European Cells and Materials Vol. 21 2011 (pages 157-176) DOI: 10.22203/eCM.v021a13 ISSN 1473-2262

CURRENT CONCEPTS AND NEW DEVELOPMENTS FOR AUTOLOGOUS *IN VIVO* ENDOTHELIALISATION OF BIOMATERIALS FOR INTRAVASCULAR APPLICATIONS

M. Avci-Adali, N. Perle, G. Ziemer and H.P. Wendel*

Clinical Research Laboratory, Department of Congenital and Paediatric Cardiac Surgery, University Children´s Hospital Tuebingen, Calwerstr. 7/1, D-72076 Tuebingen, Germany

Abstract

Circulating endothelial progenitor cells (EPCs) in the peripheral blood of adults represent an auspicious cell source for tissue engineering of an autologous endothelium on blood-contacting implants. Novel materials biofunctionalised with EPC-specific capture molecules represent an intriguing strategy for induction of selective homing of progenitor cells. The trapped EPCs can differentiate into endothelial cells and generate a non-thrombogenic surface on artificial materials. However, the success of this process mainly depends on the use of optimised capture molecules with a high selectivity and affinity. In recent years, various biomedical engineering strategies have emerged for *in situ* immobilisation of patient's own stem cells on blood contacting materials. The realisation of this *in vivo* tissue engineering concept and generation of an endothelium on artificial surfaces could exceedingly enhance the performance of not only small calibre vascular grafts and stents, but also, in general all blood-contacting medical devices, such as heart valves, artificial lungs, hearts, kidneys, and ventricular assist devices.

Keywords: Tissue engineering, biocompatibility, implants, stem cells, endothelial cells, cardiovascular tissue.

*Address for correspondence:
H.P. Wendel
Clinical Research Laboratory,
Department of Congenital and Paediatric Cardiac Surgery
University Children´s Hospital Tuebingen
Calwerstr. 7/1, D-72076 Tuebingen, Germany
Telephone Number: ++49-7071-2986605
FAX Number: ++49-7071-295369
E-mail: hans-peter.wendel@med.uni-tuebingen.de

Introduction

Atherosclerosis is a chronic inflammatory disease of the arterial wall, which leads to the asymmetric focal thickening of the intima (the innermost layer of arteries). It is the leading cause of death in the developed countries, present in almost one-third of all deaths. During atherosclerosis, the accumulation of low density lipoproteins within the intima leads to the formation of plaques that consist of lipids, inflammatory and immune cells (mainly macrophages and T cells), collagen fibres, smooth muscle cells (SMCs), debris from dead cells and calcium. Over time, the progressive plaque enlargement can cause narrowing (stenosis) or even occlusion of blood vessels. One of the treatment methods to reopen blockages in the arteries is the percutaneous transluminal angioplasty, which is mostly performed with stent deployment to keep the vessel open as long as possible.

However, if the atherosclerotic lesion in the occluded vessel is extensive and the vessel is no longer reliable, bypass surgery is performed using an autologous vascular graft. For this purpose, the patient's own internal mammary artery or saphenous vein is used to route blood flow around the obstructions. However, many patients do not have suitable autologous vessels because of previous surgical harvest or pathological vessel alterations due to progressed atherosclerosis. Furthermore, it is often difficult to obtain a sufficient graft length for effective bypass. In such cases, there is a great need for synthetic vascular grafts to replace or bypass diseased vessels. At present, the synthetic vascular grafts of choice are made of expanded-polytetrafluoroethylene (e-PTFE, Gore-Tex®) or polyethylene terephthalate (PET, Dacron®). Although these grafts exhibit long term patency for more than ten years at large graft diameters, the grafts with a less than 6 mm inner diameter are associated with high occlusion rates. The occlusion is mainly caused due to the thrombogenicity of the synthetic materials or the intimal hyperplasia at the anastomotic sites (Burkel, 1988).

Despite the tremendous progress in biomaterial development and surface modifications in recent years, the native non-thrombogenic endothelium still represents the ideal surface for blood contact. Hitherto, numerous research groups seeded synthetic material surfaces with endothelial cells (ECs) (Deutsch *et al*., 1999; Meinhart *et al*., 2001) to create an autologous endothelium *in vitro* (Fig. 1). However, this application necessitates an initial surgery to obtain a vessel biopsy from the patient and to isolate the patient's own ECs for graft seeding (Villalona *et al*., 2010). The isolated ECs are grown until the required

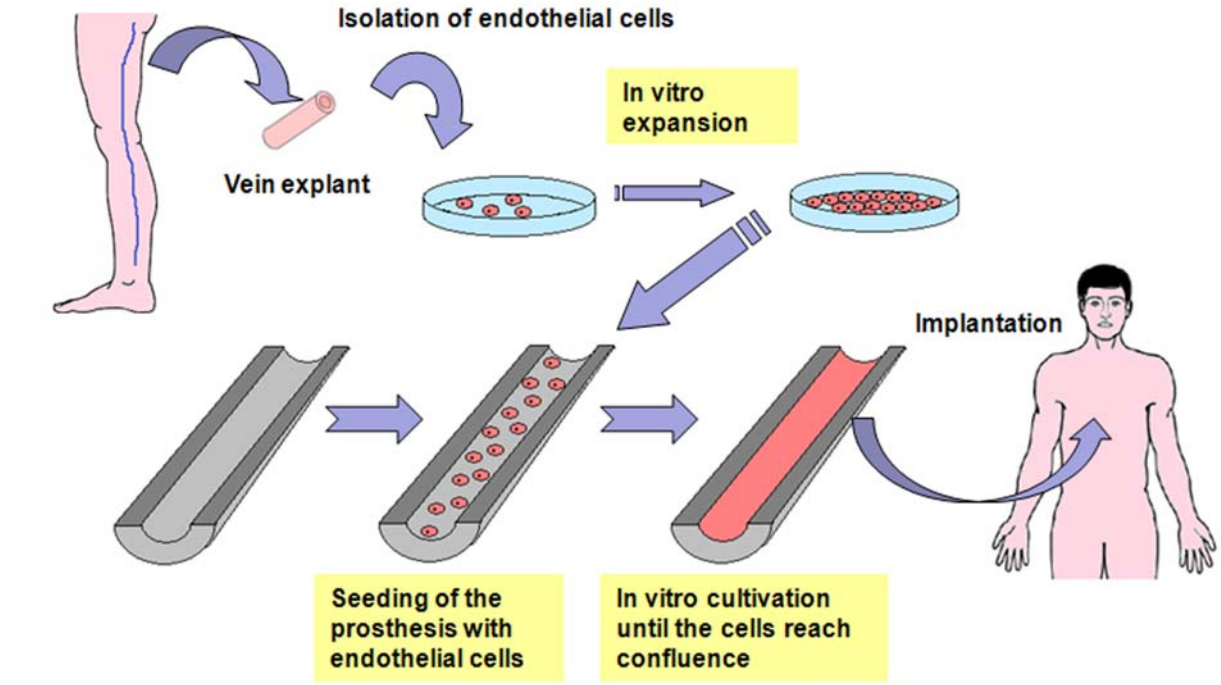

Figure 1: Schematic representation of *in vitro* endothelialisation process of artificial vascular grafts. Autologous endothelial cells are extracted from a piece of the patient's vein and expanded *in vitro* until the required cell number is achieved. Subsequently, vascular grafts are seeded with these cells and the cultivation proceeds until confluence is reached. Finally, the *in vitro* endothelialised grafts are implanted into the patient.

cell number is achieved. Thereafter, grafts are seeded with these cells and cultivated until they reach confluence. Finally, the *in vitro* endothelialised grafts are implanted into the patient. Due to this extensive procedure, the *in vitro* endothelialisation of grafts is very time-consuming and expensive, and harbours the risk of bacterial contamination and, therefore, is often unfeasible in clinical practice.

EPCs – A fascinating alternative cell source for endothelialisation of vascular grafts

In 1997, Asahara and colleagues (Asahara *et al*., 1997) identified bone marrow-derived endothelial progenitor cells (EPCs) in the circulating peripheral blood of adults that are capable of attaining EC characteristics *in vitro*. In further studies, it was demonstrated that these cells are able to repair injured blood vessels and to regenerate the function of ischaemic organs by vasculogenesis and angiogenesis (Hristov *et al*., 2003; Szmitko *et al*., 2006).

The presence of these precious endothelial progenitor cells in adults offers scientists the possibility to generate an autologous endothelium on vascular grafts or stents (Szmitko *et al*., 2006) without the need of a vessel biopsy for isolation of ECs. However, since the discovery of EPCs, there is a controversy about the identity of true circulating EPCs (Hur *et al.,* 2004; Prater *et al*., 2007; Yoder *et al*., 2007). At least 2 fundamentally different EPC populations can be obtained from peripheral blood by *in vitro* cultivation of mononuclear cells (MNCs), namely the "early" EPCs (also called colony forming unit-endothelial cells (CFU-ECs)) or "late" EPCs (also known as endothelial colony forming cells (ECFCs) or blood outgrowth endothelial cells (BOECs)) (Prater *et al*., 2007). Different cultivation methods for isolation of EPCs from MNCs are used by scientists. "Early" EPCs are similar to Asahara´s EPCs and can be obtained by using the commercially available kit EndoCult™ from StemCell Technologies (Grenoble, France). For this purpose, MNCs are isolated from the peripheral blood by density gradient centrifugation and incubated for 48 h on fibronectin-coated plates in EndoCult™ medium to remove adherent monocytes. Nonadherent cells are removed and re-plated on new fibronectin-coated dishes. CFU-EC colonies appear within 4-7 days of culture as discrete colonies comprising round cells in the centre and spindle-shaped cells sprouting at the periphery (Hill *et al*., 2003). "Late" EPCs can be obtained using the cultivation method reported by Lin and Ingram (Ingram *et al*., 2004; Lin *et al*., 2000). MNCs are incubated on collagen I-coated plates in endothelial specific growth medium. Growth medium is gently changed every day to discard non-adherent cells. ECFC colonies appear from the adherent cell population, after 2-3 weeks of culture and display typical endothelial cobblestone morphology. Hur and colleagues also demonstrated that both EPC populations can be obtained by using one culture protocol (Hur *et al*., 2004). For this purpose, they seeded MNCs on 2% gelatin-coated plates and incubated in endothelial specific growth medium. The first medium change was performed 6 days after plating. Thereafter, the medium was changed every 3 days. The "early" EPCs appeared after 3 to 5 days and their number increased for 2 weeks. Thereafter, they did not replicate

and gradually disappeared within 4 weeks after plating. The "late" EPCs appeared in 2 to 4 weeks after plating. "Early" EPCs express both endothelial and monocytic markers, such as CD14 and CD115, and exhibit a limited proliferation capacity (Rehman *et al*., 2003; Rohde *et al*., 2006; Zhang *et al*., 2006). In contrast, "late" EPCs are highly proliferative cells, which are found in lower cell densities (0.05-0.2 cells/ml) than "early" EPCs (50-500 cells/ml) in the peripheral blood of adults (Prater *et al*., 2007). Both EPC populations contribute to angiogenesis, but only "late" EPCs are able to form *de-novo* functional blood vessels *in vivo* (Critser and Yoder, 2010). However, Yoon and colleagues demonstrated that the transplantation of mixed EPCs, "late" and "early" EPCs, in athymic nude mice with hindlimb ischaemia results in synergistic augmentation of angiogenesis compared with any single type of EPC transplantation (Yoon *et al*., 2005). Hitherto, the exact *in vivo* functions of these different EPC populations are still unclear. In contrast to "early" EPCs, "late" EPCs exhibit high proliferation capacity and seem to be more appropriate for *in vitro* endothelialisation of large surfaces, such as vascular prosthesis, because of the ability to quickly generate a high number of ECs. Thus, these cells are probably best suited for rapid endothelialisation of prostheses. However, hitherto the *in vivo* endothelialisation efficiency of "late" and "early" EPCs is not well-studied. The capturing of "early" EPCs on implants could be also effective for *in vivo* endothelialisation of artificial surfaces. *In vivo*, "early" EPCs that secrete higher levels of angiogenic cytokines than "late" EPCs, may support endothelialisation by secreting proangiogenic cytokines (Hur *et al*., 2004; Zampetaki *et al*., 2008) and increase the homing of circulating EPCs. Furthermore, they may ensure the proliferation of "late" EPCs and the survival of matured ECs. Thus, the immobilisation of both types of EPCs could synergistically enhance the quality of the generated endothelium. Therefore, further *in vivo* studies are necessary to clarify the precise roles of "early" and "late" EPCs in view of the endothelialisation efficiency on implants.

A major obstacle for understanding, interpretation, and comparison of the previously published reports about EPCs is the lack of standardised methods for characterisation and naming of these cells. Research groups use the term "EPC" for a heterogeneous group of cells, such as for either "early" EPCs or "late" EPCs obtained *in vitro*. Other groups apply flow cytometry for quantification of EPCs, which are positive for CD34/CD133/VEGFR2 (Massa *et al*., 2005; Peichev *et al*., 2000), CD34/CD133 (Allanore *et al*., 2007), or CD34/VEGFR2 (Su *et al*., 2010). In turn others isolate CD34 positive cells using MACS (Magnetic Activated Cell Sorting) (Weber *et al*., 2004) and designate these cells as EPCs. Thus, for better interpretation of the data published in the literature, there is an urgent need for standardised identification and designation methods.

