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The Role of Synthetic Extracellular Matrices in Endothelial Progenitor Cell Homing for Treatment of Vascular Disease

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Abstract—Poor vascular homeostasis drives many clinical disorders including diabetes, arthritis, atherosclerosis, and peripheral artery disease. Local tissue ischemia resultant of insufficient blood flow is a potent stimulus for recruitment of endothelial progenitor cells (EPCs). This mobilization and homing is a multi-step process involving EPC detachment from their steady state bone marrow niches, entry into circulation, rolling along vessel endothelium, transmigration, and adhesion to denuded extracellular matrix (ECM) where they may participate in neovessel formation. However, these events are often interrupted in pathological conditions partly due to an imbalance in factor presentation at the tissue level. EPC number and function is impaired in patients with vascular diseases and this dysfunction has been proposed as a prominent contributor to disease pathogenesis. Research approaches aimed at providing therapeutic angiogenesis commonly involve the delivery of proangiogenic cells and/or soluble factors. Nevertheless, greater understanding of the mechanisms involved in EPC homing in both healthy and diseased states is critical for improving efficacy of such strategies. This review underscores the matrix-related signals necessary for enhancing EPC recruitment to ischemic tissue and provides an overview of the development of synthetic ECMs that aim to mimic functions of the local native microenvironment for use in therapeutic angiogenesis.

Keywords—Therapeutic angiogenesis, Ischemia, Vascular endothelial growth factor, Stromal cell-derived factor-1, Sphingosine-1-phosphate, E-selectin.

INTRODUCTION

Ischemic vascular diseases are the dominant causes of mortality worldwide and yet current therapies only delay disease progression and improve lifestyle without addressing the fundamental problem of tissue loss.[72] Insufficient vascular network formation and maintenance drives many of these clinical disorders including diabetes, arthritis, atherosclerosis, and peripheral artery disease.[20,35] Local tissue ischemia resultant of insufficient blood flow is a potent stimulus for recruitment of progenitor blood forming cells including endothelial progenitor cells (EPCs).[63] EPCs are pivotal to maintaining vascular homeostasis and repair from injury.[21,63] First identified as CD34+ mononuclear cells in adult peripheral blood, EPCs commonly express both hematopoietic and endothelial surface markers dependent on the method of isolation (Fig. 1).[3,4,22,35,50,63,87] Thus, the term actually represents a vastly heterogenous population of cells, which has led experts in the field to urge discontinuing its use.[3,29,35,63,87,89] In particular, outgrowth endothelial cells (OECs) or endothelial colony forming cells (ECFCs) are a specific subset of EPCs of solely endothelial lineage that directly participate in vessel formation in contrast to hematopoietic EPCs.[42,70,78,88,89] To date, no specific set of surface markers identifies a pure EPC population.[30,59,61,89] In fact, it has been shown that EPCs displaying the same set of surface markers contain separate subpopulations with a hierarchy of proliferative potential.[30] Therefore, it is suggested that marker identification is not sufficient for EPC classification and functional analysis should be included.[30,50,54] For the purposes of this review, we will broadly refer to all endothelial progenitors as EPCs unless further characterization is needed for clarity.

Expression of prosurvival factors and chemokines is upregulated in ischemia as a natural healing mechanism that directs EPC homing to wounded tissue for enhanced blood vessel formation and neovascularization.[31,46] EPC mobilization and homing is a multi-step process involving detachment from their steady state

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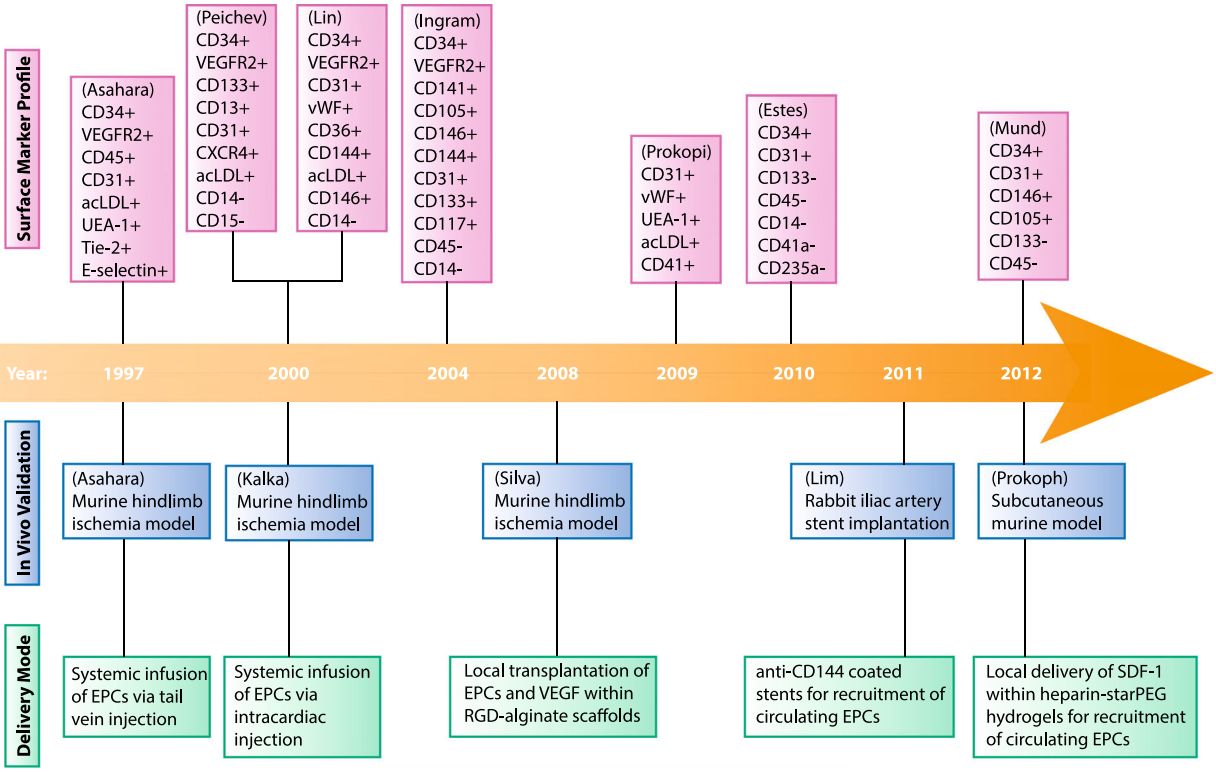

