EECP Symposium

EECP in the treatment of endothelial dysfunction: preventing progression of cardiovascular disease

John CK Hui1, William E Lawson1, Gregory W Barsness2

1 Cardiology Division, State University of New York, Stony Brook, New York, USA
2 Cardiovascular Disease and Internal Medicine, Mayo Clinic College of Medicine, Rochester, Minn, USA

Enhanced external counterpulsation

External counterpulsation (ECP) was originally conceived to be a circulatory assist device to promote blood flow to areas of the heart muscle that were lacking adequate blood supply due to obstruction of the coronary artery. During ECP the lower extremities are compressed to squeeze both arterial and venous blood back to the heart during diastole, increasing coronary perfusion pressure and right ventricular filling. The compression is released during systole, effectively increasing peripheral arterial capacitance and thereby lowering impedance to cardiac ejection and systolic workload. Enhanced external counterpulsation (EECP) was designed in the late 1970s with three pneumatic cuffs wrapped around the calves, lower and upper thighs. These cuffs are inflated sequentially to increase the volume of peripheral blood that can be effectively pumped back towards the heart. During the last four decades EECP has been demonstrated to be clinically effective in the treatment of patients with coronary artery disease. However, beside the acute hemodynamic effects that can be readily observed during EECP treatment, its mechanisms of action and its long-term effects are less well established and have only been rigorously examined within the past five years. Theoretically, EECP treatment can provide benefit through enhanced recruitment of collateral circulation and a variety of peripheral vascular effects, including improved endothelial function. Collaterals are small vessels that supply arterial blood to ischemic myocardium distal to the obstructed artery. They are created by the increased pressure gradient across a vascular stenosis and by angiogenic factors released as a result of the vascular shear forces produced during EECP treatment. In fact the most promising and beneficial effects of EECP treatment may involve these measurable and reproducible peripheral and endothelial effects of EECP treatment and will be the primary focus of this review.

Endothelial dysfunction

The functional endothelium is composed of a single layer of physiologically active endothelial cells lining the lumen of both arterial and venous conduits, although the specific paracrine activity and response vary by vascular type. The endothelial cells function as a protective barrier and facilitate bi-directional transport of macromolecules and blood gases between all tissues and circulating blood. The arterial endothelium particularly provides many different functions in cardiovascular pathophysiology, as shown in Fig.1.

Over the past 20 years, there has been intense research to clarify endothelial function and its importance in the clinical setting. It is now well recognized that endothelial dysfunction is an early finding in atherosclerotic vascular disease and a link between risk factors and the manifestation of cardiovascular disorders (Fig.2).1-4

When physical or chemical insults cause endothelial cells to become dysfunctional, their ability to produce endothelial nitric oxide synthase (eNOS), the factor necessary for the production of nitric oxide (NO), is reduced. NO is the vascular relaxation factor that, in opposition to coincident vasoconstrictive substances such as endothelin-1, regulates vasodilation and vasoconstriction, leading to arterial reactivity that controls blood flow and blood pressure. Endothelial dysfunction, therefore, results in an imbalance of vascular tone and relatively greater vasoconstriction and is one of the basic physiological pathways leading to hypertension. NO also serves as the neutralizer of oxidative agents, reducing oxidation of low-density lipoprotein cholesterol that contributes to the process of atherosclerosis. Endothelial dysfunction stimulates the expression of inflammatory cytokines, attracting inflammatory cells such as monocytes and leukocytes to the arterial wall where they penetrate into the subendothelial space and promote smooth muscle cell proliferation and migration, early events in atherogenesis and neointimalization. Therefore endothelial dysfunction is the early precursor of atherosclerosis and the cornerstone of most of the acquired cardiovascular disease.
EECP in the treatment of endothelial dysfunction

One of the most important factors that affect endothelial function is the local hemodynamic milieu; including flow-generated shear stress. Endothelial shear stress is proportional to the product of the blood viscosity and the spatial gradient of blood velocity at the arterial wall. Therefore increasing blood flow velocity (within the physiologic range) improves and maintains endothelial function and vascular health. EECP treatment increases blood flow velocity during systole by increasing cardiac output and markedly amplifies coronary blood flow during diastole by producing retrograde aortic flow. The generation of oscillatory endothelial shear stress greatly improves endothelial function.