Strategies to increase mobilisation, homing, and incorporation of EPCs

EPCs are present in low numbers in the circulating peripheral blood of adults. Thus, different strategies exist to boost the number of circulating EPCs and to increase homing and incorporation of these cells at sites of vascular injury, ischaemia, or active angiogenesis. One strategy to improve homing and incorporation of EPCs at desired tissues is the *in vitro* pre-treatment of EPCs. They can be pre-stimulated by incubation with small molecules or genetically modified to activate signalling pathways involved in survival, overexpression of antiapoptotic proteins, or expression of adhesion molecules (Seeger *et al*., 2007). Another strategy is the delivery of proteins or genes to the target tissue for *in situ* induction of chemokines and chemoattractant factors to enhance the attraction and recruitment of EPCs from the blood stream. Furthermore, the elevation of the EPC number in the peripheral blood by systemic *in vivo* application of drugs can accelerate angiogenesis, vasculogenesis, and repair of an injured endothelium. Different strategies to increase vascularisation and homing of EPCs are illustrated in Fig. 2. Since the number and function of EPCs inversely correlates with risk factors for coronary artery disease (Vasa *et al*., 2001b; Hill *et al*., 2003; Werner *et al*., 2007b),

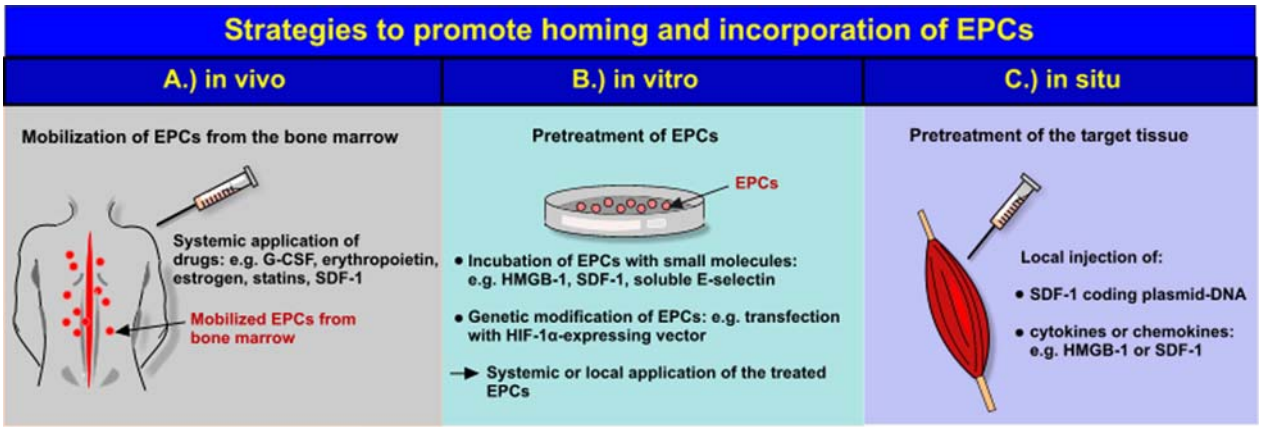

Figure 2: Different strategies for increased homing and incorporation of EPCs. (**A**) Systemic *in vivo* delivery of drugs for mobilisation of EPCs from the bone marrow into the peripheral blood. (**B**). *In vitro* pretreatment of EPCs or (**C**) pretreatment of the target tissue for *in situ* recruitment of EPCs to the target tissue.

Table 1: Strategies for increasing number and function of EPCs.

Strategies for	Drugs and Cytokines	Condition	Investigators	Effects
increasing the number of EPCs	HMG-CoA reductase inhibitors (statins) e.g. atorvastatin	*in vivo*	(Vasa *et al.*, 2001a)	Increase in number of EPCs
	ACE inhibitors e.g. ramipril	*in vivo*	(Min *et al.*, 2004)	
	AT II antagonists e.g. olmesartan, irbesartan	*in vivo*	(Bahlmann *et al.*, 2005)	
	Erythropoietin	*in vivo*	(Heeschen *et al.*, 2003)	
	G-CSF	*in vivo*	(Honold *et al.*, 2006)	
	VEGF	*in vivo*	(Asahara *et al.*, 1999)	
	PPAR-γ agonists e.g. rosiglitazone	*in vivo*	(Pistrosch *et al.*, 2005)	
increasing the function of EPCs	PPAR-γ agonists e.g. pioglitazone	*in vitro*/ *in vivo*	(Gensch *et al.*, 2007)	Prevents apoptosis of EPCs
	HMG-CoA reductase inhibitors (statins) e.g. atorvastatin, mevastatin	*in vitro*	(Assmus *et al.*, 2003)	Delay of EPC senescence
	simvastatin	*in vitro*/ *in vivo*	(Llevadot *et al.*, 2001)	Improved proliferation, enhanced migratory capacity of EPCs
	ACE inhibitors e.g. ramipril	*in vitro*	(Min *et al.*, 2004)	Increased functional activity of EPCs
	Erythropoietin	*in vitro*	(George *et al.*, 2005)	Increased proliferative and adhesive properties of EPCs
		in vitro	(Bahlmann *et al.*, 2004)	Increased number of functionally active EPCs
	Thrombin receptor activating peptide (SFLLRN)	*in vitro*	(Smadja *et al.*, 2005)	Induced proliferation, migration and increased capillary-like structure formation in Matrigel
	Resveratrol	*in vitro*	(Xia *et al.*, 2008)	Delayed the onset of senescence in EPCs
	Oestrogen	*in vitro*	(Imanishi *et al.*, 2005a)	Inhibited EPC senescence
	Puerarin	*in vitro*	(Zhu *et al.*, 2008)	Delayed the onset of senescence in EPCs and increased proliferation of EPCs

patients suffering from coronary artery disease can be medically treated to increase the number and functional activity of EPCs, thereby further improving the prospects of success for EPC based therapies.

Increasing the number and function of EPCs

Numerous cytokines, growth factors, drugs and hormones, such as vascular endothelial growth factor (VEGF) (Asahara *et al.*, 1999), angiopoietin-1, granulocyte colony-stimulating factor (G-CSF), stromal cell-derived factor-1 (SDF-1) (Askari *et al.*, 2003; Ceradini *et al.*, 2004), erythropoietin (Heeschen *et al.*, 2003), hepatocyte growth factor (HGF), oestrogen (Iwakura *et al.*, 2003; Strehlow *et al.*, 2003), and statins (Duckers *et al.*, 2007b; Vasa *et al.*, 2001a) induce the mobilisation of EPCs from the bone marrow into the circulating peripheral blood and can be used *in vivo* to boost the number of circulating EPCs in peripheral blood (Table 1). Furthermore, the *ex vivo* expansion of EPCs is often necessary for *in vitro* experiments or for potential clinical cell therapy of

ischaemic diseases. However, the *in vitro* cultivation of EPCs leads to cellular aging (senescence) and limits the proliferative capacity of these cells. Moreover, in patients with cardiovascular risk factors (Tepper *et al*., 2002; Imanishi *et al*., 2005b;) the function of EPCs is impaired. Dysfunction of EPCs may have adverse effects on EPC-based endothelialisation of biomaterials and clinical strategies that are performed to enhance the perfusion of ischaemic tissues in patients with coronary and peripheral artery disease. Fortunately, several *in vitro* studies with different drugs (Leone *et al*., 2009), such as erythropoietin (Bahlmann *et al*., 2004), statins (Assmus *et al*., 2003), and PPAR-γ (Peroxisome Proliferator-Activated Receptor-γ) agonists (Gensch *et al*., 2007; Werner *et al*., 2007a), demonstrated the ability to stimulate the function of EPCs (Table 1). Using a thrombin receptor activating peptide, SFLLRN (Serine-Phenylalanine-Leucine-Leucine-Arginine-Asparagine), Smadja and colleagues activated PAR-1 (Protease Activated Receptor-1) on human "late" EPCs, which in turn promoted EPC proliferation and enhanced SDF-1/CXCR4-mediated angiogenesis (Smadja *et al*., 2005). Other *in vitro* studies with drugs, such as HMG-CoA reductase inhibitors (Assmus *et al*., 2003), puerarin (Zhu *et al*., 2008), resveratrol (Xia *et al*., 2008), or oestrogen (Imanishi *et al*., 2005a) led to the inhibition of EPC senescence. Therefore, the application of these drugs could be useful to protect EPCs against dysfunction and to improve proliferative and functional capacity of these cells.

Increasing homing of EPCs

Prestimulation of EPCs

Homing of EPCs to the sites where vascular repair and neovascularisation are needed can be enhanced by up-regulation of adhesion molecules on EPCs. In previous studies, the pre-stimulation of EPCs with HMGB-1 (High-Mobility Group Box-1) (Chavakis *et al*., 2007), SDF-1 (Zemani *et al*., 2008), 8-pCPT-2′-O-Me-cAMP (Carmona *et al*., 2008; Patschan *et al*., 2010), activating β_2-integrin antibody (KIM185) or Mn^{2+} (Chavakis *et al*., 2005), leptin (Schroeter *et al*., 2008) and ephrin-B2-Fc chimera (Foubert *et al*., 2007) increased the homing of systemically administered EPCs. *In vivo*, the protein HMGB-1 is passively released from necrotic cells and actively secreted by activated immune cells (Rouhiainen *et al*., 2004). Thus, it serves as a chemoattractant for inflammatory and stem cells and elicits the migration of these cells to the sites of tissue damage (Palumbo *et al*., 2009). HMGB-1 transduces cellular signals via binding to RAGE (Receptor for Advanced Glycation End-products), Toll-like receptor 2 (TLR2) or 4 (TLR4) (Lotze and Tracey, 2005). From these receptors, RAGE and TLR2 are expressed on EPCs and the pre-stimulation of EPCs with HMGB-1 activates β_1 and β_2 integrins on EPCs and induces the adhesion to mature ECs, ICAM-1, and fibronectin. Using this process, Chavakis *et al*. (2007) observed improved homing and adhesion of EPCs at sites of ischaemia and active angiogenesis. Soluble E-selectin (sE-selectin) is another potent angiogenic mediator (Koch *et al*., 1995; Kumar *et al*., 2003), which was determined in elevated concentrations by Oh and colleagues (Oh *et al*., 2007) 24 h after ischaemia. The stimulation of ECs with sE-selectin induced the expression of adhesion molecules ICAM-1 and VCAM-1, which in turn contributed to EPC recruitment in ischaemic tissues and increased angiogenesis. Since EPCs have (additionally to the E-selectin) functional E-selectin ligands on their surface, the sE-selectin can also modulate EPCs. Therefore, the stimulation of EPCs with sE-selectin augmented interleukin-8 secretion and led to enhanced migration, capillary tube formation, and incorporation of EPCs into ECs.

Pretreatment of the target tissue

The local injection of cytokines or chemokines into the target tissue is another promising approach to attract progenitor cells. SDF-1 is one of the most important chemotactic and pro-migratory factors and binds to the G-protein-coupled receptor CXCR4 (CXC Chemokine Receptor 4), which is expressed on monocytes, macrophages, B and T lymphocytes, platelets, megakaryocytes, and CD34 positive cells, including both EPCs and haematopoietic stem cells (Moore *et al*., 2001). Several studies demonstrated the ability of locally administered SDF-1 to augment the EPC accumulation in a mouse model of hindlimb ischaemia (Yamaguchi *et al*., 2003; Yu *et al*., 2009). In further studies, Ceradini *et al*. (2004) showed that SDF-1 gene expression in ECs is regulated by the transcription factor HIF-1 (Hypoxia-Inducible Factor-1), which is a key mediator of cellular hypoxia response and regulates the expression of genes involved in angiogenesis, glucose metabolism, vascular tone, and oxygen transport (Frede *et al*., 2009). The induction of SDF-1 expression via HIF-1 increased the migration, homing, and adhesion of circulating CXCR4-positive cells to areas of injury. Furthermore, the *ex vivo* transfection of EPCs with an HIF-1α-expressing vector increased revascularisation efficacy in an *in vivo* mouse model of hindlimb ischaemia (Jiang *et al*., 2008). In comparison to untransfected EPCs, HIF-1α-overexpressing EPCs showed increased proliferative potential and sensitivity towards angiogenic factors. This is probably due to the recruited HIF-1α-expressing EPCs, which promote SDF-1 expression and recruit more EPCs to the injury site, and thereby lead to increased revascularisation. Furthermore, Hiasa and colleagues demonstrated that the intramuscular injection of SDF-1 coding plasmid-DNA into mice significantly augments the homing of progenitor cells to the ischaemic limb and enhances ischaemia-induced vasculogenesis and angiogenesis (Hiasa *et al*., 2004).

