Stem Cell Reviews and Reports (2018) 14:642–667
https://doi.org/10.1007/s12015-018-9825-x

Stem Cell Sources and Graft Material for Vascular Tissue Engineering

Dorothee Hielscher[1] · Constanze Kaebisch[1] · Benedikt Julius Valentin Braun[1] · Kevin Gray[2] · Edda Tobiasch[1]

Published online: 2 June 2018

Keywords Angiogenesis · Vascular cells · Graft material · Stem cells · Sprouting · Vascular grafts · Tissue engineering · Vasculature

Introduction

Coronary artery disease represents the most common type of heart disease and accounts for about 7.4 million deaths worldwide in 2012 [1]. Prognoses indicate that annual mortality from this condition will increase because the aging population also raises the prevalence of patients with multimorbidity and chronic conditions. This phenomenon already poses a great challenge to regenerative medicine approaches because these patients also often lack graft material suitable for bypass surgery [2]. For some time, clinicians have sought methods to improve the vascular regeneration of these patients by focusing on gene therapy or approaches with small molecules [3]. In the last years, researchers are increasingly focusing on stem cell-based approaches due to these cells unique ability to self-renew and differentiate into various lineages [4]. Initially, embryonic stem cells (ESCs) were the predominant stem cell source in preclinical studies due to their pluripotency, their ability to differentiate into all lineages. Scientific interest shifted as Takahashi and Yamanaka reported a method to induce pluripotency in somatic cells in 2006 giving rise to induced pluripotent stem cells (iPSCs) [5, 6]. Besides these two pluripotent stem cell types, research efforts also focus on adult, multipotent mesenchymal stem cells (MSCs) since they show, in contrast to ESC and iPSCs, no detectable teratoma formation [7]. This relative safety has brought adult stem cell approaches farther towards therapeutic application than any other stem cell type [3]. Theoretically, stem cell-based approaches have the capacity to give rise to new blood vessels. Indeed, extensive effort has been invested in tissue engineering strategies in the vascular field. However, to address the massive clinical needs to perfuse or repair tissues, vascular tissue engineering needs a scaffold platform that provides the microenvironment the cells need. This review focuses on stem cell sources and current graft materials that are favorable to promote angiogenesis within tissue engineered constructs. Further it covers the key molecules and cellular signaling involved in this essential process. Subsequently, assays to study angiogenesis, and current vascular engineering biomaterials are discussed. The intention of this review is to provide a broad overview on the field of (stem) cell sources and graft material in vascular tissue engineering. For in-depth information it is recommended to consult existing excellent works on the specific aspects [8–11].

Vascular Cells

Blood vessels provide the bodies' tissues and organs with oxygen and nutrients and are crucial for immune surveillance [12]. Essential for their proper function is the composition of the vascular wall with its distinct cell types and structural proteins. All blood vessels except capillaries comprise three layers: the innermost *Tunica intima*, the intermediate *Tunica media,* and the peripheral *Tunica adventitia* [13]. The cells that are all of mesodermic origin are organized within those layers [14]. Among them are endothelial cells (ECs), smooth muscle cells (SMCs), pericytes, fibroblasts, neurons

✉ Edda Tobiasch
edda.tobiasch@h-brs.de

Dorothee Hielscher
dorothee.hielscher@h-brs.de

Constanze Kaebisch
constanze.kaebisch@h-brs.de

Benedikt Julius Valentin Braun
benedikt.braun@smail.bcw.h-brs.de

Kevin Gray
kmgray94@uw.edu

[1] Department of Natural Sciences, Bonn-Rhine-Sieg University of Applied Sciences, Von-Liebig-Str. 20, 53359 Rheinbach, Germany

[2] Department of Bioengineering, University of Washington, Seattle, 3720 15th Ave NE, Seattle, WA 98195-5061, USA

(endings), and various inflammatory cells [15]. The following paragraphs give an overview about macro- and microvascular cells and their characteristics.