FIGURE 1. Road map of various EPC subtype identifications and strategies for *in vivo* validation. Following varying isolation protocols, different EPC subtype identifications in terms of surface marker expression have been proposed over time (top). The methodology of EPC-based therapies has concomitantly evolved over time, with brief examples provided (bottom). The last name of first author for each reference is in parentheses.

bone marrow (BM) niches, entry into circulation, rolling along vessel endothelium, transmigration, and adhesion to denuded extracellular matrix (ECM) where they may participate in neovessel formation.[45,73] All of these processes are under the tight regulation of chemokines and their receptors. However, these mechanisms are often interrupted in pathological conditions partly due to matrix modulation and an imbalance in factor presentation at the tissue level.

EPC number and function is impaired in patients with ischemic vascular diseases and EPC dysfunction has been proposed as a prominent contributor to disease pathogenesis.[35,46,55,83] Conventional therapeutic angiogenesis approaches typically involve the delivery of cells and/or soluble factors that promote blood vessel formation.[76] Herein, clinical approaches have aimed to provide exogenous EPCs within circulation to replenish necessary numbers lacked by diseased patients.[23] Other approaches focus on enhancing recruitment and retention of exogenously administered EPCs within the ischemic tissue by designing delivery vehicles that better mimic the conductive cellular milieu needed for enhanced cellular adhesion and function.[74] Implantation of OECs in ischemic tissue has demonstrated strong potential, but the poor survival, retention, and activity of transplanted cells still remains a large challenge for clinical success.[70,78] An attractive alternative that bypasses *ex vivo* cellular manipulation is to provide in situ tissue regeneration *via* controlled manipulation of endogenous EPC homing.[13] Regardless of the strategy employed, greater understanding of the mechanisms involved in EPC homing in both healthy and diseased states is critical in order to enhance efficacy of these strategies and maximize therapeutic benefit. This review highlights the matrix-related signals, including presentation of soluble cues, necessary for enhancing EPC recruitment to ischemic tissue in treatment of vascular disease.

MATRIX MODULATION IN VASCULAR DISEASES

The number of circulating EPCs is rather low under normal conditions and is increased in response to trauma or ischemia.[45] The mobilization of EPCs involves key signals that direct EPCs to migrate out of the stem cell niche of the BM and into peripheral circulation. Whereas the molecular mechanisms of leukocyte homing to sites of inflammation are well