There are over 30 papers published in peer reviewed medical journals exploring the mechanisms of action of EECP treatment. The most direct measure of the effect of EECP treatment is its ability to increase blood flow velocity. It is also well known that speeding the blood flow velocity increases shear stress on the endothelium and thereby improve endothelial function. The effectiveness of EECP therapy in improving endothelial function depends on improved hemodynamics and vascular tone and secondary cellular interactions with an intact and functional endothelial layer.

Hemodynamic effectiveness

EECP increases cardiac output by up to 60-70% and coronary blood flow velocity by approximate 109%, as demon-
EECP also results in a dramatic increase in intracoronary pressure and flow velocity measured by using a coronary sensor-tipped guidewire. Coronary diastolic pressure has been shown to increase by 93% from 71 ± 10 at baseline to 137 ± 21 mm Hg during EECP (P < 0.0001) with a decrease in coronary systolic pressure of 15% from 116 ± 22 to 99 ± 26 mm Hg, (P = 0.002). The intracoronary average peak velocity increased 109% in this study, from 11 ± 5 at baseline to 23 ± 5 cm/sec during EECP (P = 0.001).

The effect of EECP on endothelial shear stress has been reported in an animal model by Zhang and colleagues in 17 hypercholesterolemic pigs. The peak flow velocity (V) recorded in the cardiac diastolic phase by a color Doppler ultrasound system during EECP in the right brachial artery was significantly elevated compared with the pre-EECP measurement (24.62 ± 4.74 versus 59.48 ± 13.60 cm/sec, P <0.001). The internal diameter (id) of the right brachial artery was not significantly changed during EECP (1.65 ± 0.42 versus 1.68 ± 0.44 mm, P<0.05). The blood viscosity (μ) was 3.8 0.4 mPa (0.038 ± 0.004 poise). The peak diastolic endothelial shear stress (τ) during EECP was calculated using the formula: τ (dynes/cm²) = (4 μ V) / (id). Peak diastolic endothelial shear stress during EECP increased more than 2-fold compared with pre-EECP (23.92 ± 7.28 versus 49.62 ± 10.71 dynes/cm²; P<0.001).

Improve vascular tone
EECP has been shown to increase production of eNOS, thereby improving vascular tone. The endothelial response to shear stress activates a variety of signaling pathways, including eNOS, an enzyme required in the synthesis of NO, the key endothelium-derived relaxing factor that plays a pivotal role in the maintenance of vascular tone and reactivity. EECP has been shown to increase the expression of eNOS by Zhang and co-workers in a hypercholesterolemic pigs model using Western blotting technique. Expression of eNOS was significantly decreased in the group of pigs (N=28) fed with high-cholesterol diet for 15 weeks compared with the 7 pigs with normal diet served as control (P=0.009). One hour daily of EECP for 34 ± 2 hours was initiated in 17 pigs after 8 weeks of athrogenic diet and eNOS expression of the EECP treated group was 3.16 times that of the cholesterol group (P=0.023).

EECP has been shown to increase release of NO (vascular relaxation factor), and reduce endothelin-1 (a potent vasoconstrictive agent) levels. EECP treatment stimulates the production of NO. This was examined in 13 coronary disease patients receiving 1-hour daily EECP treatment for a total of 36 hours. Their ET-1 and NO were measured serially at baseline (control), after the 1st, 12th, 24th, and 36th hours of EECP, and at 1 and 3 months post-EECP. During the course of treatment, plasma NO progressively increased and plasma ET-1 progressively decreased. After 36 hours of EECP, there was a 62 ± 17% increase in plasma NO compared with baseline (43.6 ± 4.3 versus 27.1 ± 2.6 mol/L; P <0.0001), and a 36 ± 8% decrease in plasma ET-1 level (76.7 ± 9.5 versus 119.5 ± 8.5 pg/L; P<0.0001). At 3 months after completion of EECP, NO remained 13 ± 11% above baseline (P=0.002), and ET-1 remained 11 ± 10% below baseline (P=0.0068). These results demonstrate that EECP therapy has a dose related, sustained effect in stimulating endothelial cell production of the vasodilator, NO, and in decreasing production of the vasoconstrictor, ET-1, supporting the hypothesis that EECP improves endothelial function and vasomotor tone.

