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Building Blood Vessels with Vascular Progenitor Cells

Thomas Colunga^{1,2} and Stephen Dalton^{1,2,*}

¹Center for Molecular Medicine, University of Georgia, 325 Riverbend Road, Athens, GA 30605, USA

²Department of Biochemistry and Molecular Biology, University of Georgia, 325 Riverbend Road, Athens, GA 30605, USA

Abstract

Vascular progenitor cells have been identified from perivascular cell fractions and peripheral blood and bone marrow mononuclear fractions. These vascular progenitors share the ability to generate some of the vascular lineages, including endothelial cells, smooth muscle cells, and pericytes. The potential therapeutic uses for vascular progenitor cells are broad and relate to stroke, ischemic disease, and in the engineering of whole organs and tissues that require a vascular component. This review summarizes the best characterized sources of vascular progenitor cells and discusses advances in 3D printing and electrospinning using blended polymers for the creation of biomimetic vascular grafts. These advances are pushing the field of regenerative medicine closer to the creation of small diameter vascular grafts with long-term clinical utility.

Keywords

vascular progenitor cells; tissue engineering; cell therapy

Clinical Significance of Vascular Progenitor Cells

Vascular disease can be attributed to a number of factors and affects millions of patients with a broad range of clinical disorders that affect multiple organs (see Clinician's corner, box 1). In some cases, **vasculopathies** (see Glossary) arise as a consequence of other conditions. For example, peripheral vascular disease is frequently linked to complex metabolic diseases such as diabetes. Diabetes also increases the risk of amputation for patients dealing with high blood pressure-induced peripheral artery disease, which is caused by a narrowing of the artery and reduction of blood flow [1]. Other conditions such as stroke or coronary, renal, and pulmonary disease can result from blood pressure dysregulation, reduction of blood flow, and vascular occlusion [2–5]. Over 370 million people worldwide suffer from diabetes and costs related to cardiovascular disease and stroke is currently >\$300 billion annually and will exceed \$1 trillion by 2035 [6, 7]. This represents a massive public health problem and

*Correspondence: sdalton@uga.edu (S. Dalton).

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although pharmacological treatments and surgical reconstruction of large vessels (> 5mm in diameter and 2 cm in length) are commonly used to minimize disease progression, surgical replacement of vessels smaller than 5mm in diameter remains problematic [8, 9]. If synthetic, cellularized vessels can be generated that retain biological function over extended periods of time without clotting or promoting an inflammatory response, this would constitute a major advance in the treatment of vasculopathy. A potential solution is to seed either **autologous** or human pluripotent stem cell (hPSC)-derived vascular progenitor cells with significant developmental plasticity onto biological or non-biological scaffolds to generate **tissue engineered vascular grafts** (TEVGs). This topic will be addressed by discussing the developmental origins and biological properties of different vascular progenitor cells. We will then describe the progenitor cells that hold most promise for clinical use and strategies of cell delivery, including the direct transplantation of cells into diseased and injured tissue and the use of vascular progenitor cells in TEVGs. Lastly, we will cover future directions the field could take by focusing on the interface between tissue engineering and stem cell biology.

Developmental Origins of Vascular Progenitor Cells

Almost all cells with vascular potential arise from the mesoderm lineage. The first vascular progenitors identified during human **embryogenesis** give rise to blood islands - small mesoderm-derived clusters of cells in the yolk sac that contribute to the earliest vascular structure, the primitive capillary plexus [10–13]. This plexus is void of supporting stromal cells and consists of endothelium derived from the distal cells of the blood island while the inner hematopoietic population gives rise to blood cells [12]. Blood islands are not known to persist throughout development and efforts to isolate and culture this population *in vitro* has not been reported. Thus, their utility for cellular therapy or tissue engineering is unknown.

Hemangioblasts and mesoangioblasts are two other cell populations that give rise to blood vessels during early post-gastrulation development. Postnatally, both cell types disperse into multiple tissues such as the heart and bone marrow where they contribute to new blood vessel generation and can be isolated for long-term culture [14–17]. Both cell types also express vascular endothelial growth factor receptor 2/fetal liver kinase 1 (VEGFR2/Flk1), while CD34 with CD45 are associated with human hemangioblasts [15, 17]. Hemangioblasts have the inherent ability to give rise to blood cells and endothelium but are not considered a direct progenitor of vascular smooth muscle cells (VSMCs) or pericytes (Pcs) [18]. Mesoangioblasts do not directly generate blood cells but contribute to endothelium, VSMCs, Pcs and other non-vascular cell types [15, 16, 19]. The developmental contribution of mesangioblasts and hemangioblasts to blood vessel formation remains unchallenged but their potential use as cell therapeutics is less clear [16, 20].