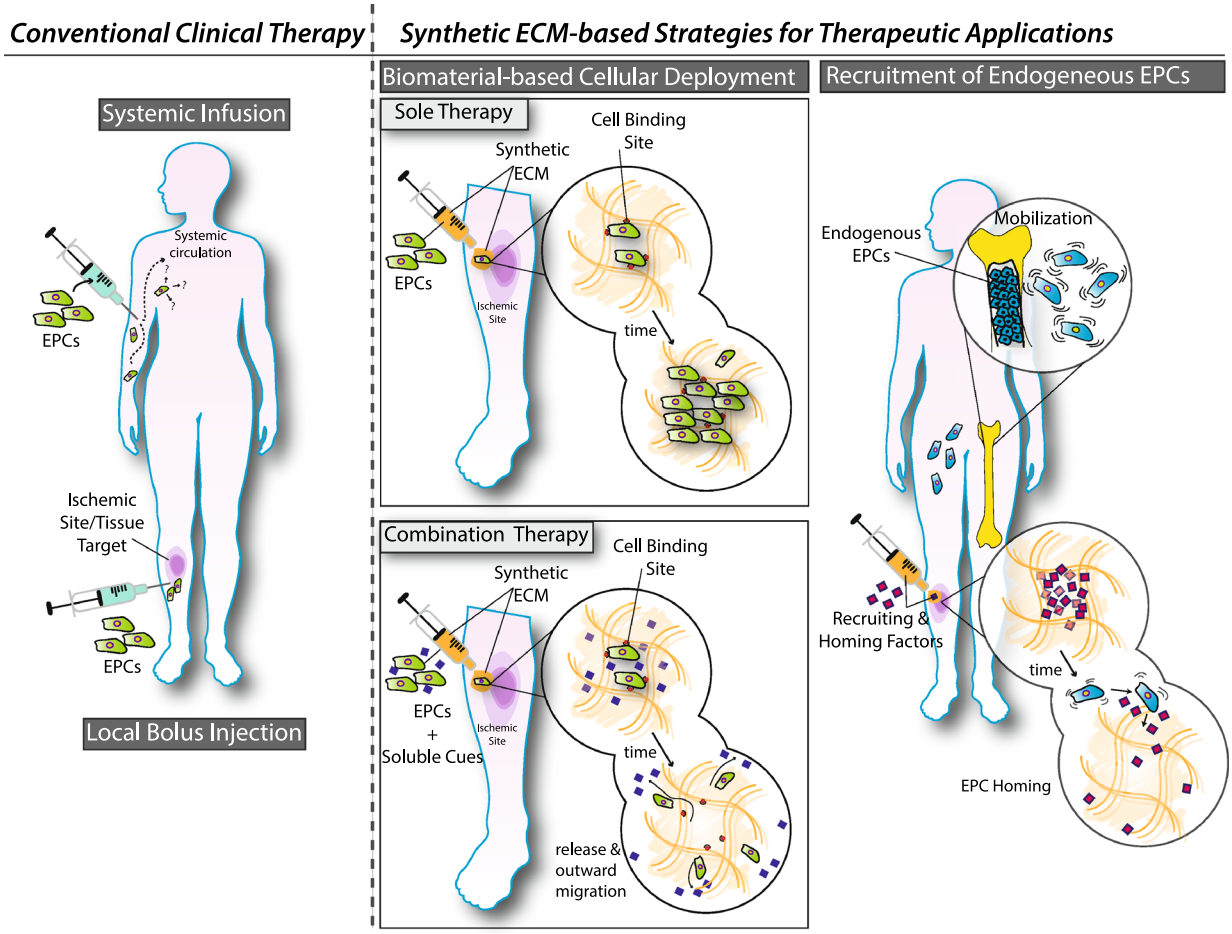

FIGURE 2. Schematic representation of EPC-based strategies for therapeutic angiogenesis. Conventional clinical therapies administer exogenously expanded and/or manipulated EPCs systemically into peripheral circulation (top left). Local bolus injection of these cells reduces the distance required for the cells to home to the target tissue site (bottom left). Delivery of EPCs within biomaterial conduits that simulate the native ECM beneficially enhances cellular survival post transplantation (top middle). Additionally, further incorporation of proangiogenic soluble cues provides a greater ECM mimic and likely enhance outward cellular migration and integration with the native tissue (bottom middle). Alternative strategies aim to eliminate *ex vivo* manipulation of cells by providing an attractive cellular milieu within the ischemic tissue that promotes endogenous EPC mobilization and recruitment *via* spatiotemporally controlled delivery of soluble factors within biomaterial conduits (right).

characterized, those of EPC migration to sites of ischemia and neovascularization are still not fully understood.[8,12,15] Nevertheless, EPC and leukocyte homing mechanisms present several similar steps as both involve a multistep cascade of adhesive and signaling events including chemotaxis, selectin-mediated tethering and rolling, and integrin-mediated firm adhesion and diapedesis.[8,12,63] Specifically, P-selectin, E-selectin, and β_2-integrins appear to be the most important adhesion molecules involved in this adhesive cascade for EPC homing.[8] For instance, previous studies demonstrated that β_2-integrins are critical for transendothelial migration of EPCs towards chemokines, including stromal cell derived factor-1 (SDF-1) and vascular endothelial growth factor (VEGF), *in vitro*.[8]

Under ischemic insult, the matrix is modulated to recruit progenitor cells to aid in tissue regeneration. For example, SDF-1, VEGF, sphingosine-1-phosphate (S1P), and other soluble prosurvival factors, are upregulated in the hypoxic tissue and have been shown to play key roles in directing EPC migration.[2,25,43,85] VEGF, a key growth factor involved in both angiogenesis and vasculogenesis, has been shown to mobilize BM-derived progenitors into circulation.[5,45] A central chemokine involved in EPC homing, SDF-1, is also produced within the hypoxic stem cell niches of the BM and mediates chemotaxis of various cell types that express the receptor CXCR4.[1,37,45,49] As such, the balance between SDF-1α concentration gradients and CXCR4 expression is thought to determine whether cells home to and stay within niches of the BM or

mobilize into peripheral circulation.[1,49] This SDF-1α/CXCR4 axis is considered one of the most broadly conserved migratory pathways involved in stem cell mobilization and homing.[37] In particular, SDF-1α has also been shown to upregulate expression of E-selectin ligands in ECFCs.[37] Further, the continuous presence of S1P in the hematopoietic microenvironment has also suggested that S1P may modulate the SDF-1/CXCR4 dependent EPC homing and lodgement.[43] While the levels of S1P are low in tissues, they are elevated in blood serum and plasma (~0.4–1 μM) where it is mainly bound to albumin and apolipoprotein M-containing high-density lipoprotein ($ApoM^+$ HDL).[43,48,80] These vascular gradients of S1P are key regulators of cell trafficking.[43]