Several studies have demonstrated the potential of EECP to improve flow-mediated vasodilation, a reflection of systemic vascular endothelial health. Reactive hyperemia-pe-
Peripheral arterial tonometry (RH-PAT), a noninvasive method to assess peripheral endothelial function, was performed in 23 patients with refractory angina undergoing 1-hour daily EECP treatment for a total 35 hour treatment course. In each patient, RH-PAT measurements were performed before and after the first, at midcourse (the 17 hour), and after the last EECP treatment. In addition, RH-PAT response was assessed one month after completion of EECP therapy. RH-PAT index, a measure of reactive hyperemia, was calculated as the ratio of the digital pulse volume during reactive hyperemia divided by that at rest. EECP was associated with a significant immediate increase in average RH-PAT index after each treatment period (P<0.05). In addition, average RH-PAT index at one-month follow-up was significantly higher than that before EECP therapy (P<0.05), demonstrating that EECP therapy provided acute endothelial benefits that accrued over a treatment course and persisted even after treatment cessation, providing long term improvement of endothelial dysfunction in patients with coronary artery disease (CAD). Endothelial function benefits mirrored improvement in anginal status. No major adverse cardiovascular events were noted during the study period.

Shechter and co-workers used the endothelium-dependent, flow-mediated dilation (FMD) of the brachial artery, as measured with high-resolution ultrasound, to assess endothelial vasomotor function changes with ECP. This was a prospective, age-matched, controlled study. The brachial reactivity in 20 consecutive CAD patients (15 males), mean age 68 ± 11 years, with refractory angina pectoris, CCS class III and IV, unsuitable for coronary revascularization was assessed by FMD before and after their ECP treatment. These measurements were compared with 20 matched control CAD patients (17 males). After completion of 35 hours of daily 1 hour ECP treatment, FMD improved significantly from baseline 3.1 ± 2.2% to 8.2 ± 2.1%; (P=0.01), whereas there were no changes in the control group within the 2-month period. ECP also improved angina symptoms by a reduction in mean number of sublingual nitrate tablets consumption per day (from 4.2 ± 2.7 to 0.4 ± 0.5; P<0.001), compared with no change in the control group (4.5 ± 2.3 to 4.4 ± 2.6; P=0.87). The mean CCS class also improved significantly in the ECP group (3.5 ± 0.5 to 1.9 ± 0.3; P<0.0001), with no change in the control group (3.3 ± 0.6 to 3.5 ± 0.5; P=0.89). This study demonstrates that ECP intervention compared with controls results in significant improvement in endothelial dysfunction and cardiac function status.

Improve arterial stiffness

Arterial stiffness is a strong independent predictor of adverse cardiovascular outcomes above and beyond traditional cardiovascular risk factors in both healthy subjects and patients with an increased risk of CAD. For relaxing arterial stiffness (reducing magnitude and altering the timing of the reflected wave), Nichols and colleagues measured the radial artery pressure waveforms of 20 patients with stable refractory angina before and after 34 1-hour EECP sessions. The heart rate and ejection duration did not change with EECP treatment. Brachial systolic and pulse pressure reduced significantly from 132 ± 18 to 121 ± 18 mmHg (P<0.001) and 61 ± 15 to 54 ± 16 mmHg (P<0.001) respectively. Aortic systolic pressure reduced from 120 ± 18 to 108 ± 18 mm Hg (P<0.001), and aortic pulse pressure from 48 ± 14 to 41 ± 16 mmHg (P<0.001). Mean blood pressure also decreased significantly from 92 ± 13 to 84 ± 9.5 mmHg (P<0.001). Associated with the fall in arterial blood pressure was an improvement in wave reflection characteristics. Compared with baseline, reflected wave augmented pressure decreased from 13 ± 7.1 to 8.7 ± 6.8 mmHg (P<0.001) after EECP treatment, and augmentation index (AIx) referenced at a heart rate of 75 beats/min reduced from 18 ± 9.6 % to 12 ± 8.4 % (P<0.01), indicating a significant reduction of arterial stiffness. The reduced arterial stiffness caused a decrease in transmission velocity and increase travel time of the reflected wave from the lower body to the heart (P<0.001), and a decrease in reflected wave systolic duration from 182 ± 33 to 168 ± 41 ms (P<0.01), thereby reducing the cardiac workload and myocardial demand.