Resident Adult Vascular Progenitors

Coelomic organs and body cavities are lined on their outside surfaces by a layer of epithelial cells called mesothelium [21]. The best characterized mesothelial layer is the epicardium, which forms around the myocardium [22]. Various mesothelium lineage-tracing studies in mouse gut, liver, lung, kidney and heart have reported contrasting results in terms of vascular

contribution by the mesothelium lineage. In some studies mesothelium contribution to the vasculature has been restricted to VSMCs and PCs while others show strong endothelial contribution [23–26]. This conflict could be representative of technical issues relating to the choice of mouse Cre-drivers or, due to differences in the role of mesothelium in vascular development of these organs. Besides vascular contribution, mesothelium-derived cells of human, rat and mouse origin can give rise to hepatic stellate cells, mesenchymal stem cells (MSCs), fibroblasts, adipocytes, chondrocytes and osteocytes both *in vitro* and *in vivo* [27–29]. Under normal physiological conditions, mesothelium in adult humans participates in organ **homeostasis** and is **quiescent** but can be reactivated following injury [29, 30]. This is exemplified in the human and mouse heart where a subpopulation of epicardium cells migrates into the subepicardial and myocardial layers as they transform into migratory epicardial-derived cells (EPDCs). In the underlying tissue, EPDCs play a role in tissue repair and/or regeneration at multiple levels [31, 32]. EPDCs support **neo-vascularization** in a zebrafish heart regeneration model by releasing angiogenic signaling molecules and deposition of extracellular matrix (ECM) which establishes a favorable microenvironment [33]. In mouse myocardial infarction models, EPDCs contribute vascular lineages such as endothelium and VSMCs to nascent coronary vessels [26]. In the heart, cells of the mesothelium lineage are a source of resident vascular progenitors. Treatment with thymosin beta 4 (Tβ4) or VEGF in mouse myocardial injury models utilized the resident EPDC population to revascularize injured tissue [30, 34]. Primary human EPDC isolation (Figure 1) is highly invasive and maintenance of vascular potential *in vitro* is limited by culture induced transdifferentiation to stromal lineages and cellular senescence, which restricts their potential clinical utility [35].

Primary Endothelium and Endothelial Colony Forming Cells

Blood vessels are typically organized so that VSMCs surround the endothelium in arteries and veins while perivascular PCs are interspersed between endothelial cells of the microvasculature. Endothelial cells (ECs) occupy the **luminal** side of vessels and express von Willebrand factor, CD31 and CD144 on their surface and have acetylated low-density lipoprotein (LDL) uptake activity in human, mouse, and rat species [36, 37]. ECs function as a selective barrier in blood vessels that modulate the diffusion of white blood cells and solutes across to the surrounding tissue and for selective transport of waste products and CO₂ from the surrounding tissue into the bloodstream. This selective barrier function is conferred by tight junctions between neighboring endothelial cells and by the presence of pores in a subset of ECs known as fenestrated endothelium [37–39]. Human umbilical vein endothelial cells (HUVECs) and microvascular endothelium (MVECs) are two of the most commonly used primary endothelial sources for TEVG construction. Although human MVECs have been shown to successfully reseed decellularized vasculature *in vitro*, the graft induced hematoma formations after transplantation and reported occlusions within capillaries after implantation for 1 week [40]. Coupled with inflammation, this constitutes a major problem encountered following transplantation of vessels, particularly those of small-medium diameter (<5mm) [41, 42]. One report utilized a decellularized porcine carotid artery cocultured with autologous saphenous vein ECs on the luminal side and carotid artery SMCs on the **adventitial** side. This system allowed the researchers to strategically place both cell types within close proximity to facilitate cell-cell signaling and minimize