Biofunctionalisation of implant surfaces to capture EPCs from blood stream

The *in vivo* endothelialisation of blood contacting prostheses inside the body is a promising alternative to the expensive and time-consuming *in vitro* generation of an endothelium on synthetic materials. For this purpose, blood contacting materials are coated with capture molecules like peptides, proteins, antibodies, magnetic

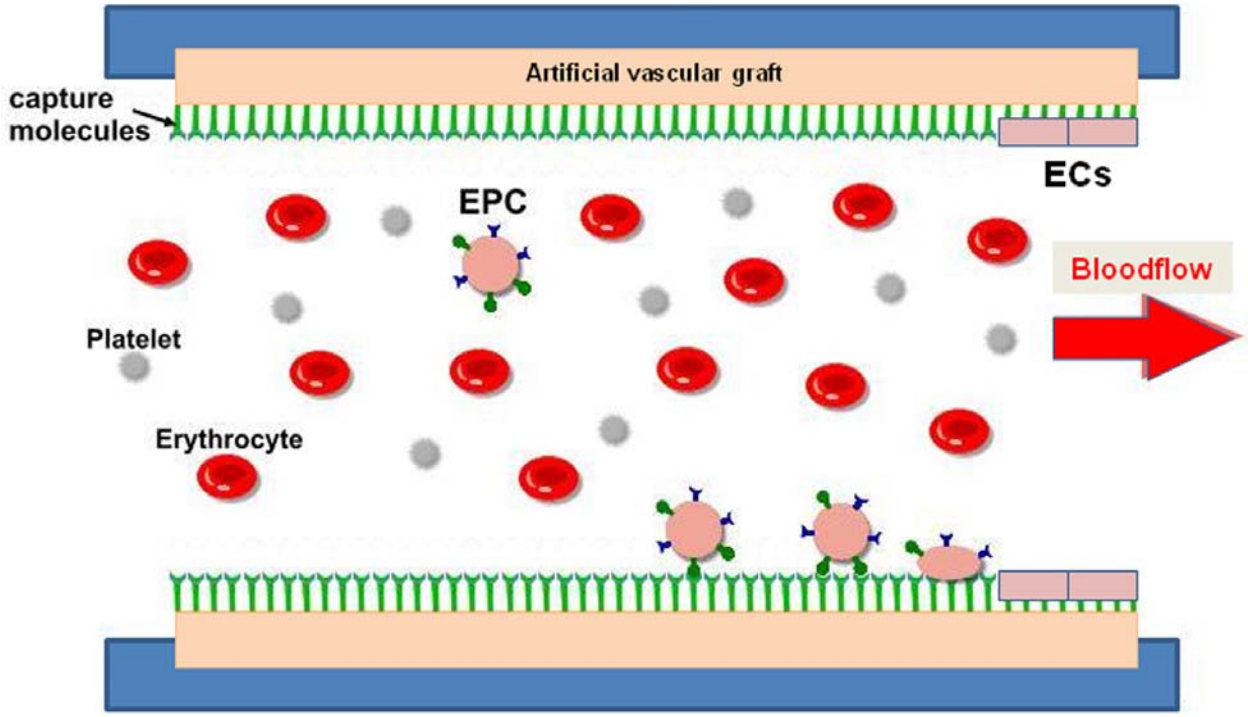

Figure 3: Immobilisation of EPCs on artificial vascular grafts coated with EPC-specific capture molecules. Peptides, proteins, antibodies, magnetic molecules, or aptamers can be used as capture molecules for EPCs. These molecules bind to their target on EPCs and immobilise the progenitor cells onto graft surfaces. Subsequently, captured EPCs can differentiate into endothelial cells and build an autologous endothelial cell layer onto artificial vascular grafts. The additional coating of the graft surface with a haemocompatible thromboresistant polymer matrix prevents the binding of other blood cells or serum proteins.

molecules, or aptamers to enable the homing of EPCs on implant surfaces (Table 2). After the implantation, EPCs are attracted directly from the blood stream to the implant surface and the endothelialisation process starts immediately. Thus, an optimal surface with high biocompatibility can be created and biomaterial induced immune reactions can be avoided. In Fig. 3, the *in vivo* endothelialisation concept of biofunctionalised implants is shown.

To ensure the successful homing of EPCs on implants, the surface coating should meet two requirements. The first requirement is the immobilisation of highly specific capture molecules. The second requirement is the prevention of unspecific adhesion of other blood cells and serum proteins, which can rapidly cover the immobilised capture molecules and impede the recognition of EPCs. Furthermore, the coatings can contain covalently immobilised growth factors, such as VEGF and Angiopoietin-1 (Chiu and Radisic, 2010) to support the differentiation of EPCs into ECs and the survival and proliferation of these cells.

Different Capture Molecules for EPCs

Monoclonal antibodies (MAB) recognizing EPC surface receptors

Circulating EPCs are characterised by expression of the early surface markers CD34 (Asahara *et al*., 1997), CD133, and VEGFR-2 (Vascular Endothelial Growth Factor Receptor-2; KDR) (Miller-Kasprzak and Jagodzinski, 2007) on their surface. Thus, several research groups are focused on creation of coatings with specific monoclonal antibodies, which recognise these main EPC surface receptors.

CD34 MAB

The Genous™ EPC capturing stent (OrbusNeich Medical Technologies (Fort Lauderdale, FL, USA) coated with anti-human CD34 monoclonal antibodies is the first biofunctionalised stent for *in vivo* endothelialisation. The safety and feasibility of this stent was demonstrated in the single-centre HEALING-FIM (Healthy Endothelial Accelerated Lining Inhibits Neointimal Growth-First In Man) study (Aoki *et al*., 2005). Later, the efficacy of the Genous™ stent to prevent in-stent restenosis was evaluated (Silber, 2006; Duckers *et al*., 2007a; Duckers *et al*., 2007b;). In this multicentre HEALING II study, patients on statins had more EPCs and less in-stent late loss than those not on statins. Therefore, patients in the HEALING IIB multicentre study were pretreated with atorvastatin prior percutaneous coronary intervention (PCI) in order to increase EPC levels and to clarify the safety and efficacy of the Genous™ stent in conjunction with statins. The statin therapy resulted in a 459% relative increase in the number of EPCs. However, the titre of circulating $CD34^+$ stem cells was elevated by only 12% (Garg *et al*., 2010), which shows that there is a discrepancy between the cells identified as EPCs and $CD34^+$ cells. Probably, due to the failure of the therapy to augment $CD34^+$ stem cell levels,

Table 2: Capture molecules for EPCs (part 1).

Capture Molecules	Application	Study	Investigators	Outcome
Monoclonal antibodies (MAB)	**CD34 MAB** coated stent (Genous™ stent)	*in vivo* (human) (HEALING-FIM)	(Aoki *et al.*, 2005)	Within 9 months : - No stent thrombosis - MACCE (Major adverse cardiac and cerebrovascular events) occured in only one patient out of 16 patients
		in vivo (human) (HEALING II)	(Duckers *et al.*, 2007a)	Patients on statins had more EPCs and less in-stent late loss than those not on statins
		in vivo (human) (HEALING IIB)	(Garg *et al.*, 2010)	The pretreatment of patients with atorvastatin prior PCI had no benefit in terms of angiographic outcomes
		in vivo (human) TRIAS	(Beijk *et al.*, 2010)	After one year : - Higher late lumen loss in the Genous™ stent compared with the Taxus stent - 4 stent thromboses with Taxus stent - No stent thrombosis with Genous™ stent
	CD34 MAB immobilised onto a sirolimus-eluting stent (SES)	*in vivo* (pig)	(Nakazawa *et al.*, 2010)	Up to 80% endothelialisation at 14 days
	CD34 MAB coated ePTFE grafts	*in vivo* (pig)	(Rotmans *et al.*, 2005)	Rapid endothelialisation Inceased neointimal hyperplasia
	CD34 MAB immobilised on electrodes of a microfluidic system	*in vitro*	(Ng *et al.*, 2010)	Trapping and accumulation of $CD34^+$ cells from PBMCs
	VEGFR-2 MAB coated solid surface	*in vitro*	(Markway *et al.*, 2008)	Ability to selectively capture flowing $VEGFR\text{-}2^+$ HUVECs
	VEGFR-2 MAB coated microfluidic device	*in vitro*	(Plouffe *et al.*, 2009)	Captured approximately 17-fold more EPCs than only BSA treated devices
	Bispecific molecule (**GPVI-CD133 construct**) immobilised on collagen coated surfaces or preincubated with EPCs	*in vitro*/*in vivo* (mouse)	(Langer *et al.*, 2010)	10-fold enhanced adhesion of human $CD34^+$ cells under flow conditions compared to collagen alone 14 days after carotid artery injury : Enhanced reendothelialisation of vascular lesions
Aptamers	**DNA-aptamers** immobilised onto PDMS or PTFE discs	*in vitro*	(Hoffmann *et al.*, 2008)	Captured EPCs from whole anticoagulated porcine blood under flow conditions

Continued on page 164.....

Table 2: Capture molecules for EPCs (part 2).

Peptides	**cRGD peptides** coated stent	*in vivo* (pig)	(Blindt *et al.*, 2006)	After 12 weeks : - Significantly reduced mean neointimal area and percent area stenosis compared with BMS
	Polyurethane vascular grafts with **YIGSR peptides**, diazeniumdiolate NO donor and PEG	*in vitro*	(Taite *et al.*, 2008)	Encouraged adhesion of ECs Reduced platelet adhesion Reduced proliferation of SMCs Enhanced proliferation of ECs
	Nanofibrous matrix with MMP-2 cleavage sites coupled to **YIGSR peptides** and NO donors	*in vitro*	(Kushwaha *et al.*, 2010)	Enhanced EC growth Inhibition of SMC growth Reduced platelet adhesion
	Metacrylic terpolymer with **TPS peptide** selected by phage display	*in vitro*	(Veleva *et al.*, 2008)	in serum free medium specific binding to HBOECs
Selectins and their ligands	**sLe^x** immobilised into a collagen matrix	*in vitro*	(Suuronen *et al.*, 2009)	Recruited more $CD133^+CD34^+$L-$selectin^+$ cells than collagen only matrix
		in vivo (Sprague-Dawley rats)	(Suuronen *et al.*, 2009)	Enhanced neovascularisation and improved tissue perfusion of ischemic hindlimbs
	P-selectin coated microtubes	*in vitro*	(Narasipura *et al.*, 2008)	significantly higher number of $CD34^+$ cells on P-selectin coated devices than on CD34 MAB coated devices
	P-selectin coated blood-compatible plastic tubes	*in vivo* (Sprague-Dawley rats)	(Wojciechowski *et al.*, 2008)	After 1 hour of blood perfusion: - Up to 7-fold enrichment of the CD34+ cells than on non-coated control tubes
Magnetic molecules	**SPIO microspheres**-loaded ECs	*in vivo* (rabbit)	(Consigny *et al.*, 1999)	Circumferential delivery of ECs to the luminal surface of injured arteries
	SPIO microspheres-loaded EPCs	*in vivo* (pig)	(Pislaru *et al.*, 2006a)	After 1 day: - Uniform cell coverage on magnetised knitted Dacron grafts
		in vivo (pig)	(Pislaru *et al.*, 2006b)	After 1 day: - Magnetised stents retained 6- to 30-fold more EPCs than non-magnetised stents
	SPIO nanoparticles-loaded EPCs	*in vivo* (Sprague-Dawley rats)	(Kyrtatos *et al.*, 2009)	After 1 day: - With magnet 5-fold enhanced cell localisation compared to cell delivery without magnet

the angiographic outcomes of Genous™ stent were not improved.

In the randomised single-centre TRIAS (TRI-stent Adjudication Study) trial, the Genous™ stent was compared with a conventional drug eluting stent (DES) (the Taxus Liberté paclitaxel-eluting stent) in patients with *de novo* coronary lesions and a high-risk of coronary restenosis (Beijk *et al*., 2010). One year outcomes of the study showed that a subgroup of patients had a significantly higher late lumen loss in the Genous™ stent compared with the Taxus stent. However, compared to four stent thromboses with Taxus stent, no stent thrombosis was observed with Genous™ stent. Prior to the completion of the 1-year follow-up, this single-centre study was transformed into a multi-centre randomised, 2-armed TRIAS study (TRIAS High Risk (HR) and TRIAS Low Risk (LR)) to increase patient recruitment. The TRIAS HR study compares the Genous™ stent with the Taxus stent in patients with a high risk of restenosis. Meanwhile, the TRIAS LR study compares the Genous™ stent with the bare metal stent (BMS) in patients with a low risk of restenosis (Klomp *et al*., 2009). The monitoring of patients will be conducted during a 5-year follow-up period.

Rotmans and colleagues implemented the coating technique, which is used for Genous™ stent, to coat ePTFE grafts with anti-human CD34 MAB (Rotmans *et al*., 2005). The implantation of these grafts in an *in vivo* porcine model resulted in rapid endothelialisation, which was verified by lectin staining (a surface antigen shared by several blood-borne cells, including monocytes). However, the endothelialisation was also associated with significantly increased neointimal hyperplasia at the venous anastomosis. This adverse effect might be caused by differentiation of attracted $CD34^+$ cells into vascular SMCs (Shimizu *et al*., 2001; Simper *et al*., 2002). Previous studies demonstrated the ability of $CD34^+$ cells to differentiate into diverse cell types, including ECs, cardiomyocytes, and vascular SMCs (Yeh *et al*., 2003). Haematopoietic stem cells (HSCs) also express CD34 on their cell surface and can differentiate into SMCs *in vitro* and *in vivo* (Sata *et al*., 2002). These cells can contribute to pathological arterial remodelling and lead to the increased proliferation index in anti-CD34 antibody coated grafts. Furthermore, the attracted EPCs can release growth factors, such as VEGF and HGF (Rehman *et al*., 2003), which can stimulate migration and proliferation of vascular SMCs (Grosskreutz *et al*., 1999; Ma *et al.,* 2003; Parenti *et al*., 2004) and lead to increased intimal hyperplasia. Therefore, a combination of the Genous™ stent and drug eluting stent has been developed. Anti-human CD34 antibodies were immobilised onto the surface of a sirolimus-eluting stent (SES) to suppress the neointimal hyperplasia while promoting EC coverage (Nakazawa *et al*., 2010). The stent deployment in pig coronary arteries resulted in increased endothelialisation compared with the standard SES alone at 3 and 14 days. The endothelialisation of the Genous™ stent was nearly completed at 14 days (98%). Although the endothelialisation rates of the Genous™ stent were approximately 2-fold greater than the SES-anti-CD34 stent at 3 days, the SES-anti-CD34 stent demonstrated up to 80% endothelialisation at 14 days. However, further studies are needed to evaluate the long term impact of these stents on neointimal proliferation.

