Endothelial Dysfunction and Cardiovascular Disease: Critical Target for Cell-Based Therapies

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Authors' contributions

This work was carried out in collaboration between all authors. All authors contributed to writing, critical review and editing of this manuscript. Author NMV created Fig. 1. All authors read and approved the final manuscript.

Review Article

ABSTRACT

This review briefly summarizes how cell-based therapeutic interventions are being developed and applied to treat endothelial dysfunction (ED) as a critical clinical target. ED directly contributes to the onset and prognosis for all of the major forms of cardiovascular diseases, including atherosclerosis, pulmonary artery hypertension, peripheral hypertension, stroke, myocardial infarction and congestive heart failure. Current pharmacological therapies used to treat ED are discussed and compared with newer strategies employing endothelial progenitor cells (EPCs) and other stem cells for tissue repair/regeneration therapies. Cell-based therapies to treat ED are still largely experimental but they are emerging in the clinic and represent a promising avenue for new interventional options to combat cardiovascular disease and improve patient outcomes.

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ABBREVIATIONS

ACEI: Angiotensin-converting zyme inhibitors; Ang-1:Angiopoietin-1; CAD:Coronary artery disease; CCBs: Calcium channel blockers; CPCs: Cardiac progenitor cells; ECs: Endothelial cells; ED: Endothelial Dysfunction; eNOS: Endothelial nitric oxide synthases; EPCs: Endothelial progenitor cells; ET1: Endothelin-1; Flk1: Fetal Liver Kinase 1; Flt-1: FMS-like tyrosine kinase-1 (Yoshida et al. 1987); GATM: Amidinotransferase; CSF: Granulocyte colony-stimulating factor; HGF: Hepatocyte growth factor; HMGCoA: 3-hydroxy-3-methyl-glutaryl-CoA; IDDM: Insulin-dependent diabetes; IGF-1:Insulin-like growth factor-1; IL-17: Interlukin- 17; iNOS: Inducible oxide synthase; MCP-1: Monocyte chemoattract protein- 1; M-CSF: Macrophage colony stimulating factor; MNCs: Mononuclear cells; NO: Nitric oxide; PAH: Pulmonary artery hypertension; PE: Proepicardium; PPARɣ: Peroxisome-proliferator activated receptor ɣ; ROS: Reactive oxygen species; SMCs: Smooth muscle cells; SP cells: Side population cells; TNF: Tumor Necrosis Factor; VCAM-1: Vascular cell adhesion molecules-1; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor; Vwf: Von Willebrand factor;

1. INTRODUCTION

From an anatomical perspective, endothelial cells essentially form a single layer of flat cells lining the circulatory system; while from a physiological viewpoint, the endothelium itself functions as an active organ. It extends from the vascular lumen to the arterial wall and into the vasa vasorum in the adventitia. Besides providing protection to the inner wall, the endothelium regulates vessel tone, permeability, coagulation, response to inflammation and angiogenesis. It produces a plethora of cell mediators. The main *vasodilating* mediators are nitric oxide (NO), prostacyclin, bradykinin and endothelium-derived hyperpolarizing factor [1,2]. The latter is an "umbrella" term for the substances and signals that induce hyperpolarization of vascular myocytes through opening of voltage channels [3,4]. Endothelial cells also produce vasoconstrictors including endothelin-1 (ET1), serotonin, and thromboxane [5].

Endothelial dysfunction (ED) can lead to pathological conditions where imbalances of substances with vasodilating, antimitogenic and antithrombogenic properties (collectively called "endothelium-derived relaxing factors") and substances with vasoconstricting, promothrombotic, and proliferative characteristics (collectively known as "endothelium-derived contracting factors") occur [3-6]. The final common end-reaction is decreased NO availability, a gas derived from l-arginine via enzymatic catalysis by endothelial NO synthases (eNOS). Reduction in NO could be due to decreased production (e.g., defective eNOS expression/activity) and/or increased degradation due to oxidative radicals or superoxides [7]. The specific mechanisms are likely variable and remain undefined in most cases.
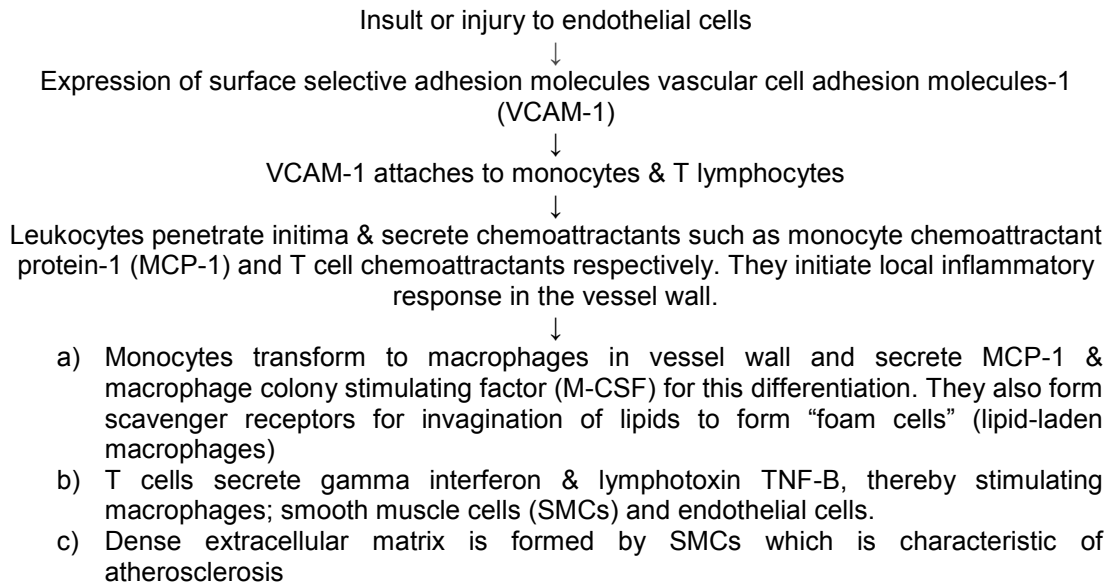
2. CLINICAL CONDITIONS ASSOCIATED WITH ENDOTHELIAL DYSFUNCTION (ED)

2.1 Atherosclerosis

It has been postulated that atherosclerosis arises as a result of injury to arterial endothelium ("Response to Injury Theory") [8]. Multiple insults can trigger these events either alone or in

combination. They include but are not necessarily limited to physical injury [4], stress (hypertension) [4], free radicals (smoking) [9], low density lipoproteins (hypercholesterolemia) [9,10], insulin resistance (diabetes) [11] and homocysteine (homocysteinemia) [9,12]. A brief outline of key steps in the onset of atherosclerotic disease is provided here as adapted from Libby et al. [6]:

Sequence of Events from Endothelial Cell Injury to Atherosclerosis

Insult or injury to endothelial cells
↓
Expression of surface selective adhesion molecules vascular cell adhesion molecules-1 (VCAM-1)
↓
VCAM-1 attaches to monocytes & T lymphocytes
↓
Leukocytes penetrate initima & secrete chemoattractants such as monocyte chemoattractant protein-1 (MCP-1) and T cell chemoattractants respectively. They initiate local inflammatory response in the vessel wall.
↓

a) Monocytes transform to macrophages in vessel wall and secrete MCP-1 & macrophage colony stimulating factor (M-CSF) for this differentiation. They also form scavenger receptors for invagination of lipids to form "foam cells" (lipid-laden macrophages)
b) T cells secrete gamma interferon & lymphotoxin TNF-B, thereby stimulating macrophages; smooth muscle cells (SMCs) and endothelial cells.
c) Dense extracellular matrix is formed by SMCs which is characteristic of atherosclerosis

ED precedes atherosclerosis, as evident by impaired vasodilation with invasive intracoronary acetylcholine testing [13]. Brachial artery ultrasound is an effective noninvasive tool to measure endothelial cell function. Using a blood pressure cuff to induce acute occlusion of the brachial artery followed by its release leads to reactive hyperemia. This causes shear stress and release of NO resulting in vasodilation. This phenomenon is called *flow mediated vasodilation* or FMD [14]. It is impaired in patients with ED even without significant coronary artery disease (CAD) [15]. ED is a marker for cardiovascular atherosclerosis risk and is observed in patients with chronic kidney disease [16], systemic hypertension [17], smoking [18], dyslipidemia [19], ageing patients [20], diabetes mellitus [12], obesity [21], hyperhomocysteinaemia [22] and patients with inflammatory or infectious diseases [23,24]. ED is probably the final biological pathway by which these cardiovascular risk factors exert their propensity to develop and progress into atherosclerosis [25]. Multivariate analysis of approximately 2500 patients with mean follow-up between 1 and 92 months for hard cardiac events (death, myocardial infarction and need for revascularization) showed a hazard ratio of +2.0 to +5.0. This analysis showed strong independent association between ED and adverse cardiovascular events [26]. Assuming ED provides an integrated functional risk assessment, the question of whether endothelial function might be a better predictor for cardiovascular events is intriguing and needs further testing with large randomized controlled studies [25].

2.2 Pulmonary Artery Hypertension (PAH)

ED also has profound effects on the development of pulmonary artery hypertension (PAH) [27,28]. Dysfunctional endothelial cell proliferation and overzealous concurrent neo-angiogenesis results in formation of plexiform lesions, a common pathological finding in PAH [28]. The mechanism responsible for ED in PAH is unknown but in genetically predisposed individuals, the process may be initiated by autoimmunity, shear stress due to high blood flow in the pulmonary area, alveolar hypoxia and viral infections [29]. NO down-regulates the production of ET-1 and is partially responsible in maintaining pulmonary vaso-relaxation [30-32]. It also enables protection with respect to the vasoconstriction in lungs due to hypoxia [31,33] and it constrains the proliferation of smooth muscle and aggregation of platelets [34]. Prostacyclin is a growth factor that may possess potent pulmonary vasodilator and anti-platelet aggregating properties [23] that have been exploited therapeutically in the treatment of this disease. Vascular endothelial growth factor (VEGF) is another growth factor specific for endothelium that has a protective effect against some of the pathological changes in PAH. It attenuates neointima formation at the site of tissue injury [35], boosts the expression of plasminogen activator [25] and reestablishes the endothelial vasoreactivity in injured tissue [35,36]. It also brings about the production of NO and prostacyclin in aortic and pulmonary vasculature [37]. Therapies that target ED may hold promise in prevention and treatment of PAH [28].

2.3 Hypertension

Abnormal endothelial function is suspected in the pathophysiology of hypertension [38,39]. Persistent decreased availability of NO was considered the central mechanism. The lack of NO induces vascular abnormalities resulting in end organ (heart, brain and kidney) damage in these patients. ED in hypertensive patients suffering from Type 2 Diabetes mellitus results in aortic stiffness as compared to the hypertensive patients not suffering from Type 2 diabetes mellitus [40]. Further, Perticone et al. [39] showed that ED has a direct link with hypertensive vascular complications. Based on this information, they proposed that abnormal FMD may be a useful marker for future cardiovascular events in subjects with untreated essential hypertension. Angiotensin-converting enzyme inhibitors (ACEI) and calcium channel blockers (CCBs) showed maximum therapeutic benefit but the effect on ED was not statistically significant [39,41]. While CCBs are widely used to treat hypertension, their efficacy with respect to ED remains an open question, though there is strong theoretical rationale for why they should be beneficial [42,43,44]. Positive benefits have been reported for newer generation beta-blockers such as nebivolol, which may exert anti-proliferative, anti-inflammatory and anti-oxidative properties via stimulation of NO release [45,46]. Nebivolol is a potent beta1-selective antagonist, which accounts for its antihypertensive effects but it also appears to have partial agonist properties with respect to beta-3 adrenergic receptors, which may explain how it affects NO release [47,48]. Such combinatorial drug activities may be desirable in a variety of clinical settings, and thus remains a strong focus of ongoing scientific and clinical investigation.

Hypertension in arteries may be related, in part, to the gain of reactive oxygen species (ROS) that can scavenge NO, thereby leading to decreased NO-induced vaso-relaxation [49]. One potential generator of ROS is NADPH oxidase since inhibition of this enzyme resulted in decreased ROS in aortas of year-old rats [49,50]. Consequently, investigations continue to explore the utility of treating ED by blocking ROS formation and enzymes such as NADPH oxidase may serve as potential therapeutic targets in the treatment of

hypertension. Another study showed that the levels of pro-inflammatory cytokine interleukin-17A (IL-17) are increased in patients with hypertensive autoimmune diseases [51]. IL - 17 decreases NO produced by endothelium and activates RhoA/Rho-kinase [51]. It was seen that by inhibiting IL- 17 or Rho- kinase, hypertension could be prevented, thereby suggesting additional novel targets for pharmacological intervention to prevent and/or treat patients with hypertensive autoimmune diseases.

2.4 Congestive Heart Failure (CHF)

Endothelium-dependent NO-mediated vasodilation is reduced in patients with congestive heart failure (CHF) specifically in areas like skeletal muscle, coronary and pulmonary circulation as compared to the test healthy individuals [52,53]. Studies show that impaired endothelial function is linked with decreased exercise capacity, impaired functional capacity and increased mortality in patients suffering from CHF. The increased mortality could be related to coronary and microvascular atherosclerotic changes leading to recurrent myocardial ischemia and necrosis [54]. The authors of this study concluded that there was an association between markers of ED and subsequent clinical outcomes in patients with CHF [54].