Macrovascular Cells

The macrovasculature encompasses the larger conduit arteries like *aorta* and *carotid* that have an internal diameter bigger than 100 μm. Acting as an elastic reservoir they store blood during systole and release it during diastole to guarantee a continuous and steady blood flow that involves large volume changes but experiences little variation in pressure [16, 17]. The structure of the muscular arteries is dominated by elastin and collagen to ensure mechanical strength [17]. The outermost layer of the vessel wall, the *Tunica adventitia*, is composed of collagen-rich extracellular matrix (ECM) that is build up by a heterogeneous population of (myo)fibroblast cells and helps to avoid ruptures within areas of high pressure [13]. The connective tissue is interspersed with nutrients-carrying microvascular vessels smaller than 100 μm (*vasa vasorum*) and autonomic nerves (*nerva vasorum*) [14]. The elastic fiber network of collagen in the *Tunica media* layer of the larger arterial wall in vertebrates is organized mainly by vascular smooth muscle cells (VSMCs). It has long been thought that the muscular layer of the *media* consists of a homogeneous population of mature, differentiated, contractile VSMCs. Nowadays more recent data point to the existence of multiple SMCs subpopulations [15]. VSMCs can be subdivided in a contractile and a synthetic phenotype. In a healthy body, the contractile phenotype is predominant and wraps circumferentially around the blood vessel to improve its mechanical properties. The cells show a spindle-shaped morphology, are characterized by a low proliferation rate, and exhibit an increased expression of contractile proteins e.g. smooth muscle myosin heavy chain. The cells switch over to a synthetic phenotype during blood vessel development, remodeling, injury, or disease. Their morphology changes to a "hill and valley" structure and the cells start to increase their proliferation rate while simultaneously decreasing their expression of contractile proteins [18]. Besides contractile and synthetic functions, they are producing a complex ECM that accounts for the elastic recoil properties of the vessel and is necessary in signaling *via* inducing, defining, or stabilizing vascular cell phenotypes [16]. The luminal part of large vertebrate arteries, the *Tunica intima*, is composed of endothelial cells (ECs) that form the inner lining of these vessels and allow the continuous traffic of plasma and cellular constituents between blood and parenchyma tissues [19]. The inner lining is encompassed by the subendothelial area of the internal elastic lamina which is build up of elastin produced by ECs – a process that is hypothesized to be a response to signals from cells residing in the *Tunica media* [20]. Whereas the endothelium has long been considered a passive barrier enabling exchange of gases, ions, and other small substances, it is now clear that ECs take over very distinct and unique functions including regulation of hemostasis, neutrophil recruitment and homing, hormone trafficking, and control of the vascular tone [19, 21, 22]. Furthermore, they play a major role in vascular development by recruiting SMCs to the vascular wall [23]. Whereas the subendothelial matrix in smaller animals is typically acellular, a population of SMCs can be found in humans and in larger animals [24].

Microvascular Cells

Accounting for over 95% of the total body vasculature, the small resistance vessels of the microvasculature are mainly precapillary arterioles with an internal diameter of less than 100 μm, capillaries (<10 μm) and venules (7 to 50 μm) [17]. A variety of large human arteries exhibit a microvasculature in their adventitial layers termed v*asa vasorum* which is mainly responsible for nutrient transport to the vessel wall [25]. In contrast to macrovessels, the endothelial cells of microvessels are surrounded by solitary VSMC-like cells called pericytes that share the basement membrane with the endothelium [26]. They are related functionally and suspected to belong to the same cell lineage than VSMCs but differ in their distance to the endothelium, in their morphology, and to some extent in their expression of specific marker. Whereas arterioles are enveloped by these VSMC-like cells and therefore primarily control the blood volume for exchange, the walls of capillaries and postcapillary venules are thin and mainly composed of ECs lacking VSMC-like cells, which give them the ideal properties for regulating the exchange process itself [27].

H- / L- type Endothelial Cells

In 2014 Adams and coworkers reported the identification of two specialized endothelial cell population in bone microvessels of mice that they termed type H and type L ECs. The terminology originates from the finding, that type H subpopulations showed a high expression of CD31 and endomucin, whereas type L cells exhibited a low expression of the two markers. While both subpopulations show signaling properties that support maturation and regeneration of bone, osteoprogenitors were preferentially positioned around type H, but not type L endothelium. The researchers also proposed that type H ECs mediate local growth of the vasculature and provide niche signals for perivascular osteoprogenitors [28, 29].