The ability of anti-CD34 antibody-coated surfaces to capture $CD34^+$ cells from a cell suspension composed of peripheral blood mononuclear cells (PBMCs) was also demonstrated by Ng and colleagues (Ng *et a*l., 2010). Anti-CD34 antibodies were immobilised on silane-PEG SAM (Polyethylene Glycol Self-Assembled Monolayer) passivated electrodes of a microfluidic system. The integrated negative dielectrophoresis (DEP) (Thomas *et al*., 2009) enabled the trapping and accumulation of $CD34^+$ cells at the centre of electrodes. Thus, this chip system can be applied for label-free quantification of $CD34^+$ cells in PBMC samples, which were termed as EPCs by Ng *et al*. (2010).

Despite promising results, the described studies should be viewed critically. CD34 is the most commonly used marker for identification and isolation of circulating EPCs. However, CD34 is not only specific for EPCs, but it is also expressed on HSCs. Peichev *et al*. demonstrated that circulating non-adherent EPCs, which have the ability to differentiate into mature adherent ECs, co-express additionally to CD34, the endothelial as well as haemangioblastic marker VEGFR-2, and the haematopoietic stem cells antigen CD133 on their surface (Peichev *et al*., 2000). Only 0.002% of the total PBMCs were CD34 positive, and only 0.4±0.2% of the total CD34+ cells were in fact EPCs (defined by additional expression of VEGFR-2 and CD133). That means that 99.6% of the anti-CD34 antibody attracted cells are not EPCs. A multitude of these cells have the ability to differentiate into proinflammatory cells. Thereby, intimal hyperplasia can be further accelerated, which is associated with deterioration of the graft patency. Furthermore, the precise characterisation of captured cells onto anti-CD34 antibody-coated implants using immunohistochemistry may improve the interpretation and understanding of the clinical outcomes.

VEGFR-2 MAB

The major mediator of the angiogenic and mitogenic effects of VEGF is the VEGFR-2, which is, in addition to mature ECs, also present on the circulating EPCs (Ferrara *et al*., 2003). Therefore, antibodies against VEGFR-2 can be also used to attract EPCs onto artificial grafts. In a model system under low shear rates, Markway and colleagues demonstrated the ability of protein G-oriented anti-VEGFR-2 MB to selectively capture flowing $VEGFR\text{-}2^+$ human umbilical vein endothelial cells (HUVECs) onto a solid surface (Markway *et al*., 2008). The protein G-orientation of antibodies enhanced the availability of the antigen-binding domains and resulted in approximately 2.5-fold higher capture efficiency than directly adsorbed antibodies did. After binding, antibody mediated adhesion was not disrupted, even if the shear rate was increased to arterial shear rate. Furthermore, Plouffe *et al*. conjugated the surface of a microfluidic device with antibodies against CD34 or VEGFR-2 to capture *in vitro* cultivated ovine peripheral blood-derived EPCs (Plouffe *et al*., 2009).

Under low shear stress, anti-CD34 antibody-coated microfluidic devices were able to capture approximately 20-fold more EPCs than only BSA treated devices. Also, the coating with anti-VEGFR-2 antibody resulted in approximately 17-fold higher EPC binding *versus* BSA controls. Thus, the ability of anti-VEGFR-2 antibody coated capture devices to bind EPCs was demonstrated. However, further studies with a mixture of cells, such as PBMCs, could be performed to ensure the specificity and to determine the efficiency of this antibody. Moreover, as an alternative to the anti-VEGFR-2 antibody, the electrostatically or covalently bound ligand of VEGFR-2 (namely, VEGF) could be used to attract EPCs on implant surfaces (Anderson *et al*., 2009).

CD133 MAB

Recently, a promising new concept for further modification of EPC specific capture molecules was developed by Langer and colleagues to guide the captured EPCs to vascular lesions (Langer *et al*., 2010). A bispecific molecule (called GPVI-CD133 construct) was designed, consisting of a recombinant form of the soluble platelet collagen receptor glycoprotein VI (GPVI) targeting collagen, which is the main component of the injured vessel wall, and a monoclonal antibody targeting CD133 on EPCs. *In vitro*, the preincubation of the collagen surface with the GPVI-CD133 construct enhanced the adhesion of human $CD34^+$ cells, which were termed as EPCs, by approximately tenfold under flow conditions compared to collagen alone. In NOD/SCID (Non-Obese Diabetic/Severe Combined Immunodeficiency) mice, 14 days after injury of the carotid artery and application of human EPCs, enhanced reendothelialisation of vascular lesions was observed when EPCs were preincubated with the bispecific construct compared to EPCs treated with the single components of the construct alone.

Despite these promising results, the use of collagen specific ligand to guide the captured EPCs to the injured endothelium should be viewed critically. Platelets have two major receptors that mediate the direct platelet-collagen interaction: the integrin $\alpha_2\beta_1$ and GPVI (Best *et al*., 2003). These receptors are involved in platelet attachment to the injured arterial wall and play a crucial role in the initiation of haemostasis and thrombosis *in vivo*. The high number of platelets (150,000-450,000 per µl blood) expressing collagen receptors enables the immediate recognition of vascular injuries. Therefore, the vascular lesions can be covered by platelets before the bispecific construct can arrive at the location in effective numbers. Because of this, it could be necessary to use very high amounts of bispecific construct to compete against platelets. Furthermore, EPCs localised in the bone marrow or immediately after migration into the circulation are CD133 positive; however, circulating EPCs obviously lose CD133 and begin to express typical mature EC surface markers (Hristov *et al*., 2003). Thus, EPCs in the peripheral blood that lost CD133 and differentiated into more mature ECs cannot be recognised by the bispecific construct. Thereby, a smaller number of EPCs can be captured. Unfortunately, it should be also mentioned that CD133 is also expressed by haematopoietic stem cells. However, the use of bispecific molecules to capture EPCs and to guide them to the desired tissues is a promising approach.

Aptamers

Due to their ability to fold into specific three-dimensional structures, short single-stranded oligonucleotides, called aptamers, can bind to a given target with high affinity and specificity. They are generally 70-90 nucleotides long and can be selected using the SELEX (Systematic Evolution of Ligands by EXponential enrichment) combinatorial chemistry process (Ellington and Szostak, 1990; Tuerk and Gold, 1990), which mimics the Darwinian evolution in test tubes (Tavitian and Haberkorn, 2009). Iterative cycles of *in vitro* selection and amplification allow the enrichment of target specific aptamers from a large chemically synthesised random sequence library composed of up to10^{15} different sequences. Typically, the selected aptamers bind their targets with dissociation constants (K_d) in the low picomolar (10^{-12} M) to low nanomolar (10^{-9} M) range (Nimjee *et al*., 2005). Since the discovery of SELEX technology in 1990, aptamers have received tremendous attention in the fields of targeted drug delivery (Chu *et al*., 2006b; Chu *et al*., 2006a; Farokhzad *et al*., 2006; Huang *et al*., 2009; McNamara *et al*., 2006), diagnosis (Shangguan *et al*., 2007; Tang *et al*., 2007), imaging (Blank *et al*., 2001; Schäfer *et al*., 2007), target validation (Blank and Blind, 2005; Cerchia and de Franciscis, 2007), therapeutics (Bock *et al*., 1992; Cosmi, 2009), biosensing (Bing *et al*., 2010; Liu *et al*., 2009), and cell fishing (Guo *et al*., 2006; Schäfer *et al*., 2007; Phillips *et al*., 2009; Pan *et al*., 2010). After 20 years of effort, aptamers have been generated against a broad range of targets, including small molecules, peptides, proteins, drugs, and even whole cells (Daniels *et al*., 2003; Cerchia *et al*., 2005; Ohuchi *et al*., 2006; Mallikaratchy *et al*., 2007; Raddatz *et al*., 2008) or organisms (Ulrich *et al*., 2002; Chen *et al*., 2007).

Using the SELEX technology, Hoffmann and colleagues were able to enrich DNA-aptamers against peripheral blood derived porcine CD31 positive EPCs (Hoffmann *et al*., 2008). The selected aptamer candidates were immobilised onto polydimethylsiloxan (PDMS) or polytetrafluoroethylene (PTFE) discs, using a haemocompatible reactive six-arm star-shaped polyethylene glycol (starPEG) coating (Hoffmann *et al*., 2006). The antithrombogenic starPEG coating prevents the undesired protein adsorption. Thereby, the covering of immobilised capture molecules by serum proteins can be avoided and the specific binding of circulating EPCs onto blood contacting implants can be significantly increased. In a modified Chandler-loop model (Chandler, 1958), discs with immobilised aptamers or control discs with nonsense aptamers were incubated with whole anticoagulated porcine blood. One of the generated aptamers was able to capture CD31 (PECAM-1) and CD144 (VE-cadherin) positive EPCs under flow conditions. These cells were cultivated for 10 days *in vitro* and exhibited properties of endothelial cells. Thus, the ability of aptamers to capture EPCs from peripheral blood was demonstrated in an *in vitro* closed-loop model under flow conditions.

Further cell-SELEX experiments are currently being performed to generate human EPC specific aptamers. Because cell-SELEX technology uses whole living cells as the target to generate cell specific aptamers (Guo *et al*., 2008), it is not necessary to know the molecular composition of the cell surface. However, it is important to perform SELEX experiments with defined and well characterised EPC populations to prevent the loss of enriched aptamers by incubation with altered cells in the next selection rounds. Furthermore, since cell-SELEX has the potential to develop aptamers for unknown molecules, the selected aptamers can then be used to purify and identify the target for aptamer recognition. In this way, new EPC specific biomarkers can be discovered.

Peptides

Extracellular matrix derived peptides

In recent years, specific peptide motifs of the extracellular matrix (ECM) components that interact with cell integrins inspired scientists to use these peptides for biofunctionalisation of biomaterial surfaces to enable accelerated *in situ* endothelialisation (de Mel *et al*., 2008).

Cyclic RGD (cRGD) peptides

Several ECM proteins, including fibronectin, vitronectin, and laminin, contain the RGD (Arg-Gly-Asp) sequence, which binds to integrin receptors on the cell surface and enables the adhesion of cells to ECM. Since the conformation of cRGD peptide mimics more closely the native ligand, this peptide is preferred for surface immobilisation. Several groups demonstrated in their studies the expression of integrins with cRGD binding motifs on EPCs (Peled *et al*., 2000; Walter *et al*., 2002). Therefore, Blindt and colleagues coated stents with a new polymer that contained cRGD peptide and examined the capability of these stents to bind EPCs and to reduce neointimal hyperplasia. These stents were implanted in porcine coronary arteries. Histomorphometric analyses after 12 weeks demonstrated that the mean neointimal area and percent area stenosis were significantly reduced in cRGD-loaded polymer stents by accelerated endothelialisation compared with the unloaded polymer or bare metal stents (Blindt *et al*., 2006). However, despite these first positive results, it should be mentioned that integrins with RGD binding motifs are not only existent on EPCs. Above all, platelets exhibit glycoprotein GPIIb-IIIa, the most abundant platelet receptor, on their surface. It recognises the RGD sequence (Sanchez-Cortes and Mrksich, 2009) in several adhesive proteins, such as fibrinogen, fibronectin, vitronectin, von Willebrand factor, and thrombospondins. Since platelets are present in a much higher number in the peripheral blood than EPCs, they can quickly bind to cRGD peptides and cover the implants within the shortest time. Therefore, capture molecules cannot bind to EPCs anymore. Moreover, the additional promotion of the platelet adhesion to the artificial grafts may lead to an enormous acceleration of the thrombus formation.

YIGSR peptides

Another peptide, which has also been used to promote endothelialisation on biomaterials, is the laminin-derived (Tyr-Ile-Gly-Ser-Arg) YIGSR peptide (Jun and West, 2004; Jun and West, 2005a; Jun and West, 2005b). Taite and colleagues incorporated this peptide, a diazeniumdiolate nitric oxide (NO) donor, and polyethylene glycol (PEG) into the backbone of polyurethane with the aim to improve the long-term patency of polyurethane vascular grafts (Taite *et al*., 2008). The modification encouraged the adhesion of ECs to the material. Simultaneously, both the incorporation of diazeniumdiolate NO donor and PEG reduced the platelet adhesion to the polyurethane surface. Additionally, the incorporated NO donor reduced proliferation of SMCs, but enhanced proliferation of ECs.

Recent tissue engineering applications are focused on mimicking biological, chemical, and nanostructural properties of natural ECM by self-assembly of peptide amphiphiles (PAs) into nanofibre networks. The viscoelastic properties, degradability, and bioactivitiy of such networks might be tuned by altering the length and chemical composition of PAs (Jun *et al*., 2008). Kushwaha and colleagues developed a native endothelial ECM mimicking nanofibrous matrix, which could be used as a self-assembled coating on vascular implants (Kushwaha *et al*., 2010). The nanofibrous matrix is formed by self-assembly of two different PAs that consist of hydrophobic tails coupled to hydrophilic functional peptide sequences. One PA (C_{16}-GTAGLIGQYIGSR) consisted of a matrix metalloproteinase-2 (MMP-2) cleavage site (GTAGLIGQ) coupled to the endothelial cell-adhesive ligand (YIGSR). The other PA (C_{16}-GTAGLIGQKKKKK) contained a matrix metalloproteinase-2 (MMP-2) cleavage site (GTAGLIGQ) that was coupled to a polylysine (KKKKK) group to form NO-donating residues. The release of NO from the nanofibrous matrix significantly enhanced EC growth and reduced SMC growth. Furthermore, a 150-fold decrease in platelet adhesion was determined on the NO-releasing nanofibrous matrix compared to the collagen-I coated surface. Such a nanofibrous matrix with multiple components can synergistically enhance the generation of an endothelium on biomaterials by supporting EC proliferation, inhibition of SMC growth, and platelet adhesion. Thus, endothelialisation is improved and the thrombosis and restenosis are prevented. However, the described studies were performed with ECs. Therefore, similar experiments might be performed with EPCs to examine whether EPCs behave similarly to ECs. Nevertheless, the modification of biomaterials by coatings with NO-releasing self-assembled PAs or by incorporation of NO donors and PEG are auspicious strategies that can be used in combination with each EPC capture molecule. Such a biomaterial could provide the optimal conditions for rapid *in vivo* endothelialisation of small-diameter vascular grafts.