Risk factors for vascular disease, including age and diabetes among others, have been shown to reduce both the quantity and functional activity of EPCs.[21,51] In such pathological conditions, the ability for EPCs to follow the natural homing cues is impaired partly due to diminished cytokine production and/or an imbalance in factor distribution.[31,45] For instance, high glucose exposure has been shown to promote EPC senescence and endothelial dysfunction.[17] SDF-1 production is also decreased in diabetic wounds, wherein exogenous administration of recombinant SDF-1 was shown to restore EPC recruitment in mice.[25,45] Additionally, while their HDL levels are high, patients with clinical evidence of vascular disease tend to have low levels of S1P in the HDL-containing fraction of serum.[84] This correlation between S1P and HDL has been suggested to contribute to the progression of atherosclerosis and may serve as a biomarker for individuals that are susceptible to ischemic heart disease but lack conventional risk factors such as low HDL-cholesterol. Oxidized low-density lipoprotein (Ox-LDL), a prominent player in the development of atherosclerosis, has further been demonstrated to impair EPC migration, adhesiveness, and vasculogenic capacity *in vitro*.[83] In accordance, previous reports have shown that circulating EPCs have decreased number and migratory function in patients with coronary artery disease (CAD) and type 1 diabetes[55] and decreased proliferative activity in patients with type 2 diabetes.[47,77] The number of circulating EPCs is inversely related to cardiovascular disease risk scores,[35] wherein such risk factors can induce a pro-inflammatory response in endothelial cells and thus lead to endothelial injury by apoptotic suicide pathways.[21]

Collectively, these impaired functions of EPCs are both (1) resultant of disease risk factors and the imbalance of soluble factor presentation at the matrix level and (2) contributing factors to disease pathogenesis as normal vessel repair and development is perturbed.[35] Therefore, control over EPC mobilization and homing presents a promising target for alleviating disease conditions.

SYNTHETIC MIMICS OF ECM FOR EPC-BASED DELIVERY STRATEGIES

Delivery of exogenous EPCs has become a hallmark in tissue engineering approaches towards therapeutic angiogenesis for regenerative medicine. In principle, these approaches boast the attractive benefit of exogenously providing the much-needed numbers and functions of EPCs lacking under vascular disease.[51,55] However, there are several unresolved aspects hampering the clinical potential of delivering exogenous EPCs, including the appropriate cellular subtype, the optimal cell dose, the administration mode, the efficiency of cell engraftment at the target tissue, and the frequency of treatment (Table 1).[11]

Systemic Infusion or Local Bolus Injection of EPCs

With over 200 clinical trials involving delivery of endothelial progenitors for treating vascular diseases, a typical approach has been to systemically infuse the cells.[2,61] Systemic infusion by intracardiac injection of culture-expanded EPCs derived from peripheral blood has been shown to enhance blood perfusion and somewhat prevent limb loss (50% recovery) in murine models of hindlimb ischemia.[33] Here, 56% of blood vessels in the ischemic tissue contained transplanted human EPCs, demonstrating the reparative function of these cells. Clinical trials for treatment of patients with CAD, including the TOPCARE-AMI,[65] REPAIR-AMI,[66] and TOPCARE-CHD[6] studies, administered heterogenous bone-marrow derived progenitor cells *via* bolus intracoronary infusion and provided proof of concept with moderate yet statistically significant improvements of left ventricular function.[62] Albeit simple delivery routes, these methods of cellular infusion often have limited efficacy due to high cell death and poor recruitment and/or retention within the ischemic target.[2,70]

In particular, the majority of systemically infused cells often get trapped in the microvasculature of other off-target organs/tissues.[27,64] For example, pulmonary passage is a major obstacle due to both the size of the cells and adhesion to the vascular endothelium.[24] Very large numbers of cells are thus required in order for enough cells to home to the target tissue for therapeutic benefit (ranging from 20 to 800 million cell/patient).[13,24,64] In particular, clinical trials for heart failure involving BM progenitor cells often require between 20 and 800 million cells per patient for a single treatment.[64] Thus, better understanding of the homing

TABLE 1. Overview of the advantages and potential disadvantages of common EPC-based strategies for therapeutic angiogenesis.[2,13,61,65,70,78]

Strategy	Advantages	Potential disadvantages
Systemic infusion of EPCs	• Simple preparation of cells in solution • Ease of administration	• Lengthy *ex vivo* expansion of cells • Large number of cells required • Poor survival of administered cells • Low engraftment efficiency • Off-target localization of administered cells
Local bolus injection of EPCs	• Simple preparation of cells in solution • Locally delivered near site of ischemia • Fewer cells required than that for systemic infusion	• Lengthy *ex vivo* expansion of cells • Poor survival of administered cells • Low engraftment efficiency • Low retention of cells within target site
Material-based deployment of EPCs	• Targeted local delivery • Enhanced survival of cells within synthetic ECM mimic • Greater engraftment efficiency • Fewer cells required than that for above methods	• Lengthy *ex vivo* expansion of cells • Complex preparation of cells within biomaterial system • Possible immune response to chosen biomaterial
Material-based deployment of EPCs and soluble factors	• Targeted local delivery • Enhanced survival of cells due to dual delivery with soluble factors • Greater engraftment efficiency • Fewer cells required than that for first two methods • Enhanced engraftment efficiency • Enhanced outward migration and function of transplanted cells	• Lengthy *ex vivo* expansion of cells • Complex preparation of cells within biomaterial system • Possible immune response to chosen biomaterial
Material-based deployment of soluble chemotactic factors	• Ease of preparation • *In situ* regeneration • No *ex vivo* manipulation of cells • Enhanced function of endogenous cells • Enhanced regenerative potential by recruitment of endogenous EPCs	• Strategy limited by capability and functional response of endogenous cells