In heart failure, the myocardium releases a number of inflammatory messengers and along with altered gene expression, triggers atherosclerosis, which leads to oxidative stress and reduction in the synthesis of NO [55,56]. This, in turn, leads to uncoupling of eNOS and enhanced release of cytokines leading to increased oxidative stress [57-59]. All of these factors together lead to heart failure and its related risk factors, including diabetes, hypertension, etc. [59]. The severity of heart failure can often be correlated with the degree of ED [59]. Factors that improve endothelial function may prevent or delay heart failure [59]. For example, it was found that the endothelium-dependent vasodilation and the availability of the NO was improved with exercise training and with the increase in peak oxygen uptake [59]. Drugs that have similar effects and have shown some benefit clinically include angiotensin-converting enzyme (ACE) inhibitors and newer generation beta-blockers such as nebivolol and carvedilol, which also have vaso-dilating properties that decrease oxidative stress and improve endothelial function [59].

3. DRUGS AFFECTING ED

While there are many drugs that affect ED, we will limit our focus to three types of widely-used clinical drugs. These include: Asprin, 3-hydroxy-3-methyl-glutaryl-CoA (HMGCoA) reductase inhibitors (commonly known as statins) and angiotensin-converting-enzyme (ACE) inhibitors.

3.1 Aspirin

Cyclooxygenase-dependent mechanisms are responsible for constriction and is a major contributor for endothelial dysfunction in atherosclerotic vascular disease [60]. It modulates the acetylcholine-induced peripheral vasodilation in patients with atherosclerosis [60]. Aspirin helps in the improvement of ED due to its ability to induce vasodilation, reduce thrombosis, and inhibit progression of atherosclerosis, thereby providing a pathophysiological basis for the beneficial effects of aspirin in atherosclerosis [60]. Many studies have confirmed that aspirin is the primary or secondary choice to assist the prevention of hypertension and other vascular diseases [61-63]. On the other hand, beneficial effects of aspirin need to be

counterbalanced with detrimental side-effects [62-64]. The detrimental effects are mainly attributed to the hemorrhagic events. Substantial improvement was seen in endothelial function and blood pressure of the patients who were co-administered aspirin and statins [65], suggesting that these drugs may act in an additive or synergistic manner to promote vascular health. This discussion leads us to another topic of drug interactions. For example, it has been said that aspirin when given in higher doses might rarefy the advantageous effects of the ACE inhibitors [66] but there has not been consistent agreement regarding this topic [67].

3.2 Statins

In addition to lowering LDL, statins are well known to boost endothelial function because of the other non-lipid lowering (pleiotropic) actions [68-70], that involve enhanced expression of eNOS, negative action of intracellular oxidative stress (on LDL and VLDL) or attenuation of proinflammatory pathways. Statins help in reducing the incidence of cardiovascular events which might be attributed to the improvement of endothelial function through the release of NO [71-73]. Along with their advantageous effects, statins are believed to cause a decrease in the coenzyme Q10 (ubiquinone) levels, which may have detrimental effects on the patients suffering from heart failure [74]; however, it is unclear if the decrease in the CoQ10 definitely limits the positive effects of the statin therapy [74]. It has also been shown that there might be an adverse effect on the function of mitochondria due to administration of statins, and this effect might be worsened due to excessive physical exercises. Thus, uncertainty remains regarding the reduction of CoQ10 in statin-associated myopathy. Consequently, there is a need for additional clinical data to clarify this issue [75]. Recent progress in this area has indicated an interesting potential link between glycine amidinotransferase (GATM) activity, which is responsible for muscle creatine synthesis, and susceptibility to statin-induced myopathy [76]. Recent studies also claim that statins increased the PPAR γ (peroxisome-proliferator activated receptor γ) activity in the macrophages just like ACE inhibitors [77]. Further study is needed to confirm and extend these results.

3.3 ACE Inhibitors

ACE inhibition helps improve the endothelial function by angiotensin II production and bradykinin-dependent NO production [78]. The latter decreases the vascular smooth muscle cell growth and migration, impedes the expression of cell adhesion molecules, prevents platelet aggregation and restores the fibrinolytic balance [37,79]. The effect of telmisartan, an angiotensin-receptor antagonist that also possesses PPAR γ (peroxisome-proliferator activated receptor γ) agonistic properties, improved insulin resistance and vascular endothelial dysfunction by boosting the production of nitric oxide in endothelium [80]. Nevertheless, many differences have been observed amongst the ACE inhibitors regarding specificity of tissue [81]. When four different ACE inhibitors were administered to about 80 patients suffering from coronary artery disease, it was observed that only quinapril showed an enhancement in the FMD of the brachial artery [81]. A recent meta-analysis of 10 clinical trials involving more than 1100 patients suggested that patients taking ACE inhibitors showed improved brachial FMD relative to controls and those receiving other antihypertensive drugs such as beta-blockers or calcium channel blockers, but were not significantly different from those taking angiotensin receptor blockers [82]. Another study showed that there was no substantial vessel dilation in patients suffering from insulin-

dependent diabetes (IDDM) when they were administered with enalapril [83]. These results suggest there are substantial differences in response to ACE inhibitors.

4. ED IN CONTEXT OF CARDIOVASCULAR REPAIR/REGENERATION THERAPIES

4.1 Stem Cell Therapy for Cardiovascular Repair/Regeneration

Cardiovascular disease is the leading cause of death in the western world [16]. The use of stem/progenitor cells to regenerate myocardium is a promising treatment therapy for curing damaged cardiac tissue due to heart failure, ischemic heart disease and many other forms of cardiovascular disorders. Embryonic stem cells are widely studied for regeneration of myocardium [84,85]. Recent findings suggest that there is a population of resident stem cells called cardiac progenitor cells (CPCs) [86-90]. These cells expressed several early cardiac markers such as Nkx2.5, GATA-4 and c-kit [86,89,91,92]. The CPCs also showed stem-cell like characteristics [90]. When injected into the regions of ischemia, these CPCs were shown to generate a new myocardium containing new blood vessels and cardiomyocytes which were derived from cardiac progenitor cells. There are also bone marrow-derived and circulating mononuclear stem cells that currently serve as the source of stem cells for most of the completed and ongoing clinical trials [93]. A major advantage provided by the use of bone marrow-derived and circulation stem cells is that they can be harvested from the same patient for autologous transfer back into that patient, thereby minimizing immune rejection.

4.2 Endothelial Progenitor Cells (EPCs)

Endothelial progenitor cells (EPCs) are a sub-type of bone marrow-derived and circulating cells (cEPCs) that have the capability to differentiate into endothelial cells [94,95]. Therefore, these progenitor cells could serve as an important tool in the process of neo-vascularization to treat ED and associated cardiovascular disease [94,96,97].

