therapeutic potential of MSCs, several challenges limit their widespread application. One major issue is the difficulty of obtaining sufficient cell numbers from a single donor source, often requiring the collection of MSCs from multiple tissues. Additionally, MSCs display restricted multipotency and tend to lose their differentiation capacity during prolonged *in vitro* culture, mainly due to cellular senescence (29, 43). Nevertheless, MSCs are considered safe for clinical use, although further investigations are required to confirm the long-term safety and functional stability of MSC-derived TEVGs before their routine application in clinical settings (50, 51). These limitations highlight the need for careful comparison between MSCs and other endothelial cell candidates to determine which populations provide the most reliable endothelial function, durability, and translational feasibility in TEVG applications (11, 12).

Embryonic stem cells (ESCs)

ESCs are pluripotent and self-renewing cells isolated from preimplantation embryos at the blastocyst stage (52, 53). Both *in vitro* and *in vivo* experiments have shown that human ESCs (hESCs) can differentiate into ECs and VSMCs through several induction methods, including stromal cell co-culture and embryoid body formation (54, 55). The differentiation potential of ESCs is regulated by multiple factors such as biomechanical stress, cytokines, and growth factors. Among these, leptin and vascular endothelial growth factor (VEGF) play an essential role in vascular permeability, cell–cell adhesion, and neovascularization through their interaction with VEGF receptor 2. Leptin has been shown to promote ESC differentiation into ECs and stimulate angiogenesis indirectly by enhancing VEGF signaling via leptin-mediated activation of VEGF receptors. Interestingly, Kurtovic *et al.* (56) suggested that leptin may induce ESC differentiation by activating the signal transducer and activator of transcription 3 (STAT3) and initiating the JAK/STAT signaling pathway, which contributes to EC and VSMC proliferation and migration.

Transforming growth factor beta (TGF-β) has also been found to play a key role in ESC differentiation. Up-regulation of TGF-β promotes ESC transition into functional VSMCs (56, 57). Researchers (58) reported a strong correlation between TGF-β1 signaling and the expression of VSMC-specific markers, with inhibition of this pathway leading to reduced VSMC differentiation. Conversely, suppression of TGF-β activity while enhancing the Notch1 pathway was shown to increase ESC differentiation into ECs and promote neovascularization (59, 60). Researchers (61) demonstrated that inhibition of the TGF-β pathway using an inactive protease-activated receptor 1 (PAR1) scaffold facilitated EC differentiation by preventing the interaction between TGF-βRI and TGF-βRII. This inhibition reduced SMAD2 phosphorylation and lowered NANOG expression levels, thereby promoting EC derivation from ESCs (62, 63).

These findings collectively indicate that TGF-β signaling plays a dual and context-dependent role in regulating ESC differentiation into ECs and VSMCs, and further studies are needed to clarify these mechanisms under different culture conditions for use in cardiovascular tissue engineering. Wang *et al.* (64) demonstrated that hESC-derived ECs implanted into severe combined immunodeficient mice successfully integrated with host vasculature and formed stable blood conduits that remained functional for nearly 150 days. Notably, their study employed a two-dimensional culture method instead of the embryoid body approach for EC differentiation. Similarly, a study (57) observed that implantation of hESC-derived ECs into ischemic hindlimb mouse models stimulated significant neovascularization. Despite these encouraging preclinical results, the application of ESCs for constructing TEVGs has not yet been explored in humans due to major safety concerns, particularly the risk of teratoma formation, as well as persistent ethical challenges surrounding the use of human embryonic material. These persistent risks substantially limit ESC suitability for TEVG endothelialization despite their robust differentiation capacity, emphasizing the need to contrast ESCs with safer, more clinically sustainable alternatives such as MSCs, EPCs, or iPSCs (11, 12).

Human umbilical vein endothelial cells (HUVECs)

HUVECs are among the most frequently used endothelial cell (EC) sources in tissue engineering research, including applications in bone, muscle, and vascular constructs, mainly due to their rapid growth and straightforward isolation procedure (9, 65). HUVECs can be obtained non-invasively from the umbilical cord after childbirth, which is generally considered biological waste (66). The umbilical cord contains approximately 30 cm of vascular tissue that provides a sufficient number of fast-growing ECs suitable for seeding scaffolds efficiently (67). HUVECs are considered promising for vascular tissue engineering because they express a wide range of EC markers such as CD31, CD36, ICAM-1, VCAM-1, and endothelin-1, all of which are essential for vascular homeostasis. In addition, HUVECs retain the functional characteristics of native ECs by producing important enzymes, including endothelial nitric oxide synthase (eNOS) and angiotensin-converting enzyme (ACE), both of which regulate vascular tone and endothelial function (10). However, despite their strong endothelial phenotype, HUVECs exhibit limited long-term stability and reduced resistance to shear stress when applied to vascular grafts, which contributes to their poor adhesion and washout from implanted TEVG surfaces (9).