That EECP treatment reduces arterial stiffness was also supported by the findings of Levenson and co-workers in a prospective, randomized, controlled study using high-resolution ultrasound echo-Doppler evaluation of the diameters of the carotid artery in 30 patients (28 males and 2 females) with stable CAD. Carotid artery blood flow was measured and carotid vascular resistance (CVR) calculated as

$$\text{CVR} = \frac{\text{mean blood pressure}}{\text{volume blood flow (ml/min)}}.$$ 

Platelet cyclic guanosine monophosphat (cGMP) content was measured at baseline and after 17 and 35 hours of EECP therapy. There were no significant between-group differences in baseline clinical characteristics or in baseline carotid hemodynamics values. There were no major complications occurring during EECP treatment. The absolute change from baseline before EECP in the stiffness index was significantly reduced at 1 hour (P<0.01), 17 hours (P<0.001) and 35 hours (P<0.01) after EECP treatment in the active EECP group, and this decrease was statistically different when compared with controls at 1 and 35 hours (P<0.05, respectively). Also, in contrast to controls, mean carotid blood flow in patients receiving active EECP increased significantly at 1 hour (P<0.05) and again more strongly at 35 hours (P<0.001), with a trend present at 17 hours (P=0.07). The difference between groups was significant at 17 hours (P<0.05) and 35 hours (P<0.01). CVR was concomitantly reduced in the active EECP treated group at 17 hours (P<0.05) and 35 hours (P<0.01), showing a significant difference between groups in CVR at 17 hours (P<0.01) and 35 hours (P<0.001). Platelet cGMP contents increased at 1, 17 and 35
hours by 0.019 ± 0.02, 0.058 ± 0.05 and 0.052 ± 0.05 pmol/10⁶ platelets in controls and by 0.097 ± 0.02, 0.059 ± 0.08 and 0.087 ± 0.09 pmol/10⁶ platelets in the active EECP treated groups respectively. Platelet cGMP in the active group negatively correlated with stiffness index (n=59, r=-0.32, P<0.05), but not in patients in the control group. Results of this study suggest that active EECP therapy relax the smooth muscle tone of the carotid artery and consequently decreases their arterial stiffness via the signaling product of NO pathways, supporting the hypothesis that EECP therapy improves endothelial dysfunction.

**Improve blood pressure**

One of the most important clinical outcomes of EECP treatment in improving endothelial function is the activation of NO release, which improves vasomotor tone. This vasodilation has the potential to manifest as a lowering of blood pressure. Campbell and co-workers examined this hypothesis. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) of 108 consecutive patients (mean age 66.4 ± 11.2 years, 81% male) had pressures recorded before the first EECP session (baseline), before and at the end of each session, and at 6 weeks after the final session, totaling 3,586 readings. Data were analyzed by stratifying patients according to baseline SBP into the following subgroups: ≤ 100 mmHg (n=16), 101 to 110 mmHg (n=26), 111 to 120 mmHg (n=20), 121 to 130 (n=27), 131 to 140 (n=7) and ≥ 141 mmHg (n=12); and baseline DBP was stratified into the following subgroups: ≤ 60 mmHg (n=37), 61 to 70 mmHg (n=39), 71 to 80 mmHg (n=22) and ≥ 81 mmHg (n=10). The average SBP at baseline was 120.4 ± 19.3 (range 88-192) mm Hg. EECP significantly lowered SBP in each baseline stratum from 101 to ≥ 141 mmHg, whereas in the lowest stratum (≤ 100 mmHg), a rise in SBP was observed. Overall there was a 6.4 ± 18.2 mmHg decrease in SBP after a course of EECP, resulting in a mean SBP of 114.0 ± 16.0 (range 86-150) mmHg (P<0.001 compared with baseline). The average DBP at baseline was 66.0 ± 10.4 (range 44-96) mmHg. There was a trend toward a decrease in DBP with a mean reduction of 1.8 ± 7.5, to 64.3 ± 9.7 (range 46-92) mm Hg. The DBP changes were very similar to those for SBP, with an increase in the 2 lowest baseline strata and a reduction in the other strata. Six-week follow-up was available in 94 patients. Overall mean SBP was 117.7 ± 17.0 (range 90-166) mmHg, 3.7 ± 17.8 mmHg lower than baseline (P=0.07). The mean DBP was 65.6 ± 9.5 (range 49-94) mmHg, 0.8 ± 13.8 mmHg lower than baseline (P=0.5). The differential blood pressure effects observed at the end of a course of EECP were maintained at 6 weeks for both SBP and DBP. Univariate and multivariate regression analysis showed no association of blood pressure changes with age, gender, baseline weight, left ventricular ejection fraction (LVEF), or medication changes. The observations that EECP lowers hypertensive BP and raises hypotensive BP are supportive of the hypothesis that EECP improves endothelial function.