inflammatory responses, leukocyte, and platelet adhesion following transplantation into a porcine model for 6 weeks [43]. Controlling biomechanical forces such as fluid shear stress, flow rate, and pressure is key to guiding ECs into quiescence thereby maturing *in vitro*-derived vessels and suppressing events such as thrombosis or inflammation after transplantation [44–46]. Shear stress in particular has been implemented as a driving force responsible for EC quiescence and can even induce an EC phenotype in mouse PSCs in the absence of VEGF [45, 47]. However, practical limitations exist for isolated primary human ECs such as culturally induced anomalies which places a restriction on their use post isolation. This has forced researchers to explore other cell sources that were collectively referred to as ‘endothelial progenitor cells’ (EPCs) until more stringent classifications that considered source, isolation methods, differentiation and growth potentials, and phenotype were applied [48]. A few important aspects of clinical utility are the ability to isolate significant numbers of a cell population, amplify said population to relevant numbers for applications such as TEVG construction, and finally the cells must maintain differentiation potential *in vitro* during prolonged culture. While most ‘EPCs’ have since been reclassified and discounted due to their inability to meet clinical hallmarks, one particular cell population that researchers routinely focus on are endothelial colony forming cells (ECFCs).

ECFCs (Figure 1) can be isolated from human peripheral blood, placenta, adipose tissue and perivascular cell fractions, and amplified for multiple passages *in vitro* while maintaining differentiation potential [48, 49]. They are characterized by their *in vitro* colony forming and clonogenic potential coupled with the ability to form vessel-like structures [48, 50]. Much controversy surrounds the definition and developmental origins of ECFCs with evidence pointing towards both a hematopoietic and endothelial origin [48, 51, 52]. ECFCs promote vascularization by contributing to the endothelium of newly-formed blood vessels following injury and can modulate the regenerating vascular microenvironment through the release of **paracrine** factors [53, 54]. As an example of the latter, ECFCs isolated from human umbilical cord blood were injected intraperitoneally in a mouse neonatal hypoxic ischemic encephalopathy model. This led to a reduction in cell apoptosis, astrocytic scar formation, and increased capillary density. No clear evidence of ECFC-derived endothelium within neo-vessels was observed in this study, however [53]. Human umbilical cord blood ECFCs injected through the femoral vein in a rat cerebral ischemia model displayed a similar reduction of apoptosis coupled with increased capillary density and functional neurological recovery but no evidence of neo-vessel contribution [54]. Supporting a direct role in neo-vascularization, one study reported that hPSC-derived ECFCs incorporate into new blood vessels in mice hind-limb ischemia and oxygen-induced retinopathy models which reduced avascular areas and increased blood perfusion, respectively [55]. Primary rat ECFCs treated with T β 4, and injected intramuscularly into a rat myocardial infarction model, also incorporate into neo-vessels and increase capillary density compared to control groups and untreated ECFCs [56]. Notwithstanding these examples, however, the vast majority of translational research surrounding vascular progenitor utility is still in the proof of concept phase and has yet to move into large animal models in which long-term efficacy must be demonstrated [57, 58]. Compelling data to support clinical utility of vascular progenitors is probably 5–10 years away but, as described, there are some encouraging reports that must be corroborated and translated into a clinical product.