EPCs express specific markers on the cell surface, including CD133, CD34 and VEGFR-2. Asahara et al. [98] showed that hematopoietic CD34+ cells have characteristic endothelial phenotypes when allowed to differentiate in ex-vivo conditions [98,99]. There has been much speculation about the types of endothelial progenitor cells and the source from which they are derived. For example, Hur et al. [100] performed an experiment where they grew the total mononuclear cells which were derived from human peripheral blood and found two different types EPCs. These two different types of EPCs were called the early EPCs and the late EPCs. They not only had differences in the morphology, proliferation rate and survival features but also showed expression of different genes. The early EPCs were found to be spindle-shaped whereas the late EPCs showed a more cobblestone-like shape. The early EPCs were quite similar to the ones reported by Asahara et al. [98] and also expressed CD45, Flt-1 (also known as the type I VEGF receptor, VEGFR1), eNOS, vWF and CD31. Despite their differences, early and late EPCs both contribute to neovasculogenesis. The early EPCs secrete angiogenic cytokines, whereas the late EPCs were responsible for the supply of endothelial cells.

Another study showed that bone marrow and the non-bone marrow cells are responsible for formation of endothelial structures. CD133/VEGFR2 cells are widely grown ex-vivo and differentiate into the endothelial progenitor cells [101]. In addition, CD14+/CD34- cells, a type of myeloid cell, differentiate to form endothelial like structures and express markers

specific of endothelial cells. Studies have also shown that non-bone marrow-derived stem cells can lead to formation of new endothelial tissue. For example, one study showed that certain stem cells are resident in the heart, which might also contribute to formation of new blood vessels [88]. However, the many potential sources that these EPCs could be derived from makes it difficult to draw firm conclusions regarding their specific origins. Nevertheless, there is some indication that the number of EPCs circulating may be associated with decreased cardiovascular risk and the migratory ability of the EPCs are affected in coronary heart diseases and other cardiovascular diseases [102,103]. EPC dysfunction might therefore lead to dysfunctional endothelium. Thus, EPC quality as well as quantity is an important factor to consider for gaining the most efficacious results.

Some studies have shown, for example, that the number of EPCs was a critical factor for neovascularization of ischemic regions in the heart [102-104]. Another study showed that transplantation of autologous EPCs helped in increasing the neovascularization in myocardial ischemia [105]. CD31+ mononuclear cells (MNCs) were isolated from the peripheral blood and transplanted in the ischemic heart. After approximately 4 weeks of transplantation the study reported that there was an improvement in ventricular ejection fraction of the rat heart. An additional study showed that gene transfer of the sp-FGF1 gene into the EPCs facilitated neovascularization of chronic myocardial ischemia in a porcine model [106]. Both studies used autologous transplantation of EPCs, which offers significant advantage for circumventing the issues of immune rejections.

4.3 EPCs and Angiogenesis

EPCs have been shown to be mobilized by tissue trauma such as hypoxia induced by myocardial infarction [107]. Mobilization of bone marrow-derived endothelial progenitor cells serves as the body's natural response to ischemia. Whereas minor burns or wounds utilize local endothelial cells for repair, bone marrow-derived endothelial progenitor cells aid in vessel growth after large-scale vascular injury. Not only do these cells play a structural role in angiogenesis but it is thought they also remain in the interstitial space and secrete growth factors, cytokines and protective proteins that promote proliferation and migration of local endothelial cells to further aid in angiogenesis. Among the list of released paracrine factors are VEGF and hepatocyte growth factor (HGF), thought to promote proliferation of endothelial cells. Angiopoietin-1 (Ang-1), which may stabilize newly formed vessels in the damaged tissue is also secreted in paracrine fashion. In addition, released eNOS and inducible nitric oxide synthase (iNOS) are thought to aid in the maintenance of perfusion. In particular, iNOS is produced during prolonged periods of hypoxia, which may occur with myocardial infarction. EPCs may also produce insulin-like growth factor-1 (IGF-1), which among other things, appeared to play an anti-apoptotic role [107].

While research is still needed to understand all the lineages of endothelial cells within the heart, recent studies have elucidated that endocardial endothelial cells appear to have more than one lineage. For example, endocardial endothelial cells originate in the mesoderm along with cardiomyocytes [108]. Studies done with zebra fish have shown endocardial cells derived from vascular lineage as well as hematopoietic lineages that migrate to the heart tube during development [109]. Though the majority of mesodermal cells within the heart field differentiate into cardiomyocytes, a minor population of these cells expresses markers of endothelial cell differentiation such as the angiogenic factor, vascular endothelial growth factor (VEGF). Additionally, the extracardiac mesoderm develops from the proepicardium (PE) population of cardiac cells that form the epicardium. Recent studies have shown that these PE cells transiently form blood islands below the epicardial space and that from these

cells originate coronary vasculature including endothelial cells and perivascular fibroblasts [108]. In addition to vessel growth, vascular endothelial cells help to derive the heart endocardium during development. In avian models, for example, endocardial cells have been shown to originate from endothelial cell populations that do not form myocardium [109].

Flk1+ (also known as the type II VEGF receptor, VEGFR-2) cardiovascular progenitor cells can differentiate into a variety of cell types through interaction with certain transcription factors such as Er71, which regulates endocardial and endothelial differentiation [110]. The ability of EPCs to differentiate into endocardium as well as promote the proliferation and regrowth of vessels makes therapeutic angiogenesis an exciting topic of research for treatment of ischemic heart disease. A number of recent studies showed promise for therapeutic angiogenesis and a new approach to treatment of ischemic heart disease. For example, transplantation of human-derived endothelial progenitor cells to ischemic rat myocardium resulted in maturation into endothelial cells with reduced infarct size and increased preservation of left ventricular function [111]. Mobilization of endothelial progenitor cells with the use of G-CSF in the FIRSTLINE-AMI trial improved cardiac function in many ways, including reduced ventricular remodeling and delaying adverse events during a follow-up period of 1 year [112]. Current research has focused attention on reasonable approaches for transplantation and introduction of angiogenic growth factor proteins. Intravenous delivery is not ideal due to decreased delivery to target tissue and potential risks of systemic effect, while localized epicardial intramyocardial injection seems invasive and impractical in the setting of human trials [113]. Determining an optimal and appropriate route for therapy is essential and in this regard, intra-arterial catheter-based introduction is one such method that appears to provide a feasible mode of therapeutic delivery [114].

Research into VEGF administration to ischemic myocardium was carried out by Lopez et al. [114], who demonstrated increased collateral circulation that resulted in improved myocardial function. The increase in collateral circulation was significant in the left anterior descending and left circumflex arteries of the heart [114]. In addition, this study demonstrated decreased resistance to flow in the left anterior descending and left circumflex arteries following VEGF administration [114]. In a separate study, Hao et al. [115] introduced VEGF in addition to platelet derived growth factor, which stimulated smooth muscle cell recruitment and improved cardiac function compared to delivery of either single agent. These studies suggest that therapeutic angiogenesis represents a promising new approach to improve endothelial function and reverse some of the effects of ischemic heart disease.