Peptides selected by phage display

Phage display technology (Smith, 1985), which uses a combinatorial peptide library to identify novel cell specific

peptide ligands, has the advantage that the selection can be performed without prior knowledge of cell-surface molecules, such as in the cell-SELEX technology. Thus, it is a promising strategy to select new EPC specific peptide ligands. Using this technique, Veleva *et al*. (2007) isolated a peptide ligand (TPSLEQRTVYAK, called TPS) that binds to human blood outgrowth endothelial cells (HBOECs). This peptide was covalently coupled to a methacrylic terpolymer matrix (Veleva *et al*., 2008). In serum free medium, TPS peptide retained the binding affinity towards HBOECs and demonstrated limited binding to HUVECs. However, in the presence of serum proteins, cell-specific binding was inhibited. It is possible that the unspecific binding of serum proteins to the methacrylic terpolymer matrix led to the covering of EPC capture molecules and prevented the specific recognition of cells. Thus, protein-resistant terpolymers should be developed to ensure selective binding of EPCs. Especially for *in vivo* applications, the inhibition of unspecific serum protein adsorption to the implant surfaces plays an important role. Moreover, the identification of cell surface molecules that are recognised by TPS peptide could help to improve the prospects of success of this EPC capture molecule.

Selectins and their ligands

The selectins, E-, L-, and P-selectin, are important cell adhesion molecules that play a key role in tethering and rolling of leukocytes on inflammated endothelium (Kansas, 1996). Several groups demonstrated that EPCs exhibit the P-selectin glycoprotein ligand-1 (PSGL-1) on their cell surface, which mediates the recruitment of EPCs on activated endothelium by binding to P- and E-selectin (Vajkoczy *et al*., 2003; Foubert *et al*., 2007; Pfosser *et al*., 2009). Additional studies also demonstrated the expression of E-selectin as well as its ligand (Oh *et al*., 2007) and L-selectin (Biancone *et al*., 2004) on EPCs.

Since several studies had verified the ability of the oligosaccharide sialyl Lewis^x (sLe^x) to bind E-selectin (Brunk and Hammer, 1997), P-selectin (Rodgers *et al*., 2000), and L-selectin (Greenberg *et al*., 2000). Suuronen and colleagues (Suuronen *et al*., 2009) immobilised sLe^x into a collagen matrix and demonstrated enhanced adhesion of EPCs. Ischaemic hindlimbs receiving this matrix revealed increased neovascularisation and improved tissue perfusion. Charles *et al*. (2007) coated surfaces with P-selectin-, E-selectin-, or L-selectin-IgG and investigated the differences in rolling velocities of CD34 expressing and non-expressing cells. For this purpose, MNCs were isolated from human adult bone marrow and separated in CD34$^+$ fraction using Dynabeads and in CD34$^-$ fraction by depletion of CD34$^+$ cells. Thereafter, cells were perfused over immobilised selectins. CD34$^+$ cells exhibited slower rolling and stronger adhesion than CD34$^-$ cells when interacting with selectins in flow microchannels. Due to distinguishable rolling behaviour of CD34$^+$ cells from that of CD34$^-$ cells on selectin coated surfaces, selectin coated stents or grafts might be developed for the selective capturing of CD34$^+$ cells from blood flow. Therefore, Narasipura and colleagues performed additional studies to determine whether CD34$^+$ cells from human bone marrow-derived MNCs could be specifically captured on P-selectin functionalised microtubes (Narasipura *et al*., 2008). Thus, flow devices were coated with recombinant human P-selectin/F_c chimera or anti-CD34 antibody to capture and enrich CD34$^+$ cells. The total number of captured CD34$^+$ cells on P-selectin coated devices was significantly higher than on antibody coated devices. It is possible that the superior binding of cells to P-selectin is induced by the preferred flow-mediated nature of selectin binding. Later, the same research group implanted blood-compatible plastic tubes coated with P-selectin into the femoral artery of rats to determine whether CD34$^+$ cells could be captured directly from circulating blood (Wojciechowski *et al*., 2008). After 1 h of blood perfusion, the percentage of captured CD34$^+$ cells to total captured MNCs on P-selectin coated tubes was enriched up to 7-fold over the CD34$^+$ population on non-coated control tubes. However, only 28% of the captured MNCs were CD34$^+$ cells.

PSGL-1 is expressed on all white blood cells and plays an important role in recruitment of white blood cells into inflamed tissue. In contrast to low EPC amounts in the peripheral blood, the number of white blood cells in 1 ml blood is approximately 4 x 10^6 - 1.1 x 10^7cells. Because of this high cell amount, white blood cells can bind rapidly to the capture molecules and block them. Thereby, efficient capturing of EPCs cannot be ensured. Also, sLe^x is constitutively expressed on granulocytes and monocytes.

Magnetic Molecules

Labelling of EPCs with superparamagnetic iron oxide (SPIO) particles offers another attractive possibility to capture EPCs on implants. Using an external magnetic device, SPIO-loaded EPCs can be attracted to the desired place *in vivo*. The first attempts in this area were performed with SPIO microspheres-loaded ECs. By applying a magnet over an injured femoral artery in combination with animal rotation, Consigny and colleagues demonstrated nearly circumferential delivery of SPIO-loaded ECs to the luminal surface of injured arteries (Consigny *et al*., 1999). Later, Pislaru *et al*. (2006a) annealed a flexible magnetic sheet to external surface of a knitted Dacron graft and filled them with SPIO microspheres-loaded porcine EPCs for 10 min before placement. Grafts precoated with EPCs, were implanted in porcine carotid arteries. After one day, the grafts were evaluated and uniform cell coverage was observed on the magnetised surfaces. In order to enable the application of this technology for stents, Pislaru and colleagues generated magnetisable stents by coating commercially available stainless-steel stents with a 10 µm thick layer of nickel (Pislaru *et al*., 2006b). Stents were magnetised and implanted for one day in porcine coronary and femoral arteries. SPIO microspheres-loaded EPCs were delivered locally during transient occlusion of blood flow. Magnetised stents retained 6- to 30-fold more labelled EPCs than non-magnetised stents. In addition, in a recent study, Kyrtatos *et al*. (2009) magnetically tagged human EPCs with a FDA-approved superparamagnetic contrast agent (Endorem®), which is clinically used for magnetic

resonance imaging of the liver and spleen. SPIO nanoparticles-loaded EPCs were administered into the injured rat carotid arteries with and without the presence of an external magnetic device for 12 min. Magnet actuator was placed at a 5 mm vertical distance from the target tissue. After one day, 5-fold enhanced cell localisation was detected at the site of carotid artery injury compared to cell delivery without the magnet. Although this application is very promising, the capturing of superparamagnetic-labelled EPCs on magnetic surfaces is time consuming and expensive. EPCs have to be isolated and cultivated for approximately 2 weeks under GMP conditions and an additional incubation for 16 h is necessary for labelling of these cells *in vitro*. However, these obstacles could be overcome by functionalisation of SPIO nanoparticles (McCarthy and Weissleder, 2008) with EPC-specific peptides, antibodies, aptamers, or proteins. Thus, the isolation of EPCs is no longer necessary. However, the success of this approach depends on the availability of highly selective EPC-specific ligands. Moreover, further studies are needed to determine the long-term influence of iron particles on proliferation and function of captured EPCs. Furthermore, it should be demonstrated that the release of iron particles from EPCs does not lead to a local inflammation, which can trigger the intimal hyperplasia. Additionally, the development of biocompatible magnetisable graft materials is required to perform long-term *in vivo* studies.

Conclusions

Rapid *in vivo* endothelialisation of intravascular implants by capturing circulating autologous EPCs directly from the blood stream via biofunctionalised implant materials is a fascinating concept to enhance the haemocompatibility of blood contacting materials. However, the success of this strategy is highly influenced by the specificity and affinity of EPC capture molecules. Although numerous approaches have already been designed to immobilise EPCs on synthetic materials, further studies, more specific capture molecules, and improvements are necessary to upgrade the *in vivo* self-endothelialisation and for it to reach clinical maturity.

References

Allanore Y, Batteux F, Avouac J, Assous N, Weill B, Kahan A (2007) Levels of circulating endothelial progenitor cells in systemic sclerosis. Clin Exp Rheumatol **25**: 60-66.

Anderson SM, Chen TT, Iruela-Arispe ML, Segura T (2009) The phosphorylation of vascular endothelial growth factor receptor-2 (VEGFR-2) by engineered surfaces with electrostatically or covalently immobilized VEGF. Biomaterials **30**: 4618-4628.

Aoki J, Serruys PW, van Beusekom H, Ong AT, McFadden EP, Sianos G, van der Giessen WJ, Regar E, de Feyter PJ, Davis HR, Rowland S, Kutryk MJ (2005) Endothelial progenitor cell capture by stents coated with antibody against CD34: the HEALING-FIM (Healthy Endothelial Accelerated Lining Inhibits Neointimal Growth-First In Man) Registry. J Am Coll Cardiol **45**: 1574-1579.

Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM (1997) Isolation of putative progenitor endothelial cells for angiogenesis. Science **275**: 964-967.

Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, Iwaguro H, Inai Y, Silver M, Isner JM (1999) VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. EMBO J **18**: 3964-3972.

Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M, Rovner A, Ellis SG, Thomas JD, DiCorleto PE, Topol EJ, Penn MS (2003) Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet **362**: 697-703.

Assmus B, Urbich C, Aicher A, Hofmann WK, Haendeler J, Rossig L, Spyridopoulos I, Zeiher AM, Dimmeler S (2003) HMG-CoA reductase inhibitors reduce senescence and increase proliferation of endothelial progenitor cells via regulation of cell cycle regulatory genes. Circ Res **92**: 1049-1055.

Bahlmann FH, De Groot K, Spandau JM, Landry AL, Hertel B, Duckert T, Boehm SM, Menne J, Haller H, Fliser D (2004) Erythropoietin regulates endothelial progenitor cells. Blood **103**: 921-926.

Beijk MA, Klomp M, Verouden NJ, van GN, Koch KT, Henriques JP, Baan J, Vis MM, Scheunhage E, Piek JJ, Tijssen JG, de Winter RJ (2010) Genous endothelial progenitor cell capturing stent *vs*. the Taxus Liberte stent in patients with *de novo* coronary lesions with a high-risk of coronary restenosis: a randomized, single-centre, pilot study. Eur Heart J **31**: 1055-1064.

Best D, Senis YA, Jarvis GE, Eagleton HJ, Roberts DJ, Saito T, Jung SM, Moroi M, Harrison P, Green FR, Watson SP (2003) GPVI levels in platelets: relationship to platelet function at high shear. Blood **102**: 2811-2818.

Biancone L, Cantaluppi V, Duo D, Deregibus MC, Torre C, Camussi G (2004) Role of L-selectin in the vascular homing of peripheral blood-derived endothelial progenitor cells. J Immunol **173**: 5268-5274.

Bing T, Liu X, Cheng X, Cao Z, Shangguan D (2010) Bifunctional combined aptamer for simultaneous separation and detection of thrombin. Biosens Bioelectron **25**: 1487-1492.

Blank M, Blind M (2005) Aptamers as tools for target validation. Curr Opin Chem Biol **9**: 336-342.

Blank M, Weinschenk T, Priemer M, Schluesener H (2001) Systematic evolution of a DNA aptamer binding to rat brain tumor microvessels. selective targeting of endothelial regulatory protein pigpen. J Biol Chem **276**: 16464-16468.

Blindt R, Vogt F, Astafieva I, Fach C, Hristov M, Krott N, Seitz B, Kapurniotu A, Kwok C, Dewor M, Bosserhoff AK, Bernhagen J, Hanrath P, Hoffmann R, Weber C (2006) A novel drug-eluting stent coated with an integrin-binding cyclic Arg-Gly-Asp peptide inhibits neointimal hyperplasia

by recruiting endothelial progenitor cells. J Am Coll Cardiol **47**: 1786-1795.

Bock LC, Griffin LC, Latham JA, Vermaas EH, Toole JJ (1992) Selection of single-stranded DNA molecules that bind and inhibit human thrombin. Nature **355**: 564-566.

Brunk DK, Hammer DA (1997) Quantifying rolling adhesion with a cell-free assay: E-selectin and its carbohydrate ligands. Biophys J **72**: 2820-2833.

Burkel WE (1988) The challenge of small diameter vascular grafts. Med Prog Technol **14**: 165-175.

Carmona G, Chavakis E, Koehl U, Zeiher AM, Dimmeler S (2008) Activation of Epac stimulates integrin-dependent homing of progenitor cells. Blood **111**: 2640-2646.

Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, Capla JM, Galiano RD, Levine JP, Gurtner GC (2004) Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med **10**: 858-864.

Cerchia L, de Franciscis V (2007) Nucleic acid-based aptamers as promising therapeutics in neoplastic diseases. Methods Mol Biol **361**: 187-200.

Cerchia L, Duconge F, Pestourie C, Boulay J, Aissouni Y, Gombert K, Tavitian B, de Franciscis V, Libri D (2005) Neutralizing aptamers from whole-cell SELEX inhibit the RET receptor tyrosine kinase. PLoS Biol **3**: e123.

Chandler AB (1958) *In vitro* thrombotic coagulation of the blood; a method for producing a thrombus. Lab Invest **7**: 110-114.

Charles N, Liesveld JL, King MR (2007) Investigating the feasibility of stem cell enrichment mediated by immobilized selectins. Biotechnol Prog **23**: 1463-1472.

Chavakis E, Aicher A, Heeschen C, Sasaki K, Kaiser R, El Makhfi N, Urbich C, Peters T, Scharffetter-Kochanek K, Zeiher AM, Chavakis T, Dimmeler S (2005) Role of beta2-integrins for homing and neovascularization capacity of endothelial progenitor cells. J Exp Med **201**: 63-72.

Chavakis E, Hain A, Vinci M, Carmona G, Bianchi ME, Vajkoczy P, Zeiher AM, Chavakis T, Dimmeler S (2007) High-mobility group box 1 activates integrin-dependent homing of endothelial progenitor cells. Circ Res **100**: 204-212.

Chen F, Zhou J, Luo F, Mohammed AB, Zhang XL (2007) Aptamer from whole-bacterium SELEX as new therapeutic reagent against virulent Mycobacterium tuberculosis. Biochem Biophys Res Commun **357**: 743-748.

Chiu LL, Radisic M (2010) Scaffolds with covalently immobilized VEGF and Angiopoietin-1 for vascularization of engineered tissues. Biomaterials **31**: 226-241.

Chu TC, Marks JW, III, Lavery LA, Faulkner S, Rosenblum MG, Ellington AD, Levy M (2006a) Aptamer:toxin conjugates that specifically target prostate tumor cells. Cancer Res **66**: 5989-5992.

Chu TC, Twu KY, Ellington AD, Levy M (2006b) Aptamer mediated siRNA delivery. Nucleic Acids Res **34**: e73.

Consigny PM, Silverberg DA, Vitali NJ (1999) Use of endothelial cells containing superparamagnetic microspheres to improve endothelial cell delivery to arterial surfaces after angioplasty. J Vasc Interv Radiol **10**: 155-163.

Cosmi B (2009) ARC-1779, a PEGylated aptamer antagonist of von Willebrand factor for potential use as an anticoagulant or antithrombotic agent. Curr Opin Mol Ther **11**: 322-328.

Critser PJ, Yoder MC (2010) Endothelial colony-forming cell role in neoangiogenesis and tissue repair. Curr Opin Organ Transplant **15**: 68-72.

Daniels DA, Chen H, Hicke BJ, Swiderek KM, Gold L (2003) A tenascin-C aptamer identified by tumor cell SELEX: systematic evolution of ligands by exponential enrichment. Proc Natl Acad Sci U S A **100**: 15416-15421.

de Mel A, Jell G, Stevens MM, Seifalian AM (2008) Biofunctionalization of biomaterials for accelerated *in situ* endothelialization: a review. Biomacromolecules **9**: 2969-2979.

Deutsch M, Meinhart J, Fischlein T, Preiss P, Zilla P (1999) Clinical autologous *in vitro* endothelialization of infrainguinal ePTFE grafts in 100 patients: a 9-year experience. Surgery **126**: 847-855.

Duckers HJ, Silber S, de Winter R, den Heijer P, Rensing B, Rau M, Mudra H, Benit E, Verheye S, Wijns W, Serruys PW (2007a) Circulating endothelial progenitor cells predict angiographic and intravascular ultrasound outcome following percutaneous coronary interventions in the HEALING-II trial: evaluation of an endothelial progenitor cell capturing stent. EuroIntervention **3**: 67-75.

Duckers HJ, Soullie T, den Heijer P, Rensing B, de Winter RJ, Rau M, Mudra H, Silber S, Benit E, Verheye S, Wijns W, Serruys PW (2007b) Accelerated vascular repair following percutaneous coronary intervention by capture of endothelial progenitor cells promotes regression of neointimal growth at long term follow-up: final results of the Healing II trial using an endothelial progenitor cell capturing stent (Genous R stent). EuroIntervention **3**: 350-358.

Ellington AD, Szostak JW (1990) *In vitro* selection of RNA molecules that bind specific ligands. Nature **346**: 818-822.

Farokhzad OC, Cheng J, Teply BA, Sherifi I, Jon S, Kantoff PW, Richie JP, Langer R (2006) Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy *in vivo*. Proc Natl Acad Sci U S A **103**: 6315-6320.

Ferrara N, Gerber HP, LeCouter J (2003) The biology of VEGF and its receptors. Nat Med **9**: 669-676.

Foubert P, Silvestre JS, Souttou B, Barateau V, Martin C, Ebrahimian TG, Lere-Dean C, Contreres JO, Sulpice E, Levy BI, Plouet J, Tobelem G, Le Ricousse-Roussanne S (2007) PSGL-1-mediated activation of EphB4 increases the proangiogenic potential of endothelial progenitor cells. J Clin Invest **117**: 1527-1537.

Frede S, Stockmann C, Winning S, Freitag P, Fandrey J (2009) Hypoxia-inducible factor (HIF) 1alpha accumulation and HIF target gene expression are impaired after induction of endotoxin tolerance. J Immunol **182**: 6470-6476.

Garg S, Duckers HJ, Serruys PW (2010) Endothelial progenitor cell capture stents: will this technology find its

niche in contemporary practice? Eur Heart J **31**: 1032-1055.

Gensch C, Clever YP, Werner C, Hanhoun M, Bohm M, Laufs U (2007) The PPAR-gamma agonist pioglitazone increases neoangiogenesis and prevents apoptosis of endothelial progenitor cells. Atherosclerosis **192**: 67-74.

Greenberg AW, Brunk DK, Hammer DA (2000) Cell-free rolling mediated by L-selectin and sialyl Lewis(x) reveals the shear threshold effect. Biophys J **79**: 2391-2402.

Grosskreutz CL, Anand-Apte B, Duplaa C, Quinn TP, Terman BI, Zetter B, D'Amore PA (1999) Vascular endothelial growth factor-induced migration of vascular smooth muscle cells *in vitro*. Microvasc Res **58**: 128-136.

Guo KT, Schäfer R, Paul A, Gerber A, Ziemer G, Wendel HP (2006) A new technique for the isolation and surface immobilization of mesenchymal stem cells from whole bone marrow using high-specific DNA aptamers. Stem Cells **24**: 2220-2231.

Guo KT, Ziemer G, Paul A, Wendel HP (2008) CELL-SELEX: Novel perspectives of aptamer-based therapeutics. Int J Mol Sci **9**: 668-678.

Heeschen C, Aicher A, Lehmann R, Fichtlscherer S, Vasa M, Urbich C, Mildner-Rihm C, Martin H, Zeiher AM, Dimmeler S (2003) Erythropoietin is a potent physiologic stimulus for endothelial progenitor cell mobilization. Blood **102**: 1340-1346.

Hiasa K, Ishibashi M, Ohtani K, Inoue S, Zhao Q, Kitamoto S, Sata M, Ichiki T, Takeshita A, Egashira K (2004) Gene transfer of stromal cell-derived factor-1alpha enhances ischemic vasculogenesis and angiogenesis via vascular endothelial growth factor/endothelial nitric oxide synthase-related pathway: next-generation chemokine therapy for therapeutic neovascularization. Circulation **109**: 2454-2461.

Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T (2003) Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med **348**: 593-600.

Hoffmann J, Groll J, Heuts J, Rong H, Klee D, Ziemer G, Moeller M, Wendel HP (2006) Blood cell and plasma protein repellent properties of star-PEG-modified surfaces. J Biomater Sci Polym Ed **17**: 985-996.

Hoffmann J, Paul A, Harwardt M, Groll J, Reeswinkel T, Klee D, Moeller M, Fischer H, Walker T, Greiner T, Ziemer G, Wendel HP (2008) Immobilized DNA aptamers used as potent attractors for porcine endothelial precursor cells. J Biomed Mater Res A **84**: 614-621.

Hristov M, Erl W, Weber PC (2003) Endothelial progenitor cells: mobilization, differentiation, and homing. Arterioscler Thromb Vasc Biol **23**: 1185-1189.

Huang YF, Shangguan D, Liu H, Phillips JA, Zhang X, Chen Y, Tan W (2009) Molecular assembly of an aptamer-drug conjugate for targeted drug delivery to tumor cells. Chembiochem **10**: 862-868.

Hur J, Yoon CH, Kim HS, Choi JH, Kang HJ, Hwang KK, Oh BH, Lee MM, Park YB (2004) Characterization of two types of endothelial progenitor cells and their different contributions to neovasculogenesis. Arterioscler Thromb Vasc Biol **24**: 288-293.

Imanishi T, Hano T, Nishio I (2005a) Estrogen reduces endothelial progenitor cell senescence through augmentation of telomerase activity. J Hypertens **23**: 1699-1706.

Imanishi T, Moriwaki C, Hano T, Nishio I (2005b) Endothelial progenitor cell senescence is accelerated in both experimental hypertensive rats and patients with essential hypertension. J Hypertens **23**: 1831-1837.

Ingram DA, Mead LE, Tanaka H, Meade V, Fenoglio A, Mortell K, Pollok K, Ferkowicz MJ, Gilley D, Yoder MC (2004) Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. Blood **104**: 2752-2760.

Iwakura A, Luedemann C, Shastry S, Hanley A, Kearney M, Aikawa R, Isner JM, Asahara T, Losordo DW (2003) Estrogen-mediated, endothelial nitric oxide synthase-dependent mobilization of bone marrow-derived endothelial progenitor cells contributes to reendothelialization after arterial injury. Circulation **108**: 3115-3121.

Jiang M, Wang B, Wang C, He B, Fan H, Guo TB, Shao Q, Gao L, Liu Y (2008) Angiogenesis by transplantation of HIF-1 alpha modified EPCs into ischemic limbs. J Cell Biochem **103**: 321-334.

Jun HW, Paramonov SE, Dong H, Forraz N, McGuckin C, Hartgerink JD (2008) Tuning the mechanical and bioresponsive properties of peptide-amphiphile nanofiber networks. J Biomater Sci Polym Ed **19**: 665-676.

Jun HW, West J (2004) Development of a YIGSR-peptide-modified polyurethaneurea to enhance endothelialization. J Biomater Sci Polym Ed **15**: 73-94.

Jun HW, West JL (2005a) Endothelialization of microporous YIGSR/PEG-modified polyurethaneurea. Tissue Eng **11**: 1133-1140.

Jun HW, West JL (2005b) Modification of polyurethaneurea with PEG and YIGSR peptide to enhance endothelialization without platelet adhesion. J Biomed Mater Res B Appl Biomater **72**: 131-139.

Kansas GS (1996) Selectins and their ligands: current concepts and controversies. Blood **88**: 3259-3287.

Klomp M, Beijk MA, Verouden NJ, Tijssen JG, de Winter RJ (2009) Design and rationale of the TRI-stent adjudication study (TRIAS) program. Am Heart J **158**: 527-532.

Koch AE, Halloran MM, Haskell CJ, Shah MR, Polverini PJ (1995) Angiogenesis mediated by soluble forms of E-selectin and vascular cell adhesion molecule-1. Nature **376**: 517-519.

Kumar P, Amin MA, Harlow LA, Polverini PJ, Koch AE (2003) Src and phosphatidylinositol 3-kinase mediate soluble E-selectin-induced angiogenesis. Blood **101**: 3960-3968.

Kushwaha M, Anderson JM, Bosworth CA, Andukuri A, Minor WP, Lancaster JR, Jr., Anderson PG, Brott BC, Jun HW (2010) A nitric oxide releasing, self assembled peptide amphiphile matrix that mimics native endothelium for coating implantable cardiovascular devices. Biomaterials **31**: 1502-1508.

Kyrtatos PG, Lehtolainen P, Junemann-Ramirez M, Garcia-Prieto A, Price AN, Martin JF, Gadian DG, Pankhurst QA, Lythgoe MF (2009) Magnetic tagging increases delivery of circulating progenitors in vascular injury. JACC Cardiovasc Interv **2**: 794-802.

Langer HF, von der Ruhr JW, Daub K, Schoenberger T, Stellos K, May AE, Schnell H, Gauss A, Hafner R, Lang P, Schumm M, Buhring HJ, Klingel K, Conrad S, Schaller M, van ZM, Jung G, Dimmeler S, Skutella T, Gawaz M (2010) Capture of endothelial progenitor cells by a bispecific protein/monoclonal antibody molecule induces reendothelialization of vascular lesions. J Mol Med **88**: 687-699.

Leone AM, Valgimigli M, Giannico MB, Zaccone V, Perfetti M, D'Amario D, Rebuzzi AG, Crea F (2009) From bone marrow to the arterial wall: the ongoing tale of endothelial progenitor cells. Eur Heart J **30**: 890-899.

Lin Y, Weisdorf DJ, Solovey A, Hebbel RP (2000) Origins of circulating endothelial cells and endothelial outgrowth from blood. J Clin Invest **105**: 71-77.