Stem Cell Sources for Vascular Tissues

Stem cells are capable of self-renewal as well as direct differentiation and are therefore able to replace aging cells or repair tissues [30]. These features have made them a promising tool for regenerative medicine. There are basically two main types

of stem cells: Embryonic stem cells which are pluripotent and thus capable of differentiating into any cell type of the adult body, and adult stem cells which can be multipotent and accordingly are able to differentiate into several cells, mainly of the germ layer of which they are derived from. Since recently, there is a new pluripotent stem cell type which is an artificially reprogrammed somatic cell, referred to as induced pluripotent stem cells (iPS) [3]. The following paragraphs provide an overview on ESCs, iPSCs, and mesenchymal and vascular stem cells (MSCs and VSCs), as well as endothelial stem and progenitor cells.

Embryonic Stem Cells

Discovered in 1998, research on human ESCs is relatively new, considering that mouse ESCs were already grown two decades earlier [31]. Since that time, there has been a growing interest in utilizing human ESCs as potential source for tissue engineering that has made them the best characterized pluripotent stem cell type [32]. Despite their auspicious potential, there are some notable hurdles to overcome before safely applying ESCs in clinics. On the one hand, there is a decades-old, still running public debate about the ethical problems concerning ESCs [33]. On the other hand, the serious risk of teratoma formation, even if only a few undifferentiated cells are transplanted into a patient, still hampers the application of ESCs [34]. ESCs can be isolated from the inner cell mass of a blastocyst. In contrast to most adult stem cells they reveal the capacity of differentiating into cells of all three germ layers. Furthermore they are defined by their potential for self-renewal [35]. Since they are established in cell culture, a significant effort has been made to find a definitive human ESC marker expression profile [36]. In 2007 the International Stem Cell Initiative investigated 59 human embryonic stem cell lines from 17 laboratories all over the world and found similar expression patterns of several marker. Among the common marker the investigated ESCs shared were SSEA3 and SSEA4, TRA-1-60, TRA-1-81, GCTM2 and GCT343, CD9, Thy1 (similar to CD90), tissue-nonspecific alkaline phosphatase, class 1 HLA, NANOG, POU5F1 (formerly known as OCT4), TDGF1, DNMT3B, GABRB3, and GDF3. However, the researchers found that the cell lines do not show exactly the same expression pattern but instead exhibit some gene-dependent variations [37]. The most common genes that are characteristic for ESCs are the transcription factors Oct-4, Sox-2, and Nanog which are typically used for verifying the uncommitted status of ESCs since each plays a role in regulating the maintenance of pluripotency [36, 38].

ESCs can be differentiated into vascular ECs and SMCs which has been described before [39–44]. ECs derived from ESCs (ESC-ECs) present endothelial surface marker like CD31 and show expression of endothelial proteins such as von-Willebrand factor and platelet endothelial cell adhesion molecule (PECAM-1 also known as CD31). In addition, they are capable of capillary tube formation in Matrigel™ and take up acetylated low-density lipoprotein, demonstrating their endothelial function [3]. The differentiation of ESCs depends on their microenvironment, which includes mechanical forces, cytokines or growth factors, ECM, and communication with adjacent cells [30]. Generation of ECs from ESCs is mainly accomplished through two approaches. One is using spontaneous differentiation of embryoid bodies (EBs), a method also called 3D differentiation [40, 45]. For this approach, the cells need to be cultured in low-attachment dishes in differentiation media, in the presence or absence of growth factors. After nine to 13 days the cells spontaneously differentiate into ECs [46]. The other method is a 2D differentiation [47], where human ESCs are cultured on mouse embryonic fibroblasts in differentiation medium containing definded FBS for ten days without supplementation of additional growth factors. Over the time a decrease in transcription of pluripotency genes such as Oct-4 is observable together with an increase in transcription of genes for hematopoietic (GATA2) and endothelial cells (PECAM1) suggesting the presence of corresponding progenitors [46]. However, a major hurdle that needs to be overcome to translate ESC-based approaches from bench to bed side is the generation of sufficient numbers of differentiated ECs, since the yield of differentiated cell in EBs ranges from 1 to 3% [40]. Xiao and coworkers reported on the successful differentiation of ESCs towards vascular smooth muscle cells using different culture conditions such as cultivating the cells on collagen type-IV or fibronectin coated plates with the addition of transforming growth factor (TGF)-β [48] or platelet-derived growth factor (PDGF) [49].