Mesenchymal Stem Cells

MSCs (Figure 1) are considered to be widely present throughout the body, including the vasculature, adipose tissue, bone marrow, placenta and cord blood, among others. Omics analyses and lineage characterization of various MSCs report them to be distinct heterogeneous populations that merely share common phenotypic characteristics [59–61]. Cells expressing CD90, CD166 and CD29 but not common vascular progenitor markers such as VEGFR2/Flk1 and CD34 are designated as MSCs in humans [62, 63]. While displaying multipotency *in vitro* and *in vivo*, their developmental origins are hotly debated and intertwined with perivascular Pcs [59, 64]. Despite a propensity for differentiation into non-vascular lineages, evidence suggests that MSCs may perform a support role in **angiogenesis** following myocardial infarction and hind-limb ischemia [65, 66]. In these latter contexts, MSCs are thought to function by a paracrine signaling mechanism that supports endothelial cell growth and differentiation but also promote the maintenance of Pc and VSMC populations [67]. MSCs also appear to serve as immunomodulators by suppressing the host immune system thereby lowering rejection incidences following **allogeneic** transplantation [68, 69]. In particular, human placenta and umbilical cord-derived MSCs have been shown to function via regulatory mechanisms such as reduction of antigen presentation by dendritic cells facilitated by the downregulation and absence of CD80, CD86 and CD40 thereby reducing T-cell proliferation and the release of cytokines such as interleukin 10 [70]. MSCs are among the few cell types that have progressed to clinical trial status for cell therapy. The TAC-HFT and PROMETHEUS clinical trials focused on the contributions of autologous bone marrow-derived MSCs in patients diagnosed with chronic ischemic cardiomyopathy [71, 72]. *In vitro*, this population generates vascular lineages such as endothelium and VSMCs but neither trial reported the contribution of MSCs to new blood vessel formation at or near the injection site. Patients treated with MSCs did, however, have increased left ventricle ejection fraction (LVEF) and suppressed scar tissue formation, compared to control patients, via paracrine mechanisms [71, 72]. However, definitive evidence that MSC-derived paracrine factors were solely responsible for improvements was lacking. While these individual studies look promising, a recent meta-analysis of 12 randomized trials showed that MSCs have no clear clinical benefit for treatment of myocardial infarction [73]. More studies are required to resolve this issue and to define in greater detail the particular population of MSCs with the most clinical utility and their mechanism of action. In addition to being a therapeutic option for tissue damage, MSCs have potential utility in conjunction with primary ECs in the engineering of TEVGs [74].

Pluripotent Stem Cells

Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), collectively referred to as hPSCs (Figure 1), can be cultured indefinitely as a self-renewing population and differentiated into all adult lineages [75–78]. This gives them many advantages and increased utility when compared to other multipotent cells that often have limited self-renewal capacity and restricted developmental potential [76, 77]. The ability of hPSCs to generate endothelium, VSMCs, and Pcs by directed differentiation provides the field with a cell source that could, in principal, generate an unlimited supply of vascular lineages [25, 36, 55, 77, 79, 80]. Molecular and functional analysis reveal that hPSC-derived

endothelium and VSMCs are analogous to their counterparts derived from tissue but now their clinical utility must be demonstrated [36, 38, 81]. Researchers have generated an “early vascular cell” by directed differentiation of hPSCs that successfully gave rise to self-assembling endothelium and Pcs into capillary-like networks *in vitro* and *in vivo* after subcutaneous implantation in mice [80]. Whether these cells are suitable for clinical use is unclear because they seem to represent an early mesoderm cell type capable of broad differentiation capacity which could possibly manifest as unwanted cell types that promote fibrosis, scar tissue formation or adipocyte deposition when used as a cellular therapeutic. A small-scale hind-limb ischemia study used hPSCs to derive a CD34⁺ early mesoderm cell: important cell signaling networks were modulated in a directed differentiation protocol followed by cell sorting to obtain an enriched population [82]. Despite a presorting differentiation efficiency of 13–20%, which is drastically lower than ideal efficiencies of >95%, clinical utility was shown by their incorporation into newly formed blood vessels and release of angiogenic cytokines when assessed 1 week after intramuscular injection [82]. The use of hiPSC-derived vascular cells (e.g. endothelium or VSMCs) also face challenges in the form of genetic instability and epigenetic alterations [83, 84]. While these cells have potential use in a range of applications, a hPSC-derived multipotent vascular progenitor that can form all vascular lineages has not been reported.

Vascular Grafts: Challenges, Properties and Future Directions

Vascular Graft Challenges

Vascular disease is frequently associated with occlusion events that restrict or completely block blood flow around the body. While **angioplasty** is generally effective it is susceptible to **stenosis** and is only a suitable option for a subset of patients [85]. TEVGs have potential utility in the treatment of a wide-range of vascular diseases including myocardial infarction, peripheral artery and venous disease, atherosclerosis, vascular aneurisms, and stroke (Figure 2, Key Figure). While the introduction of large diameter (>5mm) grafts have had some success, replacement of smaller vessels has been more problematic due to an inherent inability to remodel after transplantation which leads to occlusion and inflammation [86–88]. Autologous grafts from the saphenous vein or thoracic artery are routinely used for **vascular bypass** surgeries but suffer from high rates of failure in the first 5–10 years post-surgery and the amount of material available is limited [89, 90]. The situation is also complicated by the donor’s clinical condition that will impact tissue quality (e.g. diabetes and age) [89]. Complications associated with grafts of <5mm in diameter are generally attributed to inflammation, stenosis and neo-tissue hyperplasia mediated by macrophage infiltration which can all lead to cell death, fibrosis and chronic functional decline [42, 66, 89–92]. The challenges to engineer TEVGs that function reliably over extended periods (>10 years) is heavily impacted by the physical demands placed on such bio-constructs. Optimal tensile strength, mechanical flexibility, the ability to vasoconstrict and vaso-dilate and a consideration of burst pressures are all factored into vessel design making it a challenging process [8, 41, 93–95]. These mechanical properties are heavily influenced by choice of material and cell source and each choice appears to have its own advantages and disadvantages [41, 86, 91, 96–98].