mechanisms employed by EPCs is imperative for improving transplanted cell engraftment, especially for cells infused *via* the vascular route.[13] Alternative bolus methods use local injection within the target ischemic tissue to decrease the distance that the cells are required to navigate in order to reach the target site (Fig. 2). EPCs have also been magnetically labeled with superparamagnetic iron oxide (SPIO) nanoparticles with efforts to enhance EPC recruitment to a target location that has also been magnetized. For example, magnetized stents implanted in porcine coronary and femoral arteries captured up to 30-fold more EPCs loaded with SPIO microspheres than nonmagnetized stents.[7,56]

While moderate therapeutic benefit has been achieved with bolus infusion (systemic or local), the lack of control over post-administered EPC survival, localization, fate, and functional activity poses major challenges and severely limits the degree of therapeutic benefit and clinical success.[64,70,78] Therefore, the efficacy of administered EPCs highly depends on the mode of delivery and subsequent control over administered cell fate after transplantation.[70]

Biomaterial Based Deployment of EPCs

The use of biomaterials could enhance the efficacy of cell therapy by providing the necessary matrix-related signals required for cell engraftment, survival, and function of these adhesion-dependent primary cells.[19,32,64,78] Biomaterial based deployment of EPCs has been shown to enhance efficacy of the transplanted cells by providing a microenvironment that enhances cell survival, function, and the sustained release and repopulation of the surrounding tissue by outwardly migrating cells.[70,78] Herein, researchers strive to develop biomaterial-based, synthetic ECMs as delivery vehicles for EPCs in order to enhance survival and retention for better tissue regeneration.[32,39,79]

Biomaterials can serve to create synthetic ECM--derived delivery vehicles that mediate biological processes, including cellular alignment and migration or growth factor release.[19,28] Tissue engineering strategies typically combine cells, polymeric scaffolds, chemical cues, and/or mechanical signals to guide cell phenotype.[9] Here, isolated cells are expanded *in vitro* and incorporated within three-dimensional material conduits for subsequent transplantation into or near the wound site (Fig. 1). A major key to success lies with the biomaterial selected for use, as it must provide the cells with the ability to survive, to integrate with native tissue, and to create a functional vascular network capable of sustaining the metabolic needs of the cells.[9,72]

For biomaterials to be successful in transplantation strategies, they must be both functionally supportive to the cells and biocompatible so as to avoid immune rejection. Bioengineers have created biomaterials from synthetic polymers, ceramics, and metal alloys, as they provide substantial control to the researcher in terms of reproducibility and tunability of chemical and physical properties.[39] Biomaterial degradation is a critical property,[9] as the scaffold must be able to be remodeled and eventually dissipate in order for regenerated tissue to take its place. As discussed, recent designs are now focusing on mimicking the many functions of the extracellular matrices of native tissues to facilitate integration and help regulate host response in a well-defined manner. Naturally derived materials have thus gained much attention given their inherent biocompatibility.[39]

Scaffolds are a class of biomaterials that are created from a variety of polymers to form porous mesh-like networks and can beneficially be reliably formed with structural reproducibility that is controlled by the investigator.[16] One major area of scaffold application involves vascular grafts.[8] Surgical treatment of occluded coronary arteries often involves coronary bypass surgery, where a patient's own saphenous vein or internal mammary artery is used as an autologous vascular graft to re-route blood flow around the obstruction.[8] However, these autologous grafts are often too short or the extent of atherosclerosis is too progressed, so synthetic vascular grafts are required to bypass or replace the diseased arteries.[8] While synthetic grafts often pose high risk of thrombosis, native endothelium has been shown to inhibit this occurrence.[40,41] Thus, endothelialization of these synthetic grafts with autologous ECs has been proposed. *Ex vivo* EC seeding prior to transplantation has been pursued, but poor cell survival remains a challenge. Herein, recent approaches aim to control cell trafficking and achieve rapid endothelialization of vascular grafts by modifying the surface of scaffolds with cell-specific ligands to recruit circulating endogenous EPCs.[40,41] For example, electrospun scaffolds have been modified to present an EPC-specific peptide sequence in one approach,[40] while metal stents have been alternatively labeled with antibodies for VE-cadherin.[41] However, these strategies are largely limited by the specificity of the peptides/antibodies used, as a heterogenous population of cells may be recruited.