4.4 EPCs and Myogenesis

Regeneration of cardiac muscle tissue represents a promising treatment for the ischemic heart. There have been several studies focusing on the transplantation of the stem cells in the ischemic heart to repair the damaged myocardium. In one study [116], specialized hematopoietic cells were transplanted into the ischemic heart and their progeny were tracked. It showed that the specialized cells called the SP cells (side population cells) migrated not only to the damaged myocardium, which was ischemic but they also invaded the blood vessels. The migration of the cells was particularly found in the infarct region. The cardiomyocytes were found at a percentage of 0.02% whereas the endothelial engraftment was about 3.3%. This study showed that stem cells can be used to regenerate not only cardiac tissue, which is damaged by ischemia but also the endothelial cells which form an essential part of the heart.

Another study [117] showed that neovascularization provided an added advantage and enhanced the improvement of cardiac function. As the blood supply to the heart is restricted in ischemia, enhancing the blood supply to the tissues by neovascularization could serve to enhance cardiovascularization. In this study, the cardiomyocytes were co-cultured with endothelial cells, which resulted in the formation of endothelial junctions within the sheets of the cardiomyocytes. These networks of ECs and cardiomyocyte sheets when transplanted into ischemic heart displayed improved cardiac function compared to the ones lacking the ECs [117]. The ECs thus seem to form an important part in improving the cardiac function. The ECs not only help in improving cardiac function but also aid in the survival and spatial organization of the newly formed cardiomyocytes [118]. The study further showed that when the cardiomyocytes were transplanted on the EC network sheets, apoptosis and necrosis of the newly generated cardiomyocytes was prevented, thereby prolonging the survival of the cardiac cells. The endothelial cells thus play a pivotal role in improving the cardiac function, prolonged survival and organization of the cardiomyocytes.

Another study showed that endothelial cells, when treated with certain specific growth factors, could be differentiated into cardiomyocytes in-vitro or in-vivo [119]. The experimental results suggested that Nkx2.5, GATA4, BMP4 and FGF2 did not promote the transdifferentiation of the endothelial cells [119] but other as yet unidentified factors may have promoted the trans-differentiation. The ECs were found to express both von Willebrand factor as well as sarcomeric alpha-actinin, indicating that they express dual markers for endothelial and myocyte cells, respectively. This opens up a new avenue which highlights the plasticity of the ECs in differentiating into cardiomyocytes.

5. SUMMARY AND FUTURE DIRECTIONS

ED is widely recognized as a significant major risk factor for many of the most prominent forms of cardiovascular disease. Current pharmacological approaches for combatting cardiovascular disease show beneficial effects on endothelial function that likely account, at least in part for their clinical efficacy. These include diverse types of drugs such as aspirin, statins and ACE inhibitors. Despite much progress and success with these approaches, they each have limitations and tend to be more palliative than curative. Cell-based therapies have the potential to repair and regenerate damaged tissue and with recent advances in the field, cell-based approaches have become an intense area of basic and clinical research. One reason that EPCs are attractive candidates for therapy is because they can be readily accessed from patients and thus offers autologous sources of material, which helps to minimize immune rejection. Further, they appear to have benefit for improving ED through a variety of mechanisms including angiogenesis, myogenesis, and angiomyogenesis. Strategies that explore the use of cell-based therapies to specifically target ED may represent a particularly productive avenue for basic and clinical research since early intervention at this step has the potential to prevent the subsequent precipitation of more advanced stages of cardiovascular disease. A schematic overview of these ideas is illustrated in Fig. 1. It is anticipated that with additional focused research, cell-based therapeutic strategies will rapidly progress from bench to bedside.
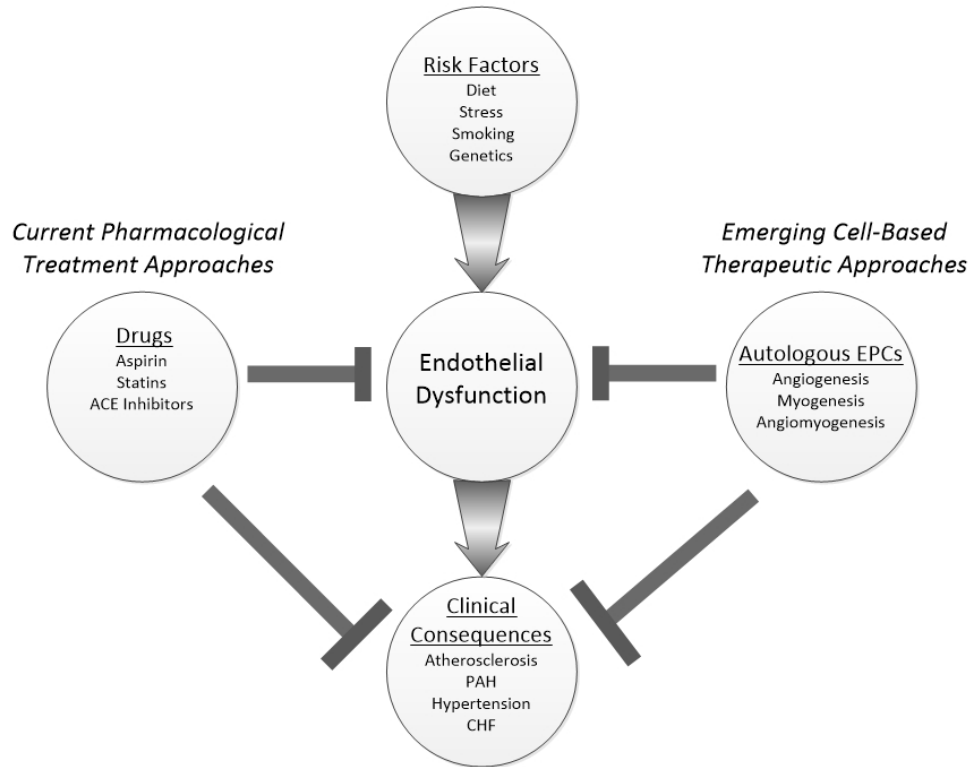
Fig. 1. Schematic overview of the role of Endothelial Dysfunction (ED) in the development of cardiovascular disease

The arrows indicate common factors leading to ED and its clinical consequences. The blocked arrows indicate how ED serves as an important target for current pharmacological and emerging cell-based therapeutic approaches to combat cardiovascular disease.