Under *in vitro* conditions, HUVECs isolated from the umbilical artery and vein display myofibroblast-like morphology and generate an extracellular matrix closely resembling that of saphenous vein cells. The derived HUVECs also exhibit similar patterns of growth and tissue organization to those observed in native venous endothelium (68). Hence, umbilical segments represent a practical and valuable autologous cell source for vascular tissue engineering with minimal invasiveness. Schechner *et al.* (69) demonstrated in an early animal study that seeding HUVECs on a collagen–fibronectin scaffold resulted in microvessel formation within 31 to 60 days post-implantation in mice. These findings are consistent with those reported previously (70), who observed enhanced vascular network formation after implanting poly (propylene fumarate)(PPF)/fibrin scaffolds seeded with HUVECs. Moreover, the co-culture of human hMSCs with HUVECs on PPF/fibrin scaffolds significantly improved scaffold vascularization (70). These results suggest that combining MSCs with HUVECs could represent a promising approach for improving graft endothelialization and vascular integration. However, clinical application of HUVEC-seeded grafts remains limited because of their poor long-term adherence to graft surfaces, which leads to cell washout and subsequent thrombosis. This limitation highlights the need to compare HUVECs with more shear-resistant endothelial cell sources, particularly those capable of sustained integration under physiological flow conditions (11).

Induced pluripotent stem cells (iPSCs)

iPSCs have been extensively investigated due to their remarkable therapeutic potential in cardiovascular regeneration and their suitability for constructing TEVGs (71). iPSCs are pluripotent cells with a high proliferative capacity, generated from adult somatic cells through reprogramming using four transcription factors: OCT-3/4, Sox2, Klf4, and c-Myc (72). These cells are considered promising candidates for vascular graft engineering because they can be obtained non-invasively from various autologous sources, such as skin fibroblasts or peripheral blood cells, and present a reduced risk of immune rejection. Moreover, iPSCs possess the ability to differentiate into several vascular cell types, including ECs and VSMCs, as demonstrated previously (73).

Several *in vitro* studies have supported the functional relevance of iPSC-derived cells in vascular grafts. A study (74) reported that grafts seeded with iPSC-derived ECs exhibited vascular architecture and anti-inflammatory profiles comparable to those of autologous vessels. Likewise, a study (75) observed that iPSC-derived ECs promoted vascularization similar to that achieved with embryonic stem cell (ESC)-derived ECs in both *in vivo* and *in vitro* conditions. Furthermore, Margariti *et al.* (76) introduced partially reprogrammed iPSCs (P-iPSCs) generated from fibroblasts using OCT4, SOX2, KLF4, and c-MYC. These P-iPSCs showed stable endothelial morphology and supported functional re-endothelialization in tissue-engineered grafts, with minimal tumor formation risk when applied to ischemic limb models and *ex vivo* systems. Further advancements have been demonstrated by Gui *et al.* (77), who developed TEVGs seeded with human iPSC-derived VSMCs capable of withstanding arterial and surgical pressures after implantation in the rat abdominal aorta. However, postoperative dilation of the grafts was reported, which was attributed to inadequate mechanical strength of the engineered tissue. In contrast, a study (78) successfully produced hiPSC-derived TEVGs with contractile properties, mechanical integrity, and patency comparable to those of native arteries, without evidence of luminal dilation following implantation. These findings collectively indicate that iPSC-derived vascular cells can achieve physiological functionality comparable to native ECs and VSMCs; however, the mechanical performance of iPSC-based TEVGs remains dependent on scaffold design and maturation processes rather than cell source alone (12).

Overall, iPSCs offer a highly versatile and patient-specific

platform for vascular tissue engineering. Nonetheless, several challenges still limit their clinical application. The most concerning issue is the potential for tumor formation resulting from genetic instability during the reprogramming process, which can cause chromosomal rearrangements and oncogenic transformation (79). Therefore, rigorous quality control, molecular screening, and long-term safety evaluation are essential prior to clinical translation, though these requirements inevitably increase the time and cost of graft fabrication. This tumorigenic risk represents a major barrier to clinical translation and necessitates direct comparison of iPSCs with more stable endothelial cell alternatives such as EPCs, MSCs, and HUVECs when selecting optimal TEVG cell sources (11). Consequently, future research should focus on developing safer and more efficient strategies for generating iPSC-derived TEVGs that combine mechanical durability with biological compatibility for clinical use.