Induced Pluripotent Stem Cells

The aforementioned drawbacks associated with ESCs motivated a far-reaching search for a more ethically and therapeutically viable pluripotent cell source. In 2006, Yamanaka and colleagues screened 24 of formerly identified key genes that seemed likely to play a role in maintenance and regulation of pluripotency in ESCs and embryos [32]. They found that the four transcription factors OCT3/4, Sox2, c-Myc, and Klf4 can induce pluripotency in somatic cells and termed these cells induced pluripotent stem cells (iPSCs) [5]. Once in a pluripotent state, these cells reveal the same characteristics as ESCs and can be differentiated into cells of all three germ layers [32]. In parallel with Takahashi and Yamanaka, Yu and Thomson successfully used Oct3/4 and Sox2 together with Nanog and Lin28 reaching the same result [6]. Later, researchers discovered that the number of acquired factors could be reduced to two factors (Oct3/4 and c-Myc or Klf4) depending on the targeted somatic (stem) cell and, in some cases such as induction of pluripotency in neural stem cells, even one factor (Oct3/4) was shown to be sufficient [50, 51].

Morphologically, iPSCs appear in a round shape with a large nucleolus and a scarce cytoplasm [31]. The reprogrammed cells grow in colonies which are tightly packed, sharp edged, flat, and mitotically very active. Their molecular profile is very similar to ESCs, expressing the markers Oct-4, Nanog, Sox2, SSEA-1, SSEA-3, SSEA-4, TRA1–60, TRA-1-81 and, ALP activity. However, Takahashi and Yamanaka found that iPS cells are very similar but not identical to ESCs [37, 52, 53].

There are two widely preferred options for introducing reprogramming factors into the somatic cells: integrating and non-integrating viral vectors. In integrating vector systems, the viral vector gets integrated into the host genome (retrovirus such as lentivirus), while non-integrating methods operate without integration into the host's genome (*i.e.* adeno virus or sendai virus) [52]. Despite their high efficacy, integrating viral vectors have been superseded by non-integrating systems because of their high risk of cancer formation and the possibility for random integration into an indispensable gene [54]. However, the tumor formation potential also originates from pluripotency [55]. Reprogramming *via* factor-containing plasmids was shown to be ineffective [56].

Since induced pluripotent stem cells can give rise to every cell of the adult body, they can be differentiated towards vascular lineages. In experiments, iPSCs were shown to be capable of differentiating into SMCs [57, 58], ECs, and vascular mural cells [59]. Park and colleagues demonstrated that functional CD34+ progenitor cells can be generated from human iPSCs by combined modulation of two signaling pathways [60]. Another group demonstrated that iPSCs are able to differentiate into CD31+ CD43- ECs and characterized the endothelial differentiation potential of seven human iPSCs lines obtained from different sources and compared it to human ESCs. The expression pattern was proven to be very similar to that observed with the human ESCs [61].

High EC and SMC differentiation efficiencies of over 80% (99% after purification) were reported by Patsch and coworkers [62]. The inhibition of GSK3 and simultaneously administration of BMP4 quickly committed hPSCs to a mesodermal fate. Subsequent exposure to VEGF or PDGF-BB facilitated the differentiation of either ECs or SMCs, respectively. With these protocols mature ECs and SMCs emerged within six days and showed surface markers and all relevant *in vitro* and *in vivo* functionalities. Global transcriptional and metabolomic studies demonstrated that the produced cells closely resemble their *in vivo* equivalent [62]. In a previous work of Sahara *et al*. it was already demonstrated that endothelial progenitors, derived from a protocol close to the one of Patsch and coworkers, implanted into immunocompromised mice form functional capillary vessels with anastomosis to the host vessels *in vivo* [63].

To date, the most efficient differentiation protocols to direct hPSCs into the endothelial lineage are based on the application of extracellular factors (mainly BMP4 and VEGF-A) [64–67]. Besides this, James and coworkers described that the TGFβ inhibitor SB431542 enhanced EC differentiation of hESCs when combined with VEGF-A administration in the first two weeks of differentiation. However, the efficiency was low (1.8% $CD31^+$ VEC^+ ECs) [68].