Liu G, Mao X, Phillips JA, Xu H, Tan W, Zeng L (2009) Aptamer-nanoparticle strip biosensor for sensitive detection of cancer cells. Anal Chem **81**: 10013-10018.

Lotze MT, Tracey KJ (2005) High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. Nat Rev Immunol **5**: 331-342.

Ma H, Calderon TM, Kessel T, Ashton AW, Berman JW (2003) Mechanisms of hepatocyte growth factor-mediated vascular smooth muscle cell migration. Circ Res **93**: 1066-1073.

Mallikaratchy P, Tang Z, Kwame S, Meng L, Shangguan D, Tan W (2007) Aptamer directly evolved from live cells recognizes membrane bound immunoglobin heavy mu chain in Burkitt's lymphoma cells. Mol Cell Proteomics **6**: 2230-2238.

Markway BD, McCarty OJ, Marzec UM, Courtman DW, Hanson SR, Hinds MT (2008) Capture of flowing endothelial cells using surface-immobilized anti-kinase insert domain receptor antibody. Tissue Eng Part C Methods **14**: 97-105.

Massa M, Rosti V, Ramajoli I, Campanelli R, Pecci A, Viarengo G, Meli V, Marchetti M, Hoffman R, Barosi G (2005) Circulating CD34+, CD133+, and vascular endothelial growth factor receptor 2-positive endothelial progenitor cells in myelofibrosis with myeloid metaplasia. J Clin Oncol **23**: 5688-5695.

McCarthy JR, Weissleder R (2008) Multifunctional magnetic nanoparticles for targeted imaging and therapy. Adv Drug Deliv Rev **60**: 1241-1251.

McNamara JO, Andrechek ER, Wang Y, Viles KD, Rempel RE, Gilboa E, Sullenger BA, Giangrande PH (2006) Cell type-specific delivery of siRNAs with aptamer-siRNA chimeras. Nat Biotechnol **24**: 1005-1015.

Meinhart JG, Deutsch M, Fischlein T, Howanietz N, Froschl A, Zilla P (2001) Clinical autologous *in vitro* endothelialization of 153 infrainguinal ePTFE grafts. Ann Thorac Surg **71**: S327-S331.

Miller-Kasprzak E, Jagodzinski PP (2007) Endothelial progenitor cells as a new agent contributing to vascular repair. Arch Immunol Ther Exp (Warsz) **55**: 247-259.

Moore MA, Hattori K, Heissig B, Shieh JH, Dias S, Crystal RG, Rafii S (2001) Mobilization of endothelial and hematopoietic stem and progenitor cells by adenovector-mediated elevation of serum levels of SDF-1, VEGF, and angiopoietin-1. Ann N Y Acad Sci **938**: 36-45.

Nakazawa G, Granada JF, Alviar CL, Tellez A, Kaluza GL, Guilhermier MY, Parker S, Rowland SM, Kolodgie FD, Leon MB, Virmani R (2010) Anti-CD34 Antibodies Immobilized on the Surface of Sirolimus-Eluting Stents Enhance Stent Endothelialization. JACC Cardiovasc Interv **3**: 68-75.

Narasipura SD, Wojciechowski JC, Charles N, Liesveld JL, King MR (2008) P-Selectin coated microtube for enrichment of CD34+ hematopoietic stem and progenitor cells from human bone marrow. Clin Chem **54**: 77-85.

Ng SY, Reboud J, Wang KY, Tang KC, Zhang L, Wong P, Moe KT, Shim W, Chen Y (2010) Label-free impedance detection of low levels of circulating endothelial progenitor cells for point-of-care diagnosis. Biosens Bioelectron **25**: 1095-1101.

Nimjee SM, Rusconi CP, Sullenger BA (2005) Aptamers: an emerging class of therapeutics. Annu Rev Med **56**: 555-583.

Oh IY, Yoon CH, Hur J, Kim JH, Kim TY, Lee CS, Park KW, Chae IH, Oh BH, Park YB, Kim HS (2007) Involvement of E-selectin in recruitment of endothelial progenitor cells and angiogenesis in ischemic muscle. Blood **110**: 3891-3899.

Ohuchi SP, Ohtsu T, Nakamura Y (2006) Selection of RNA aptamers against recombinant transforming growth factor-beta type III receptor displayed on cell surface. Biochimie **88**: 897-904.

Palumbo R, de Marchis F, Pusterla T, Conti A, Alessio M, Bianchi ME (2009) Src family kinases are necessary for cell migration induced by extracellular HMGB1. J Leukoc Biol **86**: 617-623.

Pan Y, Guo M, Nie Z, Huang Y, Pan C, Zeng K, Zhang Y, Yao S (2010) Selective collection and detection of leukemia cells on a magnet-quartz crystal microbalance system using aptamer-conjugated magnetic beads. Biosens Bioelectron **25**: 1609-1614.

Parenti A, Bellik L, Brogelli L, Filippi S, Ledda F (2004) Endogenous VEGF-A is responsible for mitogenic effects of MCP-1 on vascular smooth muscle cells. Am J Physiol Heart Circ Physiol **286**: H1978-H1984.

Patschan D, Patschan S, Wessels JT, Becker JU, David S, Henze E, Goligorsky MS, Muller GA (2010) Epac-1 activator 8-O-cAMP augments renoprotective effects of allogeneic murine EPCs in acute ischemic kidney injury. Am J Physiol Renal Physiol **298**: F78-F85.

Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, Oz MC, Hicklin DJ, Witte L, Moore MA, Rafii S (2000) Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. Blood **95**: 952-958.

Peled A, Kollet O, Ponomaryov T, Petit I, Franitza S, Grabovsky V, Slav MM, Nagler A, Lider O, Alon R, Zipori D, Lapidot T (2000) The chemokine SDF-1 activates the integrins LFA-1, VLA-4, and VLA-5 on immature human CD34(+) cells: role in transendothelial/stromal migration and engraftment of NOD/SCID mice. Blood **95**: 3289-3296.

Pfosser A, El-Aouni C, Pfisterer I, Dietz M, Globisch F, Stachel G, Trenkwalder T, Pinkenburg O, Horstkotte J, Hinkel R, Sperandio M, Hatzopoulos AK, Boekstegers P,

Bals R, Kupatt C (2009) NF kappaB Activation in Embryonic Endothelial Progenitor Cells Enhances Neovascularization via PSGL-1 Mediated Recruitment: Novel Role for LL37. Stem Cells **28**: 376-385.

Phillips JA, Xu Y, Xia Z, Fan ZH, Tan W (2009) Enrichment of cancer cells using aptamers immobilized on a microfluidic channel. Anal Chem **81**: 1033-1039.

Pislaru SV, Harbuzariu A, Agarwal G, Witt T, Gulati R, Sandhu NP, Mueske C, Kalra M, Simari RD, Sandhu GS (2006a) Magnetic forces enable rapid endothelialization of synthetic vascular grafts. Circulation **114**: I314-I318.

Pislaru SV, Harbuzariu A, Gulati R, Witt T, Sandhu NP, Simari RD, Sandhu GS (2006b) Magnetically targeted endothelial cell localization in stented vessels. J Am Coll Cardiol **48**: 1839-1845.

Plouffe BD, Kniazeva T, Mayer JE, Jr., Murthy SK, Sales VL (2009) Development of microfluidics as endothelial progenitor cell capture technology for cardiovascular tissue engineering and diagnostic medicine. FASEB J **23**: 3309-3314.

Prater DN, Case J, Ingram DA, Yoder MC (2007) Working hypothesis to redefine endothelial progenitor cells. Leukemia **21**: 1141-1149.

Raddatz MS, Dolf A, Endl E, Knolle P, Famulok M, Mayer G (2008) Enrichment of cell-targeting and population-specific aptamers by fluorescence-activated cell sorting. Angew Chem Int Ed Engl **47**: 5190-5193.

Rehman J, Li J, Orschell CM, March KL (2003) Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. Circulation **107**: 1164-1169.

Rodgers SD, Camphausen RT, Hammer DA (2000) Sialyl Lewis(x)-mediated, PSGL-1-independent rolling adhesion on P-selectin. Biophys J **79**: 694-706.

Rohde E, Malischnik C, Thaler D, Maierhofer T, Linkesch W, Lanzer G, Guelly C, Strunk D (2006) Blood monocytes mimic endothelial progenitor cells. Stem Cells **24**: 357-367.

Rotmans JI, Heyligers JM, Verhagen HJ, Velema E, Nagtegaal MM, de Kleijn DP, de Groot FG, Stroes ES, Pasterkamp G (2005) *In vivo* cell seeding with anti-CD34 antibodies successfully accelerates endothelialization but stimulates intimal hyperplasia in porcine arteriovenous expanded polytetrafluoroethylene grafts. Circulation **112**: 12-18.

Rouhiainen A, Kuja-Panula J, Wilkman E, Pakkanen J, Stenfors J, Tuominen RK, Lepantalo M, Carpen O, Parkkinen J, Rauvala H (2004) Regulation of monocyte migration by amphoterin (HMGB1). Blood **104**: 1174-1182.

Sanchez-Cortes J, Mrksich M (2009) The platelet integrin alphaIIbbeta3 binds to the RGD and AGD motifs in fibrinogen. Chem Biol **16**: 990-1000.

Sata M, Saiura A, Kunisato A, Tojo A, Okada S, Tokuhisa T, Hirai H, Makuuchi M, Hirata Y, Nagai R (2002) Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis. Nat Med **8**: 403-409.

Schäfer R, Wiskirchen J, Guo K, Neumann B, Kehlbach R, Pintaske J, Voth V, Walker T, Scheule AM, Greiner TO, Hermanutz-Klein U, Claussen CD, Northoff H, Ziemer G, Wendel HP (2007) Aptamer-based isolation and subsequent imaging of mesenchymal stem cells in ischemic myocard by magnetic resonance imaging. Rofo **179**: 1009-1015.

Schroeter MR, Leifheit M, Sudholt P, Heida NM, Dellas C, Rohm I, Alves F, Zientkowska M, Rafail S, Puls M, Hasenfuss G, Konstantinides S, Schafer K (2008) Leptin enhances the recruitment of endothelial progenitor cells into neointimal lesions after vascular injury by promoting integrin-mediated adhesion. Circ Res **103**: 536-544.

Seeger FH, Zeiher AM, Dimmeler S (2007) Cell-enhancement strategies for the treatment of ischemic heart disease. Nat Clin Pract Cardiovasc Med **4 Suppl 1**: S110-S113.

Shangguan D, Cao ZC, Li Y, Tan W (2007) Aptamers evolved from cultured cancer cells reveal molecular differences of cancer cells in patient samples. Clin Chem **53**: 1153-1155.

Shimizu K, Sugiyama S, Aikawa M, Fukumoto Y, Rabkin E, Libby P, Mitchell RN (2001) Host bone-marrow cells are a source of donor intimal smooth- muscle-like cells in murine aortic transplant arteriopathy. Nat Med **7**: 738-741.

Silber S (2006) Capturing circulating endothelial progenitor cells: a new concept tested in the HEALING studies. Minerva Cardioangiol **54**: 1-3.

Simper D, Stalboerger PG, Panetta CJ, Wang S, Caplice NM (2002) Smooth muscle progenitor cells in human blood. Circulation **106**: 1199-1204.

Smadja DM, Bieche I, Uzan G, Bompais H, Muller L, Boisson-Vidal C, Vidaud M, Aiach M, Gaussem P (2005) PAR-1 activation on human late endothelial progenitor cells enhances angiogenesis *in vitro* with upregulation of the SDF-1/CXCR4 system. Arterioscler Thromb Vasc Biol **25**: 2321-2327.

Smith GP (1985) Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. Science **228**: 1315-1317.

Strehlow K, Werner N, Berweiler J, Link A, Dirnagl U, Priller J, Laufs K, Ghaeni L, Milosevic M, Bohm M, Nickenig G (2003) Estrogen increases bone marrow-derived endothelial progenitor cell production and diminishes neointima formation. Circulation **107**: 3059-3065.

Su Y, Zheng L, Wang Q, Li W, Cai Z, Xiong S, Bao J (2010) Quantity and clinical relevance of circulating endothelial progenitor cells in human ovarian cancer. J Exp Clin Cancer Res **29**: 27.

Suuronen EJ, Zhang P, Kuraitis D, Cao X, Melhuish A, McKee D, Li F, Mesana TG, Veinot JP, Ruel M (2009) An acellular matrix-bound ligand enhances the mobilization, recruitment and therapeutic effects of circulating progenitor cells in a hindlimb ischemia model. FASEB J **23**: 1447-1458.

Szmitko PE, Kutryk MJ, Stewart DJ, Strauss MH, Verma S (2006) Endothelial progenitor cell-coated stents under scrutiny. Can J Cardiol **22**: 1117-1119.

Taite LJ, Yang P, Jun HW, West JL (2008) Nitric oxide-releasing polyurethane-PEG copolymer containing the YIGSR peptide promotes endothelialization with

decreased platelet adhesion. J Biomed Mater Res B Appl Biomater **84**: 108-116.

Tang Z, Shangguan D, Wang K, Shi H, Sefah K, Mallikratchy P, Chen HW, Li Y, Tan W (2007) Selection of aptamers for molecular recognition and characterization of cancer cells. Anal Chem **79**: 4900-4907.

Tavitian B, Haberkorn U (2009) Darwinian molecular imaging. Eur J Nucl Med Mol Imaging **36**: 1475-1482.

Tepper OM, Galiano RD, Capla JM, Kalka C, Gagne PJ, Jacobowitz GR, Levine JP, Gurtner GC (2002) Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. Circulation **106**: 2781-2786.