Biomechanical Properties of Synthetic Polymers

Synthetic polymers such as poly-lactic acid (PLA), poly-glycolic acid (PGA) and poly-ε-caprolactone (PCL) are commonly used to generate biodegradable TEVGs [41, 86, 91]. These polymers can generate TEVGs with varying degrees of porosity, tensile strength, and flexibility via modulation of polymer concentration, choice of organic solvents, and crosslinking [99, 100]. This is evident for PLA which has reported tensile strengths ranging from 38–77 MPa (psi) prior to vascular graft applications [101]. PLA is also classified as a hydrophobic polymer that lacks cell binding sites which could result in lower seeding efficiencies and possible immune responses [102]. PGA is a particularly attractive choice for TEVGs due to its degrading time of 2–3 months *in vivo* which allows the graft to remodel after deposition of ECM by seeded or recruited autologous cells [103, 104]. However, this can lead to rapid structural integrity loss and acute inflammatory responses thereby minimizing clinical utility [103, 104]. While PCL tensile strength is higher than PLA, it suffers from longer degrading times compared to PGA [104, 105]. Researchers have now started to exploit the various biomechanical properties of synthetic polymers to generate blended TEVGs that provide tensile strength comparable to native vessels but allow for optimal degradation times to ensure remodeling after transplantation.

Biomechanical Maturation of Vascular Grafts

Biomechanical forces are routinely applied to TEVGs to quantify their metrics but they also play a huge role in facilitating cell and graft maturation. A recent report showed that hydrostatic pressures applied to vascular grafts constructed of fibronectin and seeded with rat VSMCs influenced stress fiber formation, fibronectin fibrillogenesis, ECM formation, and cell proliferation to various degrees indicating the delicate balance between graft development and cell phenotypes [106]. The graft was sutured to the rat abdominal aorta for 10 weeks and not only withstood physiological pressure but successfully re-endothelialized [106]. Ciliated ECs can also react to **mechanotransduction** by upregulation of the transcription factor Krüppel-like factor 2 (KLF2) and VEGFR2 [45, 107].

Emerging Technologies

A range of progenitor and differentiated cells have been described that are currently in use for TEVG development. Their use in conjunction with biological and fabricated scaffolds for development of TEVGs will now be discussed, beginning with acellular biological scaffolds.

Decellularized vascular scaffolds have been prepared from various vascular sources and anatomical locations to serve as a test-bed for clinical development and therapeutic applications [108–111]. Decellularized scaffolds are a desirable platform on which to base TEVGs as they retain a native ECM onto which vascular lineages can be assembled [112–114]. The decellularization of vessel scaffolds requires detergents and enzymes and the cellularization step optimally requires a **bioreactor** to control growth conditions and to modulate perfusion rates [109, 110, 112]. A porcine liver that was decellularized via mechanical and enzymatic treatment was seeded with autologous endothelial cells for 3 weeks. Following **anastomosis**, the vasculature was able to perfuse blood for 3 days but still remained susceptible to thrombosis and failure in the acute stage immediately following transplantation [115].

While the development of next-generation decellularized constructs is ongoing, tissue engineers have developed fabricated scaffolds to overcome mechanical and cell seeding problems associated with existing TEVGs. As a way to reproduce biological scaffolds, but with the advantage of customizing vessel properties, natural components such as human, porcine or rat collagen, gelatin and fibrin are used for bioscaffold fabrication [116]. These proteins can be used for applications such as 3D printing and **electrospinning technologies** to generate scaffolds that can be cellularized with vascular progenitors or mixtures of mature human vascular cells. As an alternative to natural materials that are found in blood vessel walls, tissue engineers have adopted the use of synthetic polymers such as those previously described [93, 117–119]. 3D printed and electrospun TEVGs can also be loaded with growth factors and small molecules that promote cellular maturation. These technologies can be used for the fabrication of precise, personalized constructs based on patient vascular defect size and location in contrast to decellularized constructs, which are more limited in shape, size and branching [120].