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) were first described by Friedenstein and colleagues in 1966 who isolated these adult stem cells from human bone marrow [69]. In the following years, MSCs from other sources including dental pulp, skin, tendon, muscle, peripheral blood, umbilical cord blood, and adipose tissue could be isolated of which umbilical cord blood, bone marrow, and fat tissue represent the most prominent sources [70–72]. MSCs are multipotent stem cells and can be differentiated into the chrondrogenic, osteogenic, adipogenic, and myogenic lineages [73–75]. Fundamental aspects like the embryonic origin of MSCs and their niche *in vivo* is still a highly debated question. Furthermore, MSC populations exhibit considerable donor-to-donor and intrapopulation heterogeneity [76]. This is the main reason why MSCs do not possess a reliable unique marker, although the International Society for Cellular Therapy defined several minimal criteria for defining these cells. First, cultured MSCs should adhere to plastic surfaces. Second, ≥95% of the population must express CD105, CD73 and CD90 while lacking (≤2% positive) the expression of CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA class II surface molecules. Third, the differentiation capacity of MSCs must include osteoblast, adipocyte, and chondroblast differentiation *in vitro* as demonstrated by staining [77]. Morphologically, MSCs appear fibroblast-like and spindle-shaped [78]. The isolation of MSCs can basically be divided into bone marrow-derived (BM-MSCs) and non-bone marrow-derived sources. Bone marrow-derived MSCs are harvested *via* aspiration of the iliac crest. However, their use is limited due to low numbers, low isolation volumes, pain, and to some extent ethical concerns. Additionally, their usability is further impeded by underlying diseases and stem cell populations that diminish with age [79]. Non-bone marrow-derived sources include adipose-derived stem cells that are easy to obtain in large amounts from liposuction surgeries of healthy individuals [70, 80].

The capacity of BM-MSCs to differentiate into cells of the vascular lineages has been widely investigated. Researchers could demonstrate that these cells are able to differentiate towards endothelial cells expressing typical marker like von-Willebrand factor (vWF), vascular endothelial growth factor receptor1/2 (VEGFR1/2), and vascular endothelial (VE)-cadherin *in vitro* [81]. In another study Janeczek Portalska and colleagues could observe that endothelial like-MSCs derived from bone marrow showed doubled numbers of vessel-ingrowth in PLLA/PLGA constructs two weeks after

subcutaneous implantation into mice [82]. However, MSCs are a highly heterogeneous population and it cannot be fully excluded, that a contamination by endothelial (progenitor) cells upon isolation leads to enrichement of those cells upon culture. Indeed, EC marker have been found on mRNA level in cell suspensions directly after isolation, although they were not detected as proteins [83]. Other studies indicated a heterogeneous angiogenic gene expression in different MSC types [84, 85] as well as a paracrine influence of VEGF secretion by MSCs on the differentiation of endothelial progenitor cells [86]. Summing up, data on the derivation of ECs from MSCs are poor and controversly discussed among the scientific community so that considerable skepticism remains concerning the applicability of EC differentiation from MSCs.

Besides MSCs, a small subpopulation of very small embryonic-like cells (VSELs) were found in adult bone marrow and other adult tissues, contributing to cardiac and endothelial repair [87, 88]. VSELs express some pluripotency markers and can differentiate into cells of all three germ layers. However, there are publications refering to VSELs as multipotent stem cells [89]. They are thought to be mobilized into peripheral blood in response to injury, as shown in critical limb ischemia patients [88, 89]. Furthermore, Guerin and coworkers demonstrated that VSELs are capable of endothelial lineage differentiation *in vitro* and *in vivo*. In a mouse model, human VSELs were shown to trigger postischemic revascularization and human CD31+ cells were found in neovessels of plug sections. Supporting these results, Ratajczak *et al.* detected high expression levels of the Flk2 transcript in highly purified VSELs [90]. Hence, VSELs might display another potential new source for endothelial cells.

In contrast to EC differentiation, SMC differentiation from MSCs is well described and reproduced in many laboratories [91–93]. Human BM-MSCs have been exploited as source of SMCs and were used, together with ECs, in engineered small-diameter vessel grafts [92].