Thomas RS, Morgan H, Green NG (2009) Negative DEP traps for single cell immobilisation. Lab Chip **9**: 1534-1540.

Tuerk C, Gold L (1990) Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science **249**: 505-510.

Ulrich H, Magdesian MH, Alves MJ, Colli W (2002) *In vitro* selection of RNA aptamers that bind to cell adhesion receptors of Trypanosoma cruzi and inhibit cell invasion. J Biol Chem **277**: 20756-20762.

Vajkoczy P, Blum S, Lamparter M, Mailhammer R, Erber R, Engelhardt B, Vestweber D, Hatzopoulos AK (2003) Multistep nature of microvascular recruitment of *ex vivo*-expanded embryonic endothelial progenitor cells during tumor angiogenesis. J Exp Med **197**: 1755-1765.

Vasa M, Fichtlscherer S, Adler K, Aicher A, Martin H, Zeiher AM, Dimmeler S (2001a) Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. Circulation **103**: 2885-2890.

Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H, Zeiher AM, Dimmeler S (2001b) Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. Circ Res **89**: E1-E7.

Veleva AN, Cooper SL, Patterson C (2007) Selection and initial characterization of novel peptide ligands that bind specifically to human blood outgrowth endothelial cells. Biotechnol Bioeng **98**: 306-312.

Veleva AN, Heath DE, Cooper SL, Patterson C (2008) Selective endothelial cell attachment to peptide-modified terpolymers. Biomaterials **29**: 3656-3661.

Villalona GA, Udelsman B, Duncan DR, McGillicuddy E, Sawh-Martinez R, Mirensky T, Hibino N, Painter C, Erickson B, Shinoka T, Breuer C (2010) Cell Seeding Techniques in Vascular Tissue Engineering. Tissue Eng Part B Rev **16**: 341-350.

Walter DH, Rittig K, Bahlmann FH, Kirchmair R, Silver M, Murayama T, Nishimura H, Losordo DW, Asahara T, Isner JM (2002) Statin therapy accelerates reendothelialization: a novel effect involving mobilization and incorporation of bone marrow-derived endothelial progenitor cells. Circulation **105**: 3017-3024.

Weber A, Pedrosa I, Kawamoto A, Himes N, Munasinghe J, Asahara T, Rofsky NM, Losordo DW (2004) Magnetic resonance mapping of transplanted endothelial progenitor cells for therapeutic neovascularization in ischemic heart disease. Eur J Cardiothorac Surg **26**: 137-143.

Werner C, Kamani CH, Gensch C, Bohm M, Laufs U (2007a) The peroxisome proliferator-activated receptor-gamma agonist pioglitazone increases number and function of endothelial progenitor cells in patients with coronary artery disease and normal glucose tolerance. Diabetes **56**: 2609-2615.

Werner N, Wassmann S, Ahlers P, Schiegl T, Kosiol S, Link A, Walenta K, Nickenig G (2007b) Endothelial progenitor cells correlate with endothelial function in patients with coronary artery disease. Basic Res Cardiol **102**: 565-571.

Wojciechowski JC, Narasipura SD, Charles N, Mickelsen D, Rana K, Blair ML, King MR (2008) Capture and enrichment of CD34-positive haematopoietic stem and progenitor cells from blood circulation using P-selectin in an implantable device. Br J Haematol **140**: 673-681.

Xia L, Wang XX, Hu XS, Guo XG, Shang YP, Chen HJ, Zeng CL, Zhang FR, Chen JZ (2008) Resveratrol reduces endothelial progenitor cells senescence through augmentation of telomerase activity by Akt-dependent mechanisms. Br J Pharmacol **155**: 387-394.

Yamaguchi J, Kusano KF, Masuo O, Kawamoto A, Silver M, Murasawa S, Bosch-Marce M, Masuda H, Losordo DW, Isner JM, Asahara T (2003) Stromal cell-derived factor-1 effects on *ex vivo* expanded endothelial progenitor cell recruitment for ischemic neovascularization. Circulation **107**: 1322-1328.

Yeh ET, Zhang S, Wu HD, Korbling M, Willerson JT, Estrov Z (2003) Transdifferentiation of human peripheral blood CD34+-enriched cell population into cardiomyocytes, endothelial cells, and smooth muscle cells *in vivo*. Circulation **108**: 2070-2073.

Yoder MC, Mead LE, Prater D, Krier TR, Mroueh KN, Li F, Krasich R, Temm CJ, Prchal JT, Ingram DA (2007) Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. Blood **109**: 1801-1809.

Yoon CH, Hur J, Park KW, Kim JH, Lee CS, Oh IY, Kim TY, Cho HJ, Kang HJ, Chae IH, Yang HK, Oh BH, Park YB, Kim HS (2005) Synergistic neovascularization by mixed transplantation of early endothelial progenitor cells and late outgrowth endothelial cells: the role of angiogenic cytokines and matrix metalloproteinases. Circulation **112**: 1618-1627.

Yu JX, Huang XF, Lv WM, Ye CS, Peng XZ, Zhang H, Xiao LB, Wang SM (2009) Combination of stromal-derived factor-1alpha and vascular endothelial growth factor gene-modified endothelial progenitor cells is more effective for ischemic neovascularization. J Vasc Surg **50**: 608-616.

Zampetaki A, Kirton JP, Xu Q (2008) Vascular repair by endothelial progenitor cells. Cardiovasc Res **78**: 413-421.

Zemani F, Silvestre JS, Fauvel-Lafeve F, Bruel A, Vilar J, Bieche I, Laurendeau I, Galy-Fauroux I, Fischer AM, Boisson-Vidal C (2008) *Ex vivo* priming of endothelial progenitor cells with SDF-1 before transplantation could increase their proangiogenic potential. Arterioscler Thromb Vasc Biol **28**: 644-650.

Zhang SJ, Zhang H, Wei YJ, Su WJ, Liao ZK, Hou M, Zhou JY, Hu SS (2006) Adult endothelial progenitor cells from human peripheral blood maintain monocyte/macrophage function throughout *in vitro* culture. Cell Res **16**: 577-584.

Zhu J, Wang X, Shang Y, Xie X, Zhang F, Chen J, Fu G (2008) Puerarin reduces endothelial progenitor cells senescence through augmentation of telomerase activity. Vascul Pharmacol **49**: 106-110.

Discussion with Reviewers

Reviewer I: What is in your opinion the most specific approach for the *in vivo* vascularisation of biomaterials in terms of EPC?

Authors: The success of the *in vivo* endothelialisation depends on the haemocompatibility of the coating technology and the specificity of the used EPC capture molecules. Any class of capture molecules such as peptides, antibodies or aptamers, with a high biocompatibility, which meet the specificity requirement, is appropriate for the biofunctionalisation of biomaterials for *in vivo* tissue engineering of an endothelium. Despite the previous developments in this area, more specific capture molecules are necessary that exclusively target EPCs in the circulating peripheral blood. In addition, standardised definitions for EPC subpopulations and characterisation methods are necessary for an accurate assessment of the *in vivo* endothelialisation on implants.

Reviewer I: In extreme injuries cases, other tissues also required tissue-engineered constructs to be repaired (e.g. bone). In these cases, one of the unsolved problems is the promotion of a micro-vascular network within a tissue-engineered implant that also involves other cell types. Do you think that the methods you described could be transposed to other applications and promote the formation of such a capillary-like network within porous biomaterials without ending with a full endothelialisation of the material?

Authors: The major obstacle in generating artificial three dimensional (3D) large structures, such as engineered tissues or organs, is the efficient oxygen and nutrient supply to cells within 3D constructs. Because of the thickness, the diffusion is limited. Thus, a microvascular network is necessary for sufficient perfusion of the tissue. Furthermore, the repopulation of the construct with different types of cells is another challenge for tissue engineers. There are different approaches to improve the success of engineered tissue constructs. Heike Mertsching and colleagues demonstrated that the preserved vessel network of decellularised biological vascularised matrixes (BioVaM) from porcine small intestine segments can be reseeded with EPCs to line the graft vessel bed with a functional endothelium. Harald Ott and Doris Taylor have even managed to create a beating heart in the laboratory. Using cells from newborn rats, they were able to repopulate acellularised rat hearts. For the repopulation, a mix of heart- and endothelial cells as well as stem- and progenitor cells was prepared by mincing hearts from newborn rats. Four days after injection of these cells into the left ventricles of the decellularised hearts and pumping a solution of oxygen and nutrients through the network of preserved blood vessels, the previously nonliving heart-matrix began to beat. These fascinating results demonstrate the feasibility to recellularise even whole organ matrixes with different types of cells. The results also show that the generation of artificial tissues requires well-defined scaffold geometries within a tissue engineered matrix. Furthermore, for targeted cell-homing in three dimensional constructs, cell specific capture molecules can be immobilised at defined positions. For example, by using the cell-SELEX technology, cell type specific aptamers can be selected. These capture molecules can be immobilised in 3D constructs to catch the cells at desired places. Additionally, the incorporation of growth factors and degradable polymers, which prevent during the repopulation the migration of cells to undesired locations, can be used to control cell growth and migration.

Reviewer I: In terms of the different possibilities to achieve an *in vivo* vascularisation, which of these approaches might have the potential to result in a clinical application.

Authors: Hitherto, the most progressed approach to capture circulating EPCs from the peripheral blood is the use of monoclonal antibodies (MABs), namely antibodies targeting CD34. Stents coated with anti-CD34 MABs are already in clinical use for *in vivo* endothelialisation. However, CD34 is not only expressed on EPCs but also on haematopoietic stem cells. Thus, anti-CD34 MABs are not selective for only EPCs and therefore, in our opinion, this approach is not convincing. In our very own ranking for capture molecules that have the potential to result in a clinical application, aptamers take the first place, peptides are on the second position, subsequently come MABs and finally magnetic beads. However, it should be mentioned that the best *in vivo* endothelialisation outcome and long-term biocompatibility depends on the specificity of EPC capture molecules that are used for biofunctionalisation. Despite the current developments in this area, several research groups are still searching for new more specific EPC markers and capture molecules that exclusively target EPCs in the circulating peripheral blood.

Reviewer II: Taking into account the low frequency of EPC within the circulation and the unsolved question of specific markers to identify these cells, how would the authors rank the individual methods they summarise in this review in terms of specificity and efficacy or outcome *in vivo*?

Authors: The best *in vivo* endothelialisation outcome and long-term safety stands or falls with the specificity of the used EPC capture molecules. Hitherto, the major limitation of the used capture molecules that are reviewed in this manuscript is that they are not highly selective for EPCs. In this regard, cell-SELEX and phage display technologies are auspicious methods to select highly specific EPC capture molecules. Using these methods, new up to now undiscovered markers on circulating EPCs can be

discovered. Especially SELEX technology, which contains approximately 10^{15} different oligonucleotides in the start library as possible target binding ligands, has the ability to select EPC specific capture molecules by using EPCs as target cells. Furthermore, the use of anti-VEGFR2 antibodies could be more selective for circulating EPCs than anti-CD34 or anti-CD133 antibodies, because CD34 is also expressed by haematopoietic stem cells, and CD133 expression decreases after mobilisation of the EPCs from bone marrow into the peripheral blood. Also the magnetic labelling of EPCs is a promising approach, however it needs the previous isolation of EPCs and the effect of iron particles on survival of EPCs is not well studied. Also the incorporation of additional molecules, such as NO donors, PEG, or growth factors, into the backbone of antithrombogenic biomaterials is a promising approach to enhance the adhesion of EPCs and the proliferation of captured EPCs on biomaterials. Using these modifications, the adhesion of platelets and serum proteins can be also reduced, which in turn can enhance the capturing efficacy of capture molecules. Despite the present developments in this area, more specific capture molecules are necessary that exclusively target EPCs in the circulating peripheral blood. In addition, standardised definitions for EPC subpopulations and characterisation methods are required for an accurate evaluation and comparability of the used EPC capturing methods in regard to specificity and *in vivo* endothelialisation efficiency on implants.

Reviewer II: The procedure for endothelialisation of grafts described (Introduction) is indeed time consuming, expensive and possibly leading to contamination, but what also about the viability of the endothelial cells when the implant is introduced *in vivo*, especially at long term? Was this ever tested? Couldn't this be an additional possibility of drawback for this *ex vivo* approach which even further validates the presented approach of EPC recruitment?

Authors: Thank you for this interesting comment. A detailed study of the long-term viability of endothelial cells (ECs) on *in vitro* endothelialised and implanted grafts is so far unknown to us. But, a study, which determines the *in vivo* viability of these ECs and their ability to maintain a confluent EC-layer after implantation, would be very interesting. It is known that after a finite number of divisions *in vitro*, mature ECs undergo senescence, like most human somatic cells. The EC senescence in long-term *in vitro* cell cultures may limit initially the ability to produce sufficient number of cells to seed the implants. Due to aging of the vasculature or pathological changes of the ECs caused by cardiovascular diseases, the population doublings can differ from one patient to another patient. On the other hand, the *in vitro* generated endothelium on grafts can contain after a long time of cultivation several senescent ECs. After implantation, the ECs are exposed to enormous continuous sheer stress of blood flow, which can cause the partial loss of ECs. The proliferation of neighbouring ECs is necessary to compensate the cell loss and to fill the gaps. Therefore, the use of cells with high-proliferative potential and low senescence is required to enable the long-term patency and biocompatibility of grafts. Thus, the direct capturing of highly proliferative EPCs without the need of isolation procedure and long *in vitro* cultivation periods has a huge potential to maintain a confluent endothelium over a long time. Thereby, the long-term safety of implants could be ensured.