Scaffolds may also be used to deliver incorporated growth factor(s) with known release profiles. For example, VEGF and PDGF delivery with spatiotemporal control from layered poly-lactide-co-glycolide (PLG) scaffolds enhanced vascular recovery in murine ischemic hindlimbs.[16] Scaffolds also provide a 3D structural network upon which cells may be seeded and cultured *in vitro*. One vascular tissue engineering strategy is to create blood vessels and primitive

vascular networks within biodegradable scaffolds *ex vivo* for subsequent implantation *in vivo*.[86] This approach typically involves seeding and culturing the cells on an appropriate scaffold that stimulates cell growth/differentiation and blood vessel formation *in vitro*. The rationale for this approach is that engineered microvessels might readily form anastomoses with existing vessels in the host and thus accelerate vascularization and improve construct viability.[86] However, although cellularized vessels grown and cultured within biomaterial scaffolds have been shown to have strong mechanical properties *in vitro,* they often lead to thrombosis shortly after implantation *in vivo*.[34]

Hydrogels are a useful class of cell delivery vehicles as they can be delivered in a minimally invasive manner and used to fill irregularly shaped defects, as opposed to scaffold transplantation.[9,71] Similarly to scaffolds, hydrogels are used to deliver cells to the desired tissue site, to provide a space for new tissue formation, and to control the structure and function of the regenerated tissue.[39] Beneficially, these highly hydrated networks strongly resemble the native ECM of tissues, facilitating better integration given their inherent biocompatibility.[39] Furthermore, homogenous cell seeding is easily established as the cells may be mixed with the various component solutions prior to gelation.

Several biomaterials have been used for hydrogel formation in vascular engineering.[38] Here, we highlight advances using naturally derived polymers in hydrogel formation, such as fibrin and alginate. Fibrin is a naturally derived and US FDA-approved biomaterial that is commercially available[28] and has been extensively used for a variety of therapeutic applications.[10] Fibrin hydrogels are advantageous cell delivery vehicles in terms of biocompatibility, biodegradation, and hemostasis.[90] Fibrin is formed through the polymerization of fibrinogen, a process that is initialized by activation of the serine protease thrombin in response to tissue injury.[10] As fibrin forms to coagulate the wound and stop bleeding, it serves as a natural scaffold for tissue regeneration and repair following injury. Products of fibrin degradation also activate wound repair, making fibrin a prime biomaterial that both provides a highly characterized porous matrix and stimulates surrounding cells.[10,28] Fibrin has a multitude of binding sites for growth factors, integrins, and additional ECM components that aid cell infiltration.[10] Furthermore, fibrin augments angiogenesis both *in vitro* and *in vivo*,[28,75] wherein transplantation of endogenous endothelial cells within fibrin hydrogels promotes capillary formation in ischemic myocardium in sheep.[14]

Alginate, a naturally occurring anionic polymer typically obtained from brown seaweed, is another hydrogel-forming biomaterial that has been extensively studied for many biomedical applications.[39] Benefits to using alginate include its high biocompatibility, structural similarities to ECMs, low toxicity, relatively low cost, and mild gelation by addition of divalent cations.[39] Alginates are polysaccharide copolymers that contain blocks of (1,4)-linked β-D-mannuronate and α-L-guluronate residues that vary in size and distribution depending on the source. Alginate is inherently non-degradable in mammals given the lack of alginase, but ionically cross-linked gels are somewhat dissolved by releasing divalent ions into the surrounding media or tissue.[39] Investigators may beneficially control the degradation rate by varying the degree of oxidation and/or molecular weight.[9] Partial periodate oxidation creates hydrolytically labile bonds that accelerates degradation *via* hydrolytic scission. Alternatively, decreasing the molecular weight (MW) distribution of the polymer chains used to form the gels also permits rapid degradation, but leads to mechanical weakness.[9] Investigators have previously shown that bimodal gels created from a mixture of high MW polymers and low MW polymers (prepared *via* gamma irradiation[71]) maintain mechanical stability while simultaneously providing control over degradation.[9] Such bimodal gels have been used to provide spatiotemporal control of release of multiple proangiogenic factors including VEGF, platelet-derived growth factor-BB, and S1P for therapeutic angiogenesis.[26,71,85]

Alginates used for cell delivery must be chemically modified to present cell adhesion ligands, such as peptides including the sequence arginine-lysine-aspartic acid (RGD).[9,39] The incorporation of EPCs within RGD-modified alginate was shown to enhance both $\alpha v\beta 5$ integrin expression and epidermal growth factor (EGF) secretion as opposed to EPCs delivered without the scaffold.[78] Alternatively, alginate mixed with Matrigel to provide cell adhesion sites may be 3D printed with precisely controlled porosity for enhanced EPC incorporation.[57] Further, EPCs housed within hydrogels may also be co-delivered with growth factors that further mimic healthy ECM and promote cellular survival and infiltration.[57,70] In one approach, spatially controlled incorporation of $VEGF_{165}$-loaded gelatin microparticles within EPC-laden, 3D printed hydrogels was shown to create regionally defined vascularized zones *in vivo*.[57] Alternatively, EPCs transplanted within RGD-alginate hydrogels that released $VEGF_{121}$ to promote outward cell migration were shown to alleviate many of the limitations of cell-based therapies, such as cell death, low engraftment efficiency, and lack of control over transplanted cell fate.[70] This EPC delivery approach dramatically improved vascularization and rescued murine ischemic hindlimbs from toe and foot necrosis and autoamputation.