**Promoting neovascularization (angiogenesis) and collateral competence**

**Increase release of angiogenic factors**

Factors contributing to the development of collateral circulation include the mechanical pressure gradient associated with an ischemic arterial lesion, as well as the activation of angiogenic factors triggered by the increased shear stress exerted at the endothelial surface. Masuda and co-workers reported an increase in growth factors: vasoendothelial growth factor (VEGF) (15.6%), hematopoietic growth factor (HGF) (26.6%), and basal fibroblast growth factor (bFGF) (18.8%), after EECP therapy compared with baseline, assessed by radioimmunoassay from blood samples collected from the brachial vein of 11 chronic stable angina patients (P<0.04). These results suggest that EECP therapy promote angiogenic factors, presumably through increased shear stress, which in turn are associated with the development of functional collateral vessels as demonstrated by an improvement in N-ammonia positron emission tomography perfusion scan and coronary flow reserve.

**Increase collateral flow index**

Two studies done in Europe documented the promotion of coronary collateral circulation after EECP treatment. The first one is the Art. Net.2 trial testing the hypothesis that a treatment course with EECP can induce the growth of myocardial collateral arteries. This study was designed as a prospective, controlled, proof-of-concept study. Inclusion criteria were: age 40 to 80 years, stable coronary disease, a residual significant stenosis of at least one epicardial artery and a positive ischemic stress-test for the region of interest. The primary endpoint was the pressure-derived collateral flow index (CFIp), determined by simultaneous measurement of mean aortic pressure (Pa, mmHg), distal coronary occlusive wedge pressure (Pw, mmHg) and central venous pressure (Pv, mmHg). The index was calculated as CFIp = (Pw-Pv) / (Pa-Pv). The pressure derived fractional flow reserve (FFR) and the indexes of microcirculatory resistance (IMR) were also assessed. A total of 23 patients (age 61 ± 2.5 years) were prospectively recruited into the two study groups: EECP group (n=16) and the control group (n=7). All patients underwent a cardiac catheterization at baseline and after 7 weeks. In the EECP group, the CFIp (from 0.08 ± 0.01 to 0.15 ± 0.02; P<0.001) and FFR (from 0.68 ± 0.03 to 0.79 ± 0.03; P=0.001) improved significantly, while in the control group no change was observed. The non-invasive secondary endpoints included symptoms Canadian Cardiovascular Society (CCS) and New York Heart Association (NYHA) classification, treadmill-testing and analysis of shear-stress related soluble proteins. Only the EECP group showed a reduction of the CCS (P=0.008) and NYHA (P=0.001) classifications.

The second study was done by Gloekler and colleagues of Bern, Switzerland in twenty patients with chronic stable...
coronary artery disease in a single-blinded, sham-controlled study.\textsuperscript{23} Collateral flow index (CFI) was determined by the ratio of mean distal coronary occlusive pressure to mean aortic pressure with central venous pressure subtracted from both. Additionally, coronary collateral conductance (occlusive myocardial blood flow per aortic-coronary pressure drop) was determined by myocardial contrast echocardiography and brachial artery flow-mediated dilation was obtained. CFI changed from 0.125 (0.073; interquartile range) at baseline to 0.174 (0.104) at follow-up in the EECP group ($P=0.006$), and from 0.129 (0.122) to 0.111 (0.125) in the sham group ($P=0.14$). Baseline to follow-up change in coronary collateral conductance was from 0.365 (0.268) to 0.568 (0.585) ml/min/100 mm Hg in the EECP group ($P=0.072$), and from 0.229 (0.212) to 0.305 (0.422) ml/min/100 mm Hg in the sham group ($P=0.45$). The study concluded that the clinical benefit of EECP for CAD patients might be explained by its arteriogenic effect due to increased laminar shear stress at the endothelial cell layer, improving endothelial function.