6. CONCLUSION

ED is an underlying contributing factor for all major forms of cardiovascular disease, and currently serves as a therapeutic target for some of the most commonly used drugs today including, aspirin, statins, ACE inhibitors, and many others. While effective at treating ED, pharmacological interventions alone have limitations, and there is always a risk of negative side effects. Cell-based therapies to repair and regenerate damaged endothelium in ED provide an attractive alternative/adjunct to pharmacological approaches, and they are likely to remain an intense area of research focus for many years to come.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bauersachs J, Hecker M, Sauer E, Fleming I, Busse R. Nitric oxide attenuates the release of endothelium-derived hyperpolarizing factor. Circulation. 1996;94:3341-3347.
2. Furchgott RF, Vanhoutte PM. Endothelium-derived relaxing and contracting factors. The FASEB Journal. 1989;3:2007-2018.
3. Flammer AJ, Luscher TF. Human endothelial dysfunction: EDRFs. Pflugers Archiv : European Journal of Physiology. 2010;459:1005-1013.
4. Edwards G, Feletou M, Weston AH. Endothelium-derived hyperpolarising factors and associated pathways: a synopsis. Pflugers Archiv : European Journal of Physiology. 2010;459:863-879.
5. Virdis A, Ghiadoni L, Taddei S. Human endothelial dysfunction: EDCFs. Pflugers Archiv : European Journal of Physiology. 2010;459:1015-1023.
6. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105:1135-1143.
7. Moncada S, Palmer R, Higgs E. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacological Reviews. 1991;43:109-142.
8. Ross R, Glomset J, Harker L. Response to injury and atherogenesis. The American Journal of Pathology. 1977;86:675.
9. Libby P. Inflammation in atherosclerosis. Nature. 2002;420:868-874.
10. Rubbo H, Trostchansky A, Botti H, Batthyany C. Interactions of nitric oxide and peroxynitrite with low-density lipoprotein. Biol Chem. 2002;383:547-552.
11. Cubbon RM, Kahn MB, Wheatcroft SB. Effects of insulin resistance on endothelial progenitor cells and vascular repair. Clin Sci (Lond). 2009;117:173-190.
12. Makimattila S, et al. Chronic hyperglycemia impairs endothelial function and insulin sensitivity via different mechanisms in insulin-dependent diabetes mellitus. Circulation. 1996;94:1276-1282.
13. Reddy KG, Nair RN, Sheehan HM, Hodgson JM. Evidence that selective endothelial dysfunction may occur in the absence of angiographic or ultrasound atherosclerosis in patients with risk factors for atherosclerosis. Journal of the American College of Cardiology. 1994;23:833-843.
14. Corretti MC, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002;39:257-265.
15. Kinlay S, Libby P, Ganz P. Endothelial function and coronary artery disease. Current Opinion in Lipidology. 2001;12:383-389.
16. Roger VL, et al. Heart disease and stroke statistics--2012 update: a report from the American Heart Association. Circulation. 2012;125:e2-e220.
17. Panza JA, Quyyumi AA, Brush Jr JE, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. New England Journal of Medicine. 1990;323:22-27.
18. Celermajer D, et al. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. Circulation. 1993;88:2149-2155.

19. Spieker LE, et al. High-density lipoprotein restores endothelial function in hypercholesterolemic men. Circulation. 2002;105:1399-1402.
20. Linder L, Kiowski W, Bühler F, Lüscher T. Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation *In vivo*. Blunted response in essential hypertension. Circulation. 1990;81:1762-1767.
21. Steinberg HO, et al. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. Journal of Clinical Investigation. 1996;97:2601.
22. Virdis A, et al. Mechanisms responsible for endothelial dysfunction induced by fasting hyperhomocystinemia in normotensive subjects and patients with essential hypertension. Journal of the American College of Cardiology. 2001;38:1106-1115.
23. Hürlimann D, et al. Anti–tumor necrosis factor-α treatment improves endothelial function in patients with rheumatoid arthritis. Circulation. 2002;106:2184-2187.
24. Paoletti R, Gotto AM, Hajjar DP. Inflammation in atherosclerosis and implications for therapy. Circulation. 2004;109:III-20-III-26.
25. Mandriota SJ, et al. Vascular endothelial growth factor increases urokinase receptor expression in vascular endothelial cells. Journal of Biological Chemistry. 1995;270: 9709-9716.
26. Lerman A, Zeiher AM. Endothelial function: cardiac events. Circulation. 2005;111: 363-368.
27. Humbert M, et al. Endothelial cell dysfunction and cross talk between endothelium and smooth muscle cells in pulmonary arterial hypertension. Vascular Pharmacology. 2008;49:113-118.
28. Budhiraja R, Tuder RM, Hassoun PM. Endothelial dysfunction in pulmonary hypertension. Circulation. 2004;109:159-165.
29. Farha S, et al. Hypoxia-inducible factors in human pulmonary arterial hypertension: a link to the intrinsic myeloid abnormalities. Blood. 2011;117:3485-3493.
30. Cooper CJ, et al. Role of nitric oxide in the local regulation of pulmonary vascular resistance in humans. Circulation. 1996;93:266-271.
31. Frasch HF, Marshall C, Marshall BE. Endothelin-1 is elevated in monocrotaline pulmonary hypertension. The American journal of physiology. 1999;276:304-310.
32. Klinger JR, Abman SH, Gladwin MT. Nitric oxide deficiency and endothelial dysfunction in pulmonary arterial hypertension. Am J Respir Crit Care Med. 2013;188:639-646.
33. Perrella MA, Edell ES, Krowka MJ, Cortese DA, Burnett JC Jr. Endothelium-derived relaxing factor in pulmonary and renal circulations during hypoxia. The American Journal of Physiology. 1992;263:45-50.
34. Dinh-Xuan A. Endothelial modulation of pulmonary vascular tone. European Respiratory Journal. 1992;5:757-762.
35. Asahara T, et al. Local delivery of vascular endothelial growth factor accelerates reendothelialization and attenuates intimal hyperplasia in balloon-injured rat carotid artery. Circulation. 1995;91:2793-2801.
36. Epstein FH, Vane JR, Änggård EE, Botting RM. Regulatory functions of the vascular endothelium. New England Journal of Medicine. 1990;323:27-36.
37. Unemori EN, Ferrara N, Bauer EA, Amento EP. Vascular endothelial growth factor induces interstitial collagenase expression in human endothelial cells. Journal of Cellular Physiology. 1992;153:557-562.
38. Flammer AJ, et al. The Assessment of Endothelial Function From research into clinical practice. Circulation. 2012;126:753-767.
39. Perticone F, et al. Prognostic significance of endothelial dysfunction in hypertensive patients. Circulation. 2001;104:191-196.