Limitations to Transplantation Strategies

While stem cell-based therapy is one of the most documented approaches in regenerative medicine, the requirement for *ex vivo* expansion of cells for subsequent transplantation *in vivo* poses major difficulties and controversy.[15] Not only is this approach largely restricted by the limited availability of cell sources and numbers, but the *ex vivo* manipulation of cells requires labor-intensive procedures and excessive cost, and leads to anticipated difficulties in clinical translation and regulatory approval. There is also a great possibility for as of yet undetermined, undesired, and/or poorly controlled consequences from administered stem/progenitor cells.[15,64] For instance, cell transplantation poses risks of immune rejection and pathogen transmission. Collectively, these challenging limitations rationalize the development of strategies that facilitate reactivation of endogenous stem cell potential and homing as an appealing and conceptually simpler alternative to stem cell transplantation.[15,64]

BIOMATERIAL DELIVERY OF SOLUBLE FACTORS FOR MANIPULATING ENDOGENOUS EPC HOMING

Manipulating endogenous EPC homing beneficially reduces the risks and costs incurred by *ex vivo* cellular manipulation.[15] In order to recruit endogenous EPCs to diseased tissues, the necessary matrix characteristics of healthy recruitment-promoting tissue must be restored. Herein, certain matrix effectors, cytokines, and chemokines may be delivered to shift the balance towards promoting EPC homing to the target tissue.[73,81] Direct and indirect interactions between such factors and components of the ECM dictate how they are presented to and subsequently stimulate the surrounding cells.[67] Therefore, control over the spatiotemporal presentation of factors is crucial for the intended biological effect to be achieved. The design of synthetic ECMs using biomaterials is again important for controlling the locally sustained release of these factors to induce similar homing responses that healthy tissues would for enhanced wound healing. Herein, the binding affinity, loading concentration, and diffusion kinetics of soluble factors within biomaterial conduits are tuned to modify/control release and create sustainable concentration gradients. Here, we highlight some of the possible target factors for delivery with the goal of enhancing EPC recruitment within ischemic and diseased tissue.

Delivery of Soluble Factors for Enhanced Recruitment

E-selectin, an inducible cell adhesion molecule solely expressed on endothelial cells (ECs), mediates adhesive interactions between circulating cells (leukocytes, progenitors) and the endothelium, an essential initial step of the homing process.[53] Furthermore, soluble E-selectin, which is a cleavage form of membrane-bound E-selectin, has been shown to stimulate migration and angiogenic tube formation of human umbilical vein endothelial cells (HUVECs).[36] Both membrane-bound E-selectin in vessels and soluble E-selectin in serum are elevated after ischemia is induced in a mouse model of hindlimb ischemia.[53] Furthermore, the number of administered EPCs that home to ischemic tissue is significantly reduced when E-selectin is blocked with antibodies or when E-Sel$^{-/-}$ mice are used.[53] Interestingly, the decreased homing to the ischemic limb in E-sel$^{-/-}$ mice was rescued by injecting soluble E-selectin into the ischemic limb. This points to an important role of soluble E-selectin in EPC homing towards ischemic tissue and provides a possible target for delivery to enhance this phenomenon in ischemic tissue hampered by vascular diseases.[44]

SDF-1 has also been shown to increase EC-EPC adhesion by specifically upregulating E-selectin expression by microvascular ECs.[44] Delivery of SDF-1 within ischemic tissue boasts a possible method to recruit circulating progenitors for enhanced tissue regeneration given its prominent role in stem cell trafficking.[18,69] Herein, temporally sustained gradients are essential for SDF-1-mediated chemotaxis. Since small proteins such as SDF-1 and VEGF can readily diffuse through the ECM, strategies that promote controlled delivery may be essential for effectively directing cellular trafficking.[69] Binding to sulfated glycosaminoglycans (GAGs) in the ECM, such as heparin, is pivotal to locally accumulating and protecting such proteins against degradation.[58] While alginate is known to also have an affinity for heparin-binding proteins, other biomaterials are modified by incorporating heparin to tune protein binding and control release.[19,32] Herein, chemokine gradients were sustainably generated by incorporating SDF-1 within StarPEG-heparin hydrogels.[58] Here, the release profile of SDF-1 may be altered by precisely varying the loading concentration and by matrix metalloprotease (MMP)-mediated hydrogel cleavage. Implantation of these biofunctionalized hydrogels led to increased homing of systemically administered CellTracker CM-DIL-labaled human EPCs along the established gradient of SDF-1.[58] However, SDF-1 is also cleaved by exopeptidases and MMP-2, local proteases that are activated in the inflammatory environment of injured tissue.[69] Therefore, other strategies have employed delivery of a protease-resistant SDF-1 designed to retain *in vivo* bioactivity upon controlled nanofiber-mediated delivery within infarcted myocardium in rats.[69]

Nonetheless, the efficacy of approaches aimed at delivering SDF-1 for enhanced progenitor cell homing is limited by cellular desensitization to gradients of SDF-1. For example, it has been shown that EPCs from patients with CAD have significantly reduced CXCR4-mediated basal Janus kinase (JAK)-2 phosphorylation and are less responsive to SDF-1 compared with healthy controls.[81] Therefore, a possible solution is to provide factors and/or chemical cues that promote and enhance sensitivity of diseased EPCs to the provided SDF-1 stimulation.