**Inhibiting intimal hyperplasia and smooth muscle cell proliferation and migration**

Endothelial dysfunction is a common link in the pathophysiologic progression of CVD. As EECP therapy has been shown to improve endothelial function, possibly through a mechanism mediated by increased shear stress forces acting at the endothelial surface, it is intriguing to consider possible associated palliative effects on vascular health. This hypothesis was tested in a hypercholesterolemic pig model by Zhang and co-workers.\textsuperscript{8} Thirty-five male pigs were randomly assigned to 3 groups: control group with usual diet ($n=7$), high-cholesterol diet (CHOL, $n=11$), and CHOL+EECP ($n=17$). Intima hyperplasia was observed in the coronary arteries of the high-cholesterol diet group, whereas in animals receiving EECP therapy, the intima-to-media area ratio was significantly decreased by 41.59% (21.27% $\pm$ 10.0% versus 36.41% $\pm$ 16.69%, $P=0.008$) and was not significantly different from the control group with normal diet (19.70 $\pm$ 9.01%). Similarly, the wall-to-lumen ratio, another measure of intima hyperplasia, was significantly decreased in the CHOL+EECP group compared with the CHOL group (17.57 $\pm$ 4.70% versus 23.95 $\pm$ 4.44%, $P=0.004$), reversing the hyperplastic effect and not significantly different from the control group with normal diet (18.04 $\pm$ 8.23%). This study clearly demonstrates EECP therapy reduces hypercholesterolemia-induced endothelial damage, arrests vascular smooth muscle cell proliferation and migration and eventually inhibits intimal hyperplasia and the development of atherosclerosis by increasing the arterial wall shear stress, which in turn activates the endothelial NO synthase/NO pathway.

**Modulating inflammatory processes**

Since EECp therapy increases shear stress in both the central and peripheral beds, including coronary arteries, Casey and colleagues hypothesized that EECP would decrease circulating levels of selected proinflammatory markers and adhesion molecules in patients with angina pectoris.\textsuperscript{24} Twenty-one consecutive patients with angina pectoris were randomly assigned to either 35 1-hour full pressure EECP treatments (EECP group, $n=12$), or 75 mm Hg EECP (sham group, $n=9$). Plasma tumor necrosis factor-α (TNF-α), monocyte chemotactic protein (MCP-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1) from venous blood samples collected before and after EECP treatment were determined using enzyme-linked immunosorbent assay. After 35 hours of active EECP treatment, there were significant reductions of circulating levels of TNF-α (29%) and MCP-1 (20%) compared with baseline, while there were no changes in the sham group. There were no changes for sVCAM-1 in either the EECP group (776 $\pm$ 280 vs 726 $\pm$ 278 ng/ml, $P=0.14$) or the sham group (847 $\pm$ 177 vs 859 $\pm$ 160 ng/ml, $P=0.81$). Increased plasma levels of TNF-α and MCP-1 have been shown to predict future coronary events.

The effect of exposure to increased shear stress promoted by EECP on the progression of atherosclerosis and the underlying inflammation-related molecular mechanisms in a porcine model of hypercholesterolemia has also been investigated.\textsuperscript{25} EECP treatment of hypercholesterolemic pigs resulted in a 34.38% increase in mean wall shear stress and a significantly lower pulsatility index in the brachial artery. The animals receiving EECP showed a marked reduction in atherosclerotic lesion size in the coronary artery and abdominal aorta compared with the hypercholesterolemic control group, associated with a decrease in macrophage accumulation. The expression of a set of genes involved in inflammation [including C-reactive protein (CRP), complement 3a, vascular cell adhesion molecule-1 (VCAM-1), and inducible nitric oxide synthase], mitogen-activated protein kinase (MAPK)-p38 phosphorylation, and nuclear factor-kappa B (NF-κappa B) activation, was attenuated. These findings suggested that EECP treatment exerts a retarding effect on atherosclerosis by downregulating proinflammatory gene expression. The underlying mechanisms are related to chronic exposure to increased pulsatile shear stress promoted by EECP; this exposure suppresses the over-activation of the MAPK-P38/NF-κappa B/VCAM-1 signaling pathway induced by hypercholesterolemia.