Discussion

The findings presented in this review highlight the considerable progress made in cardiovascular tissue engineering, particularly in understanding how different cell types contribute to the development of functional and clinically relevant TEVGs. Although several approaches have been explored, the central challenge remains achieving a stable and fully functional endothelial lining capable of regulating thrombosis, inflammation, vascular tone and long-term graft remodeling. Therefore, the key question is not simply whether a cell type can express endothelial markers, but whether it can generate a durable, hemocompatible and clinically scalable endothelial interface under physiological flow conditions.

Criteria for selecting and evaluating endothelializing cell sources

The selection of cells for TEVG endothelialization should be based on functional performance rather than endothelial marker expression alone. Candidate cells should first demonstrate a stable endothelial phenotype, the ability to form a confluent monolayer, nitric oxide production and anti-thrombotic behavior. This distinction is important because recent preclinical work on fully biological endothelialized vascular conduits showed that hiPSC-derived endothelialization can provide antithrombotic function and graft patency, indicating that functional outcomes are more informative than marker expression alone (15). Cells intended for vascular graft applications must also remain attached and functional under physiological flow. Abutaleb and Truskey directly addressed this issue by differentiating hiPSC-derived vascular endothelial cells and characterizing them under physiological shear stress, supporting the need to test endothelial maturity under hemodynamic conditions relevant to vascular grafts (20). Translational criteria are equally important. Soares *et al.* emphasized that engineered vascular grafts are intended to act as living conduits capable of remodeling and self-repair, but their clinical value depends on overcoming limitations in reproducibility, durability and manufacturability (13). Similarly, it is reported human iPSC-derived TEVGs as a potentially more readily available graft platform, although such approaches still require careful assessment of scalability, safety and long-term function (14). Therefore, cell selection should integrate endothelial function, hemocompatibility, scaffold retention, flow resistance, safety and manufacturing feasibility rather than relying only on cell origin.

Mechanistic differences between candidate cell types

The major cell sources used for TEVG endothelialization differ in their mechanisms of action and should not be considered interchangeable. Mature endothelial cells and HUVECs provide a direct endothelial lining and remain useful for testing endothelial behavior on scaffolds, but their direct clinical use is limited by donor specificity, limited autologous availability and vulnerability to detachment under arterial shear stress. EPCs and endothelial colony-forming cells (ECFCs), in contrast, are more closely associated with endothelial repair and *in situ* endothelialization. This is supported by Tang *et al.*, who developed peptide-functionalized small-diameter vascular grafts designed to capture ECFCs and promote endothelialization, and by Tian *et al.*, who showed that peptide-grafted hydrogels can support ECFC rolling and adhesion (17, 19). These studies suggest that EPC/ECFC-based strategies may be particularly relevant when scaffold surfaces are engineered to recruit circulating endothelializing cells. MSCs may contribute less reliably as true endothelial replacements, but their paracrine and immunomodulatory properties may support remodeling and reduce inflammatory activation. iPSC-derived endothelial cells offer a scalable and potentially patient-specific endothelial source, particularly when differentiation protocols include functional characterization under shear stress, as shown by Abutaleb and Truskey (20). Supporting vascular cells are also important: Duan *et al.* showed that hiPSC-derived VSMCs in a fibronectin-functionalized collagen hydrogel augmented endothelial cell morphogenesis, indicating that VSMCs and pericyte-like support cells may contribute to matrix organization and vascular maturation rather than replacing the endothelial layer itself (22).

Application-specific suitability of different cell sources

The most appropriate cell type is likely to depend on the intended TEVG application. For small-diameter grafts exposed to high thrombogenic risk, the priority is rapid formation of a stable and hemocompatible endothelial layer that can withstand arterial shear stress. Where rapid luminal endothelial coverage is needed, mature endothelial cells, EPCs or ECFCs may be useful, provided that sufficient cell numbers and durable retention can be achieved. For pediatric or congenital cardiovascular applications, grafts with growth and remodeling potential are particularly attractive; Soares *et al.* highlighted this broader goal of engineered vascular grafts as living conduits capable of growth, remodeling and self-repair, while also noting that clinical translation remains challenging (13). In urgent vascular reconstruction, patient-specific cell expansion may be impractical because isolation, differentiation and quality control require time. In that context, host-cell-recruiting or acellular approaches may be more realistic. A study showed that circulating monocytes can contribute directly to endothelialization of acellular arterial grafts under suitable chemical and biomechanical cues, while researchers reported monocyte-associated vascular-like tissue reconstruction on peptide-modified acellular grafts (16, 21). Conversely, for planned reconstructive procedures,

iPSC-derived endothelial and smooth muscle cells may offer a more personalized or standardized vascular graft platform, as illustrated by Luo *et al.*'s report of human iPSC-derived TEVGs (14). These examples indicate that cell-seeded and in situ endothelialization strategies should be viewed as complementary rather than competing approaches.