Locally sustained delivery of exogenous VEGF in combination with SDF-1 from alginate hydrogels was shown to increase recruitment of systemically infused human endothelial progenitors to murine ischemic tissue.[2] Earlier studies also showed that daily systemic administration of recombinant human $VEGF_{165}$ isoform alone over the period of a week mobilized and increased the number of circulating BM-derived EPCs in mice.[5] However, although bolus VEGF injection provided therapeutic benefit in animal studies, there was no significant improvement in clinical trials presumably due to rapid washout of the protein from bolus delivery strategies.[71] Spatiotemporal delivery of human $VEGF_{121}$ isoform within RGD-modified alginate hydrogels was shown to improve outward migration and invasion of OECs also housed within the hydrogel.[70,78] This isoform readily diffuses through the surrounding ECM unlike the $VEGF_{165}$ isoform, which binds heparin with high affinity and thus traverses more slowly.[57,70] Gelatin microparticles (GMPs), which form electrostatic interactions with VEGF, incorporated within 3D-printed hydrogels have also been used to provide spatiotemporal control of release with regionally sustained $VEGF_{165}$ release.[57] Herein, the synthetic ECM of the hydrogel carrier can manipulate factor release and signaling.

Alike SDF-1, EPCs derived from patients with CAD have impaired migratory responsiveness to VEGF stimulation as compared with healthy controls.[81] VEGF-induced migration was also reduced by a similar degree in CXCR4 antibody-treated EPCs from healthy volunteers. S1P has not only been shown to enhance EPC sensitivity to SDF-1 stimulation,[82] but is also a potent regulator of cell trafficking in both physiological and pathological conditions.[43] EPCs derived from patients with CAD and stimulated for 2 h with S1P prior to intravenous infusion in a mouse model of hindlimb ischemia were shown to enhance vascular restoration similar to that of EPCs from healthy patients.[82] Importantly, this suggests that S1P stimulation may alleviate or reverse the SDF-1/CXCR4 functional impairment exerted on patient-derived EPCs due to vascular disease risk factors.[82] Microspheres made from PLG are biocompatible and bioabsorbable, and have been used for sustained S1P delivery in murine ischemic hindlimbs.[52,60,68,76] Interestingly, the combined delivery of S1P and VEGF from PLG was shown to prevent VEGF-induced edema over an extended period of time.[60] Furthermore, local transplantation of S1P-loaded PLG microspheres within ischemic murine muscle tissue led to increased expression of several angiogenic factors, including Angiopoietin-1 (Ang-1), SDF-1, hepatocyte growth factor (HGF), and interleukin-1 beta (IL-1β). In addition, it has been shown that hypoxia augments the angiogenic and migratory response of EPCs to S1P stimulation *in vitro*, further highlighting the impact of the extracellular milieu within ischemic tissue on the function of these cells.[85]

Limitations to Endogenous Recruitment Strategies

One possible concern involving approaches designed to enhance EPC homing is the lack of specificity towards recruiting a specific cell type.[7] EPCs are a heterogenous population of cells and many of the proposed factors may also recruit circulating hematopoietic progenitors, resident mesenchymal stem cells, and other cell types. However, these strategies are still valid and potentially clinically beneficial towards the end-point goal of enhancing vascularization and recovery from ischemic vascular diseases. In fact, it has been shown that delivery of hematopoietic and non-hematopoietic EPCs together led to greater benefit than delivery of either population alone.[70] Here, the rationale is that hematopoietic EPCs may have an indirect contribution of secreting soluble pro-angiogenic factors that further entice non-hematopoietic EPCs to directly participate in the formation of new blood vessels.

CONCLUSIONS AND FUTURE DIRECTIONS

EPC-based therapies hold tremendous therapeutic and curative potential for vascular ischemic diseases.[13,61] Manipulation of EPC homing provides further promise based on the inherent correlation between dysfunctional EPCs and vascular disorders.[13] Enhancing the endogenous homing capacity of these diseased cells is a growing area of research that may either surpass or improve approaches involving cellular transplantation. New discoveries of biomaterial systems that enable spatiotemporal control of chemokines and chemical cues within the field of bioengineering fuel further investigation into the kinetic delivery profiles required for optimal EPC recruitment and neovascularization at sites of ischemia. These biomaterials may be used as synthetic ECMs to either

(1) enhance cellular adhesion, survival, and function upon transplantation or (2) appropriately provide soluble factors and chemokines with spatiotemporal control that enables enhanced recruitment of circulating progenitors. Given that the ability for EPCs to follow natural homing cues is impaired in pathological conditions, it is important to further elucidate these mechanisms and how they are particularly disrupted so that they may thus be therapeutically reversed in diseased states. Finally, the materials and approaches discussed here may also ultimately find application outside of the context of vascular diseases with a better understanding of the general mechanisms governing cell trafficking and homing.

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