**Activating endothelial progenitor stem cells for enhanced reparative effects**

Increase circulating endothelial progenitor cells Endothelial progenitor cells (EPCs) are bone marrow cells that have the potential to proliferate, migrate, and differentiate into mature endothelial cells. Risk factors such as obesity, insulin resistance, diabetes, smoking, hypertension, hyperlipidemia, and increasing age lead to damage of endothelial cells. EPCs are capable of vascular regeneration to repair...
endothelial cell injury.26 EPCs are characterized via expression of CD34 and kinase insert domain receptor (KDR) and can also be assessed by EPC colony-forming units (CFUs) assay. Increased levels of circulating CD34+/KDR+EPCs have been associated with reduced risk of death and major adverse cardiovascular events.27 Since ECP therapy improves endothelial function, Barsheshet and colleagues hypothesized that some of the beneficial effects of ECP may be attributed to the effect on circulating EPCs.28 Twenty-five patients with symptomatic CAD were enrolled in this study, 15 treated with ECP and 10 served as the control group who refused ECP. Venous blood samples were taken 1 week before and 1 week after a full 7-week ECP course, or after a 9-week follow-up period in the control group to determine level of circulating EPCs by immunoassay expressed as the number of CD34+/KDR+EPCs/105 peripheral blood mononuclear cells, or by the number of CFUs assay. Endothelial function was also assessed by brachial artery flow-mediated dilatation (FMD). ECP significantly increased the median number of CD34+/KDR+EPCs from 10.2 to 17.8, \( P=0.049 \), but not in the Control group (from 10.0 to 14.0, \( P=0.430 \)). ECP also increased the number of EPC-CFUs from 3.5 to 11.0, \( P=0.010 \), and not in the control group from 6.9 to 8.2, \( P=0.557 \).

Parallel to the increase in the number of circulating EPCs was the significant improvement in endothelial function documented by FMD from 7.4% to 12.2% in the ECP group, \( P<0.001 \); with no change in the control group (10.5% at baseline to 8.4% at 9-week follow-up, \( P=0.696 \)). Similarly clinical outcome assessed by the reduction of CCS angina class was reduced from 3.0 to 2.0, \( P<0.001 \) in the ECP group but remain unchanged in the Control group. There was a significant correlation between EPC-CFUs and FMD (\( r=0.46, P=0.027 \)), showing that ECP therapy increased the number of circulating EPCs and improved endothelial function.

**EECP as a regenerative therapy** In the United States, EECP is used for the treatment of severe angina and heart failure in patients who are not candidates for revascularization. The clinical benefits of EECP extend well beyond the time period of any hemodynamic effects, but the cause of this prolonged effect is not understood. Dr Jewell and co-workers hypothesized that the prolonged clinical benefits suggest EECP be a regenerative therapy.29 The proposed mechanism for the increase in regenerative circulating stem cells is the enhanced shear forces induced on the endothelial boundary by the flow reversal produced by the sequential inflation of the pneumatic cuffs during EECP therapy. Nine patients were recruited from those referred for EEC. There were 8 men and 1 woman with a mean age 70.2 ± 10.2 years. All patients had limiting angina (CCS class II or greater), receiving appropriate medical therapy and were not considered candidates for revascularization. There were no significant differences in the counts for either progenitor cell line comparing the values for the samples obtained 5-7 days before treatment with the values obtained immediately before the first treatment. The total number of cells increased progressively during the 4 weeks of therapy. After 4 weeks, there was a significant increase in EPCs (\( P=0.014 \) compared with the baseline value). Likewise, the number of HPCs also increased significantly (\( P=0.008 \) compared with baseline) during treatment. Before EECP, the median CCS class was 3.0 and decreased to 1.5 after 4 weeks of therapy (\( P<0.01 \)). In conclusion, the present study demonstrates an increase in progenitor cells during EECP therapy in patients with severe CAD. The mechanism whereby EECP causes this increase is unknown, but this observation could provide an explanation for the angiogenesis and improvements in left ventricular function observed with EECP and the long-lasting benefits following EECP therapy. By increasing circulating progenitor cells EECP could be considered a regenerative therapy and further proof of this concept may be warranted in the treatment of other degenerative diseases including vascular ulcers, peripheral neuropathy, Parkinson’s disease, stroke, and myocardial infarction. Other methods of mechanically increasing circulating stem cells could also be investigated.