3D printing and electrospinning matrices are frequently designed to orientate cells individually or in layers to form vessel structures that will support blood flow following transplantation [41, 96, 117–119]. These scaffolds are made with high technical specifications and can incorporate multiple materials assembled into multiple layers with different physical and biochemical characteristics. The rationale in the design of TEVGs is to facilitate assembly of cells on the tunica intima and the tunica externa and throughout the tunica media by introducing porous materials that allow for cell migration, attachment and maturation [91, 118, 119, 121, 122]. Potential for this technology is underscored by recent work by Mirabella and co-workers who used transplanted TEVGs comprised of 3D printed grafts seeded with HUVECs [118]. Here, low passage HUVECs were selected and grafts were subjected to shear stress conditioning prior to implantation which significantly improved blood perfusion and provided a therapeutic benefit in hind-limb ischemia and myocardial infarction models.

Concluding Remarks

Vascular disease is one of the most significant medical problems facing our society. A potentially effective way to address this problem is to utilize stem cell biology with tissue engineering. In this review, we discussed how vascular progenitors can be used in conjunction with scaffolding technologies to generate blood vessels that can replace diseased or damaged vasculature. The introduction of new materials from an engineering perspective combined with the derivation of vascular progenitor cells, that have developmental plasticity and which can assemble into functional vessels, are likely to play an important role in this area. When choosing a vascular progenitor for cellular therapy or tissue engineering, a multitude of parameters including cell type, cell number, delivery method (i.e. use of scaffold) and location, patient diagnosis, age and paracrine versus autocrine factors must be considered. Biomechanical forces applied to the cells within TEVGs must also be considered as they directly influence cell maturation, signaling, and ultimately inflammation susceptibility. Inflammation is one of the leading causes for graft failures and this is partly driven by an immature cellular phenotype. The ultimate goal of manufacturing an 'off-the-shelf' TEVG for vascular disease and corrective surgery has not

yet been achieved (see Outstanding Questions). However, stem cell technology, materials science and bioengineering techniques such as 3D printing and electrospinning offer a potential solution to this biomedical problem.

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Glossary

Adventitial

the outer layer of veins and arteries, also called tunica externa, that is comprised of connective fibers such as collagen that is deposited by vascular smooth muscle cells.

Allogeneic

relates to cells or other biological material that are from the same species but transplanted into other individuals (e.g. bone marrow transplantations).

Anastomosis

surgical procedure attaching a vessel (synthetic or autologous) to an existing blood vessel for the purpose of restoring blood flow to a damaged or diseased area.

Angiogenesis

formation of new blood vessels that branch-off from existing ones.

Angioplasty

surgical procedure to open blocked arteries. This procedure is frequently used to clear coronary vessels.

Autologous

transplantation of cells or other biological material that are derived from the recipient.

Bioreactor

apparatus in which cells can be grown to industrial scale or used to perfuse media through a closed system with stringent control of cell culture parameters such as temperature, CO₂, pressure or flow rate.

Electrospinning technologies

application of an electrostatic charge to natural or synthetic polymers pumped through a syringe and needle to a spinning mandrel or collection plate to generate a scaffold onto which cells can be seeded for generation of vascular grafts.

Embryogenesis

early development in humans that covers the first eight weeks post fertilization and encompasses formation of the three germ layers.

Exosomes

membrane bound vesicles released by cells. These vesicles carry cargo such as growth factors, proteins, microRNA and lipids that impact function of other cell types.

Homeostasis

the process by which tissues and organs self-regulate to maintain function.

Luminal

the inner cavity of a blood vessel that is typically covered with endothelium and is the conduit for flow of blood cells.

Mechanotransduction

a cell sensory mechanism by which they sense and convert mechanical stimulations to biological processes such as gene expression.