40. Bruno R, et al. Type 2 diabetes mellitus worsens arterial stiffness in hypertensive patients through endothelial dysfunction. Diabetologia. 2012;55:1847-1855.
41. Taddei S, Virdis A, Ghiadoni L, Mattei P, Salvetti A. Effects of angiotensin converting enzyme inhibition on endothelium-dependent vasodilatation in essential hypertensive patients. J Hypertens. 1998;16:447-456.
42. Tang EH, Vanhoutte PM. Endothelial dysfunction: a strategic target in the treatment of hypertension? Pflugers Archiv : European Journal of Physiology. 2010;459:995-1004.
43. Mason RP. A rationale for combined therapy with a calcium channel blocker and a statin: evaluation of basic and clinical evidence. Current drug targets. Cardiovascular & Haematological Disorders. 2005;5:489-501.
44. Schulman IH, Zachariah M, Raij L. Calcium channel blockers, endothelial dysfunction, and combination therapy. Aging Clinical and Experimental Research. 2005;17:40-45.
45. Wehland M, et al. The effects of newer beta-adrenoceptor antagonists on vascular function in cardiovascular disease. Current Vascular Pharmacology. 2012;10:378-390.
46. Bakris GL, Basile JN, Giles TD, Taylor AA. The role of nitric oxide in improving endothelial function and cardiovascular health: focus on nebivolol. The American Journal of Medicine. 2010;123:2-8.
47. Cockcroft JR, Pedersen ME. Beta-blockade: benefits beyond blood pressure reduction? Journal of Clinical Hypertension (Greenwich, Conn.). 2012;14:112-120.
48. Munzel T, Gori T. Nebivolol: the somewhat-different beta-adrenergic receptor blocker. J Am Coll Cardiol. 2009;54:1491-1499.
49. Wind S, et al. Oxidative stress and endothelial dysfunction in aortas of aged spontaneously hypertensive rats by NOX1/2 is reversed by NADPH oxidase inhibition. Hypertension. 2010;56:490-497.
50. Michell DL, Andrews KL, Chin-Dusting JP. Endothelial dysfunction in hypertension: the role of arginase. Frontiers in Bioscience (Scholar edition). 2011;3:946-960.
51. Nguyen H, et al. Interleukin-17 causes Rho-kinase-mediated endothelial dysfunction and hypertension. Cardiovascular Research. 2013;97:696-704.
52. Colombo PC, Onat D, Sabbah HN. Acute heart failure as "acute endothelitis"— Interaction of fluid overload and endothelial dysfunction. European Journal of Heart Failure. 2008;10:170-175.
53. Shechter M, Matetzky S, Arad M, Feinberg MS, Freimark D. Vascular endothelial function predicts mortality risk in patients with advanced ischaemic chronic heart failure†. European Journal of Heart Failure. 2009;11:588-593.
54. Katz SD, et al. Vascular endothelial dysfunction and mortality risk in patients with chronic heart failure. Circulation. 2005;111:310-314.
55. Vanhoutte PM, Shimokawa H, Tang EH, Feletou M. Endothelial dysfunction and vascular disease. Acta Physiologica (Oxford, England). 2009;196:193-222.
56. Ontkean M, Gay R, Greenberg B. Diminished endothelium-derived relaxing factor activity in an experimental model of chronic heart failure. Circulation Research 1991;69:1088-1096.
57. Bauersachs J, et al. Endothelial dysfunction in chronic myocardial infarction despite increased vascular endothelial nitric oxide synthase and soluble guanylate cyclase expression: role of enhanced vascular superoxide production. Circulation. 1999;100: 292-298.
58. Munzel T, Harrison DG. Increased superoxide in heart failure: a biochemical baroreflex gone awry. Circulation. 1999;100:216-218.
59. Marti CN, et al. Endothelial dysfunction, arterial stiffness, and heart failure. Journal of the American College of Cardiology. 2012;60:1455-1469.
60. Husain S, Andrews NP, Mulcahy D, Panza JA, Quyyumi AA. Aspirin improves endothelial dysfunction in atherosclerosis. Circulation. 1998;97:716-720.

61. Hennekens CH. Aspirin in the treatment and prevention of cardiovascular disease. Annual Review of Public Health. 1997;18:37-49.

62. Felmeden D, Lip G. Antithrombotic therapy in hypertension: a cochrane systematic review. Journal of Human Hypertension. 2005;19:185-196.

63. Trialists'Collaboration A. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. Bmj. 2002;324:71-86.

64. Hansson L, et al. Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomised trial. The Lancet. 1998;351:1755-1762.

65. Magen E, et al. Effects of low-dose aspirin on blood pressure and endothelial function of treated hypertensive hypercholesterolaemic subjects. Journal of Human Hypertension. 2005;19:667-673.

66. Dubey K, Balani D, Pillai K. Potential adverse interaction between aspirin and lisinopril in hypertensive rats. Human & Experimental Toxicology. 2003;22:143-147.

67. Zhu B-q, et al. Comparative effects of aspirin with ACE inhibitor or angiotensin receptor blocker on myocardial infarction and vascular function. Journal of Renin-Angiotensin-Aldosterone System. 2003;4:31-37.

68. Beckman JA, Creager MA. The nonlipid effects of statins on endothelial function. Trends Cardiovasc Med. 2006;16:156-162.

69. Bonetti P, Lerman L, Napoli C, Lerman A. Statin effects beyond lipid lowering—are they clinically relevant? European Heart Journal. 2003;24:225-248.

70. Laufs U. Beyond lipid-lowering: effects of statins on endothelial nitric oxide. European Journal of Clinical Pharmacology. 2003;58:719-731.

71. Romano M, et al. Fluvastatin reduces soluble P-selectin and ICAM-1 levels in hypercholesterolemic patients: role of nitric oxide. Journal of Investigative Medicine: the Official Publication of the American Federation for Clinical Research. 2000;48:183-189.

72. O'Driscoll G, Green D, Taylor RR. Simvastatin, an HMG–coenzyme A reductase inhibitor, improves endothelial function within 1 month. Circulation. 1997;95:1126-1131.

73. Suzumura K, Yasuhara M, Tanaka K, Suzuki T. Protective effect of fluvastatin sodium (XU-62-320), a 3-hydroxy-3-methylglutaryl coenzyme a (HMG-CoA) reductase inhibitor, on oxidative modification of human low-density lipoprotein< i> in vitro</i>. Biochemical Pharmacology. 1999;57:697-703.

74. Strey CH, et al. Endothelium-ameliorating effects of statin therapy and coenzyme Q< sub> 10</sub> reductions in chronic heart failure. Atherosclerosis. 2005;179:201-206.

75. Marcoff L, Thompson PD. The role of coenzyme Q10 in statin-associated myopathy: a systematic review. J Am Coll Cardiol. 2007;49:2231-2237.

76. Mangravite LM, et al. A statin-dependent QTL for GATM expression is associated with statin-induced myopathy. Nature; 2013.

77. Yano M, et al. Statins activate peroxisome proliferator-activated receptor gamma through extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinase-dependent cyclooxygenase-2 expression in macrophages. Circulation Research. 2007;100:1442-1451.

78. Lee R, Channon KM, Antoniades C. Therapeutic strategies targeting endothelial function in humans: clinical implications. Current Vascular Pharmacology. 2012;10:77-93.