**Summary** Endothelial dysfunction is a syndrome that encompasses interruption in the barrier function of the vascular endothelium, impairment of control of vascular tone in vasodilation and constriction, failure to provide angiogenic competence, disturbances of antithrombogenic and anti-inflammatory properties, inappropriate regulation of vascular smooth cell proliferation and migration, and breakdown in the reduction of oxidative stress. It is an early marker of cardiovascular events and death, as well as a predictor of atherosclerosis. There are numerous publications in the medical literature documenting endothelial dysfunction and its association with CAD, peripheral vascular disease, hypertension, stroke, heart failure (HF) and diabetes, and it is the link between risk factors such as obesity, insulin resistance, diabetes, smoking, hypertension, hyperlipidemia, inactivity, ageing and genetic predisposition to cardiovascular disease such as CAD, peripheral vascular disease, stroke, HF, renal failure, and complications from diabetes mellitus. Because of the overwhelming evidence associating endothelial dysfunction as precursor of cardiovascular disease, it is extremely important to have an intervention that can provide improvement of endothelial dysfunction and protects against progression of vascular disease processes.

Vascular health is preserved through a carefully regulated mechanism that maintains endothelial surface shear forces within a narrow “healthy” physiologic range. Similarly, it has been documented that certain activities, such as exercise, and perhaps EECP, encourage improved endothelial function through modulation of flow-generated endot-
helical shear stress on the arterial wall. It is the mechanical dragging force acting on the wall of the vessel derived from the friction of the flowing blood, and is proportional to the velocity of blood flow. The shear stress modulates endothelial gene expression through complex mechanoreception and mechanotransduction process, controlling vasomotor tone and other pathophysiological processes responsible for atherosclerosis and vascular remodeling.

EECP is a noninvasive circulation assist device for the treatment of patients with CAD. EECP has been shown to be effective in improving exercise capacity, angina functional classes, myocardial perfusion, and quality of life in patient suffering from refractory angina pectoris. EECP therapy has also been shown to be safe and effective in the treatment of patients suffering from HF. The hemodynamic effects during EECP treatment have been documented to significantly increase coronary blood flow as well as blood flow in the descending aorta bidirectionally. The increased blood flow increases endothelial shear stress, activating eNOS, an enzyme essential in the synthesis of NO, a powerful endothelium-derived relaxing factor responsible for vascular dilation, and endothelin-1, a vasoconstrictor. Clinical outcomes of the effects of EECP therapy in the activation of NO have been documented by the increase in RH-PAT and brachial artery flow-mediated dilation in patients with parallel improvement in their angina functional class and status. Improvement in endothelial dysfunction by increasing NO release as a consequence of EECP therapy also leads to the reduction of arterial stiffness documented by decreased pulse wave velocity generated by cardiac output and the magnitude and arrival time of the reflected wave from peripheral vascular sites to the root of the aorta. The reduction of arterial stiffness together with vasodilation of microvasculature leads to a reduction of vascular resistance, SBP and reduced cardiac energy requirements. Improved endothelial function due to EECP therapy also activates angiogenesis growth factors leading to neovascularization with recruitment of collateral circulation, bringing blood flow to regions lacking perfusion due to atherosclerotic occlusion, restoring organ function. EECP therapy also inhibits smooth muscle cell proliferation and migration, reducing intimal hyperplasia, reversing progression of the atherosclerotic process. In addition, EECP therapy reduces plasma inflammatory cytokines and adhesion molecules, markers of future cardiovascular events. All of the research evidence to support the hypothesis that EECP therapy increases shear stress on the endothelium and activates the mechanoreceptors and their signaling pathways that modulates endothelial function and morphology, can prevent future cardiovascular events.

Risk factors, genetic predisposition, and low or high shear stress acting on the endothelial cells can all contribute to the development of endothelial dysfunction, leading to premature cell senescence and apoptosis. In addition to increased shear stress to stimulate endothelial cell function, EECP therapy also repairs the damages to the endothelium by mobilizing bone marrow EPC to the peripheral circulation and replacing injured intima.

Endothelial dysfunction is the precursor of cardiovascular disease. EECP therapy has been shown to improve various endothelial functions including managing vasomotor control, relaxing arterial stiffness, decreasing vascular resistance and hypertension, promoting angiogenesis competence, inhibiting intimal hyperplasia and smooth muscle cells proliferation and migration, reducing inflammatory and adhesion processes and activating endothelial progenitor stem cells replacing damaged endothelial cells. EECP therapy should be considered as a noninvasive intervention for endothelial dysfunction, and as a powerful preventive therapy for cardiovascular disease.

**References**