Translational bottlenecks limiting clinical application

Despite substantial preclinical progress, several barriers continue to limit the clinical translation of cell-based TEVG endothelialization. Many experimental grafts can achieve endothelial marker expression or early luminal coverage, but long-term patency under arterial flow remains difficult to reproduce consistently. Recently fully biological endothelialized vascular conduits was reported that provided antithrombotic function and maintained graft patency in a preclinical setting, but the study also illustrates that such approaches still require further validation before routine clinical application (15). iPSC-derived vascular grafts are similarly promising but technically demanding. Luo *et al.* reported readily available TEVGs derived from human iPSCs, while Abutaleb and Truskey showed that hiPSC-derived vascular endothelial cells require functional characterization under physiological shear stress (14, 20). Together, these studies indicate that iPSC-based strategies require robust differentiation, purification, hemodynamic testing and safety assessment before translation. Broader translational barriers also remain, including patient-to-patient variability in autologous cells, impaired cell function in older or diseased patients, immune compatibility, genetic stability, tumorigenic risk, cost-effective manufacturing, good manufacturing practice compliance and regulatory approval. As a study emphasized, engineered vascular grafts have substantial promise, but reproducibility, long-term durability and clinical scalability remain central obstacles (13).

Future strategies to improve cell performance and safety

Future TEVG development should focus on integrating cell performance, scaffold design and biophysical conditioning. For iPSC-derived endothelial cells, directed differentiation should be accompanied by functional testing under flow; Abutaleb and Truskey's protocol for generating and characterizing hiPSC-derived vascular endothelial cells under physiological shear stress provides one example of how endothelial maturity can be assessed under hemodynamic conditions (20). Scaffold functionalization is another important direction. Tang *et al.* developed a peptide-functionalized small-diameter vascular graft to capture ECFCs, while Tian *et al.* demonstrated that peptide-grafted hydrogels can support ECFC rolling and adhesion, suggesting that surface chemistry can be used to direct early endothelial recruitment (17, 19). Biomaterial composition may also be used to regulate endothelial behavior: Researchers fabricated copper-doped bioactive glass-containing small-diameter grafts and reported enhanced angiogenic activity and endothelial regeneration-related responses, although further long-term validation is required (18). Future designs should also consider host-mediated remodeling, as researchers showed that monocytes or monocyte subpopulations can contribute to endothelialization or vascular-like tissue reconstruction under appropriate scaffold and signaling conditions (16, 21). Finally, multicellular strategies may improve vascular maturation; Duan *et al.* showed that hiPSC-derived VSMCs can augment endothelial morphogenesis in a fibronectin-functionalized collagen hydrogel, supporting the inclusion of VSMCs or pericyte-like support cells in future graft designs (22). Together, these studies suggest that TEVG endothelialization may be improved by combining directed differentiation, endothelial purification, shear-stress conditioning, scaffold functionalization, host-cell recruitment and standardized potency assays before implantation.

Conclusion

Over the past few decades, cardiovascular tissue engineering has been extensively investigated with the aim of improving graft surface characteristics, mechanical strength, and biological functionality. The ideal coronary tissue-engineered vascular graft (TEVG) should not only exhibit strong biocompatibility and mechanical resistance to physiological stress but also possess the capacity for self-repair and remodeling to prevent thrombosis and long-term graft failure. Achieving these characteristics largely depends on identifying suitable seed cells that can differentiate into both VSMCs and ECs without compromising safety or multipotency. Although several cell types, including MSCs, iPSCs, and ESCs, have shown promising potential in *in vitro* studies, the absence of clinical trials remains the primary limitation preventing their translation to clinical use. Moreover, preclinical evidence consistently shows that the long-term performance of TEVGs is determined not only by scaffold design but also by the endothelial phenotype achieved by the selected cell source, reinforcing the importance of rigorous comparative evaluation among available cellular candidates. Therefore, future research should prioritize conducting well-designed clinical studies to evaluate the safety, functionality, and long-term outcomes of cell-derived TEVGs. Furthermore, developing safer, more efficient, and cost-effective grafts that can be produced in a shorter time frame will be essential to meet increasing clinical demand and advance the practical application of TEVGs in cardiovascular surgery. Such advancements will require integrating insights from vascular biology, stem cell engineering, and biomaterials science to establish clinically viable TEVGs capable of matching or surpassing the performance of current autologous grafts.

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Authors' Contributions

MKA E, S E, A K, AA E, S A and M A and made substantial contributions to the concept and design of this review and participated in the acquisition, analysis, and interpretation of the literature. All authors contributed to drafting the manuscript and critically revising it for important intellectual content. All authors approved the final version of the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest related to the content of this review.

Declaration

During the preparation of this manuscript, the authors used an AI-assisted tool (Chat-GPT) to support language editing and organization of the manuscript. The authors reviewed and edited the content after using this tool and take full responsibility for the accuracy, integrity and final content of the manuscript.

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