79. Hornig B, Kohler C, Drexler H. Role of bradykinin in mediating vascular effects of angiotensin-converting enzyme inhibitors in humans. Circulation. 1997;95:1115-1118.

80. Sugawara A, et al. PPARγ agonist beyond glucose lowering effect. The Korean Journal of Internal Medicine. 2011;26:19-24.

81. Anderson TJ. Assessment and treatment of endothelial dysfunction in humans. J Am Coll Cardiol. 1999;34:631-638.

82. Shahin Y, Khan JA, Samuel N, Chetter I. Angiotensin converting enzyme inhibitors effect on endothelial dysfunction: a meta-analysis of randomised controlled trials. Atherosclerosis. 2011;216:7-16.

83. Mullen MJ, et al. Effect of enalapril on endothelial function in young insulin-dependent diabetic patients: a randomized, double-blind study. J Am Coll Cardiol. 1998;31:1330-1335.

84. Kudo M, et al. Implantation of bone marrow stem cells reduces the infarction and fibrosis in ischemic mouse heart. J Mol Cell Cardiol. 2003;35:1113-1119.

85. Koudstaal S, et al. Concise review: heart regeneration and the role of cardiac stem cells. Stem Cells Transl Med. 2013;2:434-443.

86. Beltrami AP, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell. 2003;114:763-776.

87. Oh H, et al. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. Proc Natl Acad Sci USA. 2003;100:12313-12318.

88. Torella D, Ellison GM, Mendez-Ferrer S, Ibanez B, Nadal-Ginard B. Resident human cardiac stem cells: role in cardiac cellular homeostasis and potential for myocardial regeneration. Nat Clin Pract Cardiovasc Med. 2006;3(Suppl 1):8-13.

89. Barile L, Messina E, Giacomello A, Marban E. Endogenous cardiac stem cells. Prog Cardiovasc Dis. 2007;50:31-48.

90. Lyngbaek S, Schneider M, Hansen JL, Sheikh SP. Cardiac regeneration by resident stem and progenitor cells in the adult heart. Basic Res Cardiol. 2007;102:101-114.

91. Kattman SJ, Huber TL, Keller GM. Multipotent flk-1+ cardiovascular progenitor cells give rise to the cardiomyocyte, endothelial, and vascular smooth muscle lineages. Dev Cell. 2006;11:723-732.

92. Moretti A, et al. Multipotent embryonic isl1+ progenitor cells lead to cardiac, smooth muscle, and endothelial cell diversification. Cell. 2006;127:1151-1165.

93. Raval Z, Losordo DW. Cell therapy of peripheral arterial disease: from experimental findings to clinical trials. Circulation Research. 2013;112:1288-1302.

94. Shi Q, et al. Evidence for circulating bone marrow-derived endothelial cells. Blood. 1998;92:362-367.

95. Reyes M, et al. Origin of endothelial progenitors in human postnatal bone marrow. J Clin Invest. 2002;109:337-346.

96. Kienstra KA, Hirschi KK. Vascular progenitor cell mobilization. Methods Mol Biol. 2012;904:155-164.

97. Hunting CB, Noort WA, Zwaginga JJ. Circulating endothelial (progenitor) cells reflect the state of the endothelium: vascular injury, repair and neovascularization. Vox Sang. 2005;88:1-9.

98. Asahara T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997;275;964-967.

99. Harraz M, Jiao C, Hanlon HD, Hartley RS, Schatteman GC. CD34- blood-derived human endothelial cell progenitors. Stem Cells. 2001;19:304-312.

100. Hur J, et al. Characterization of two types of endothelial progenitor cells and their different contributions to neovasculogenesis. Arterioscler Thromb Vasc Biol. 2004;24:288-293.

101. Urbich C, Dimmeler S. Endothelial progenitor cells: characterization and role in vascular biology. Circulation Research. 2004;95:343-353.

102. Mobius-Winkler S, Hollriegel R, Schuler G, Adams V. Endothelial progenitor cells: implications for cardiovascular disease. Cytometry A. 2009;75:25-37.
103. Shantsila E, Watson T, Lip GY. Endothelial progenitor cells in cardiovascular disorders. J Am Coll Cardiol. 2007;49:741-752.
104. Ding DC, Shyu WC, Lin SZ, Li H. The role of endothelial progenitor cells in ischemic cerebral and heart diseases. Cell Transplant. 2007;16:273-284.
105. Kawamoto A, et al. Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. Circulation. 2003;107:461-468.
106. Chen SY, et al. Autologous transplantation of EPCs encoding FGF1 gene promotes neovascularization in a porcine model of chronic myocardial ischemia. Int J Cardiol. 2009;135:223-232.
107. Jujo K, Ii M, Losordo DW. Endothelial progenitor cells in neovascularization of infarcted myocardium. Journal of Molecular and Cellular Cardiology. 2008;45:530-544.
108. Ishii Y, Langberg J, Rosborough K, Mikawa T. Endothelial cell lineages of the heart. Cell and Tissue Research. 2009;335:67-73.
109. Milgrom-Hoffman M, et al. The heart endocardium is derived from vascular endothelial progenitors. Development. 2011;138:4777-4787.
110. Misfeldt AM, et al. Endocardial cells are a distinct endothelial lineage derived from Flk1+ multipotent cardiovascular progenitors. Developmental Biology. 2009;333:78-89.
111. Kawamoto A, et al. Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. Circulation. 2001;103:634-637.
112. Ince H, et al. Preservation from left ventricular remodeling by front-integrated revascularization and stem cell liberation in evolving acute myocardial infarction by use of granulocyte-colony-stimulating factor (FIRSTLINE-AMI). Circulation. 2005;112:3097-3106.
113. Haider H, Akbar SA, Ashraf M. Angiomyogenesis for myocardial repair. Antioxid Redox Signal. 2009;11:1929-1944.
114. Lopez JJ, et al. VEGF administration in chronic myocardial ischemia in pigs. Cardiovasc Res. 1998;40:272-281.
115. Hao X, et al. Angiogenic effects of sequential release of VEGF-A165 and PDGF-BB with alginate hydrogels after myocardial infarction. Cardiovasc Res. 2007;75:178-185.
116. Jackson KA, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest. 2001;107:1395-1402.
117. Sekine H, et al. Endothelial cell coculture within tissue-engineered cardiomyocyte sheets enhances neovascularization and improves cardiac function of ischemic hearts. Circulation. 2008;118:145-152.
118. Narmoneva DA, Vukmirovic R, Davis ME, Kamm RD, Lee RT. Endothelial cells promote cardiac myocyte survival and spatial reorganization: implications for cardiac regeneration. Circulation. 2004;110:962-968.
119. Condorelli G, et al. Cardiomyocytes induce endothelial cells to trans-differentiate into cardiac muscle: implications for myocardium regeneration. Proc Natl Acad Sci USA. 2001;98:10733-10738.