Adipose tissue-derived stem cells also offer a source for vascular cells [94] and were shown to be capable of differentiating towards endothelial cells *in vitro* when cultured with vascular endothelial growth factor (VEGF) and/or b-fibroblast growth factors (b-FGF) on Matrigel™ coated coverslips or in cell culture dishes [95, 96]. EC specific marker such as CD31 (PECAM-1), CD34, CD144 (VE-cadherin), and endothelial cell nitric oxide synthase (eNOS) were proven to be expressed in those cells. Other studies demonstrated the successful differentiation into contractile SMCs that adhered and proliferated on vascular grafts [97, 98].

Endothelial Progenitor Cells

The existence of endothelial progenitor cells was postulated since the middle of the twentieth century, but evidence was provided as recently as 1997 when Asahara *et al.* reported the isolation of putative CD34+ endothelial progenitor cells from human peripheral blood [91]. At that time, the field was dominated by studies suggesting that endothelial progenitor cells in adults are circulating bone marrow-derived cells [91–93] sharing a common hemangioblast precursor with hematopoietic stem cells. Although the work of Asahara and coworkers was promising, EPC identity was not thoroughly confirmed [99]. As a result, various studies in the next decades followed, trying to obtain a population of true EPCs. Since then, a variety of different cell types has been named EPCs because the theoretical definition that "EPCs are able to differentiate towards endothelial cells and contribute to the formation of new blood vessels" [100] lacked specificity. Until today there is considerable confusion about the definition of EPCs since various cell types emerged in scientific literature due to unresolved issues with EPC identity and characterization. However, it is now generally accepted that basically two different approaches to study EPCs exist that result in distinct cell populations. Flow cytometry-based assays of blood samples are using CD34, VEGFR, and sometimes CD133 [101] to identify mononuclear circulating EPCs. However, data are controversial on the use of CD34 and CD133. Regarding cell culture-based methods to isolate EPCs, there is now consent that two distinct populations are obtained. Originally, they were referred to as early or hematopoiectic and late or nonhematopoietic EPCs. Later, the name circulating angiogenic cells (CACs) was replaced by the more common name myeloid angiogenic cells (MACs) for early EPCs. These spindle-shaped cells appear within one week in culture and show AcLDL uptake, isolectin binding, and VEGFR2/CD31 expression. Although they are not able to become endothelial cells they promote angiogenesis through paracrine mechanisms [102]. The other known subtype, formerly referred to as late EPCs, nonhematopoietic EPCs, or blood outgrowth endothelial cells (BOECs), are commonly known as endothelial colony forming cells (ECFCs) today. Appearing within four weeks of culture they show a cobblestone-like morphology together with a high proliferation potential. Many studies confirmed a high expression of the endothelial markers VE-cadherin, vWF, CD31, CD36, CD105, CD146, VEGF2, and Tie2 as well as they were described negative for the haematopoietic markers CD45 and CD14 [103]. ECFCs have an intrinsic angiogenic capacity, can contribute to vascular repair of injured endothelium and *de novo* blood vessel formation [104]. Several preclinical studies could demonstrate a therapeutic potential of ECFCs, including endothelialization of cardiovascular grafts to ensure antithrombogenicity and vascular patency *in vivo* [105]. Transplanted in mice, the ECFCs could self-assemble into long-lasting microvascular networks that anastomosed with the host vasculature. At present, only ECFCs are referred to as true EPCs and are recommended for use in vascularization of engineered tissues [99, 106, 107].

Angiogenetic Sprouting

Signalling and Molecules in Angiogenic Sprouting

For stable and long-term *in vivo* survival, tissue-engineered grafts need vascular ingrowth to ensure nutrient and oxygen supply as well as the removal of metabolic waste products [108, 109]. Angiogenic sprouting is defined as the branching of new blood vessels from existing ones mainly by migration and proliferation of endothelial cells from pre-existing vessels in the direction of hypoxic perfused tissue, a fundamental process for vertebrate development, tissue maintenance and repair [110]. Several types of specialized endothelial cells are required during vessel branching: polarized tip cells with their spike-like filopodia participate in the initiating events, highly proliferative stalk cells elongate the stalk of the sprout and wall-like phalanx cells are engaged in the perfusion of the newly formed branch [111]. In response to stimuli from growth factors, extracellular matrix (ECM) proteins, and integrins, the respective vascular cells migrate, proliferate, and assemble into a vascular loop in order to establish a perfused vessel branch [112]. The process occurs in a series of tightly-regulated steps, which are discussed henceforth in the context of the molecules and signalling mechanisms that guide them (Fig. 1).