Neo-vascularization

formation of new blood vessels in areas that previously had no vascular networks or in tissues that experienced trauma (e.g. myocardial infarction).

Paracrine

release of cell signaling molecules, growth factors, cytokines and chemokines by one cell that only influence nearby or distant cells.

Quiescent

a cellular state that is marked by temporary exit from the cell cycle but allows for re-entry to actively divide and proliferate.

Stenosis

the narrowing of a blood vessel causing restricted blood flow. In tissue engineered vascular grafts this can be caused by calcification and inflammation related to immune cell invasion.

Tissue engineered vascular graft (TEVG)

constructed by decellularization of explanted vascular scaffolds, 3D printing or electrospinning technologies using natural or synthetic polymers and seeded with vascular cell types to create vessels for *in vivo* transplantation and repair of vascular structures.

Vascular Bypass

surgical procedure typically associated with blockage or narrowing due to plaque build-up. Arteries or veins from another part of the body or synthetic vessels are grafted to bypass the blocked area to restore blood flow.

Vasculopathies

any number of diseases or defects of the blood vessels that leads to functional decline.

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Highlights

- Regenerative medicine is uncovering potential therapies for ischemic diseases by using vascular progenitors for tissue engineering applications.
- Genetic manipulation with tools like CRISPR-Cas9 in diseased patient-derived induced pluripotent stem cells allows for the use of autologous cells in vascular therapy.
- Biological and fabricated scaffolds are viable alternatives that can be cellularized for the purpose of generating transplantable vascular grafts.
- Bioengineering of small diameter multi-branching vascular grafts that can grow *in vivo* is being pioneered by 3D printing and electrospinning.

Outstanding Questions

- A majority of *in vivo* studies involving murine models are carried out in immune compromised animals. Granted that these studies can show proof of concept, should researchers move to animal studies with intact immune systems to get a clearer understanding of how the immune system would factor into patency or thrombosis of tissue engineered vascular grafts?
- Paracrine effects are often reported in studies with direct cell injections into the tissue for vascular repair. Should the approach be to parse out individual cellular components for testing such as mRNA, growth factor or exosomes to gain a clearer picture regarding mechanism of action? What would be the proper delivery method (intramuscular, intravenous or nanoporation)?
- Which cells are going to prove of most utility for vascular graft engineering, endothelial colony forming cells (ECFCs) or epicardial-derived cells (EPDCs)? Will these cell types be generated from human pluripotent stem cells or isolated from patients? Are ECFCs lineage restricted to only endothelium or can they also generate smooth muscle to form invested vessels?
- Will pre-seeded TEVGs have more clinical utility than acellular grafts?
- Small diameter synthetic vessels are surely becoming a pressing need for vascular repair. Could they also have use as an *in vitro* model system for edema and drug discovery/delivery?
- Is an off-the-shelf cellular TEVG attainable given cell culture requirements and timely and unique patient specific needs?

Clinician's Corner (box 1)

- Vascular injuries result in an upregulation of molecular markers that bind and recruit thrombotic and inflammatory platelets, leukocytes and macrophages.
- Vascular grafts designed for pediatric use should be biodegradable and allow for remodeling to provide longevity especially in cardiovascular applications.
- Mesothelium is present on all coelomic organs but its developmental program and full contribution to organ specific vasculature is still not fully understood.
- The development of a human pluripotent stem cell-derived or primary vascular progenitor that can give rise to all the required vascular lineages such as endothelium, smooth muscle and pericytes has yet to be reported.

Figure 1. Cell types used for vascular repair and for vascular engineering applications

Primary cells from individuals can be used directly or following cellular reprogramming to generate vascular cell types. Reprogrammed cells offer a potentially unlimited supply of autologous cells for implantation which could minimize rejection. Primary cells such as endothelial progenitor cells, endothelial cells, vascular smooth muscle cells, pericytes and mesenchymal stem cells can be routinely harvested for expansion and use in cellular therapy and tissue engineering applications.

Figure 2. Tissue engineered vascular grafts and their potential use in vascular engineering
Tissue engineered vascular graft (TEVG) scaffolds can be generated from biological material such as decellularized arteries and veins or from fabricated materials that can be assembled into scaffolds using 3D-printing or electrospinning technology. These scaffolds are then seeded with vascular progenitor cells for the generation of transplantable TEVGs.