The first phase of angiogenic sprouting is the permeabilization of the parent vessel wall in the direction of hypoxic tissue to give migrating and proliferating ECs access to the connective tissue [113]. To accomplish this, various classes of pericellular proteases lyse through the endothelial basement membrane [114]. Among the broad spectrum of proteases in this context well-studied are matrix metalloproteases (MMPs) and serine proteases.

In vertebrates, twenty-three different MMPs have been identified [115]. Mature ECs synthesize only little or no active MMPs, but their production is strongly induced in capillary sprouts [116]. Notably, proteases are also important for the activation and modification of pro-angiogenic growth factors and receptors that usher ECs into the next step of angiogenesis. While MMPs are typically secreted, considerable data suggest that the plasma membrane-bound membrane-type matrix metalloproteases (MT-MMPs) also play a critical role in EC tubular morphogenesis in three-dimensional extracellular matrices [117]. MT1-MMP has been reported to be an especially important regulator of angiogenesis [114, 116, 118]. A serine protease that plays a crucial role in the degradation of endothelial ECM, the urokinase-type plasminogen activator (u-PA), is part of a complex system of serine proteases that is strongly involved in angiogenesis [119]. In 2014 Stojkovic and co-workers reported that u-PA gene and protein expression in human ECs can be upregulated by interleukin (IL)-33 whereby vessel sprouting and the formation of tubular structures was induced [120]. Also fibroblast growth factors (FGFs) promote EC migration by inducing the synthesis of ECM degrading enzymes such as collagenase and uPA [112, 121].

The second and most significant phase of angiogenic sprouting comprises the weakening of EC-EC contacts, EC

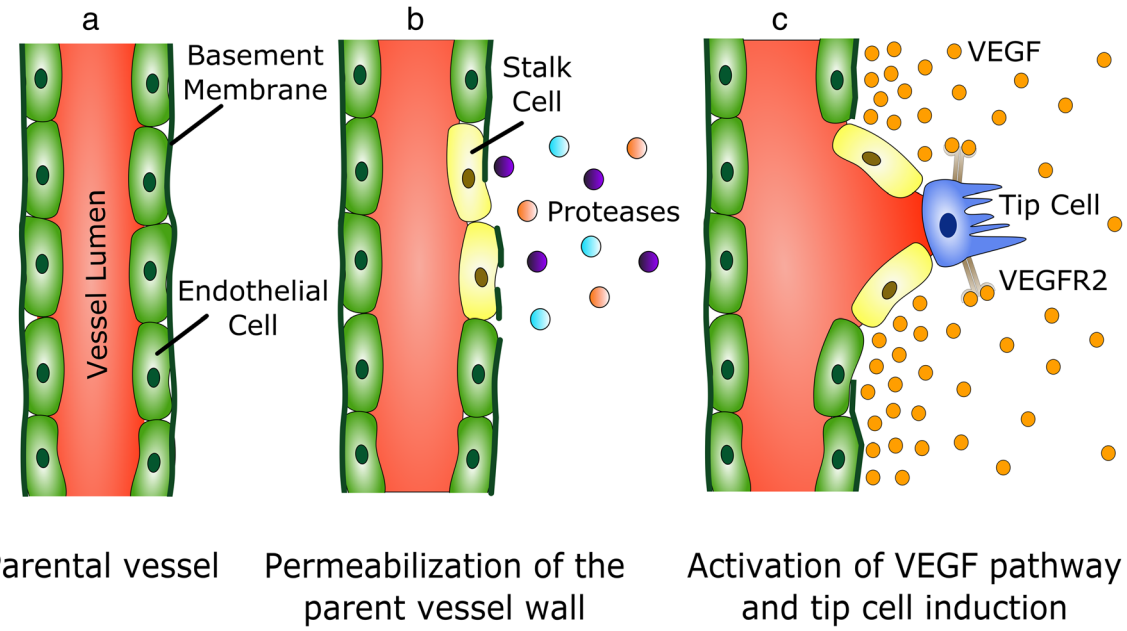


Fig. 1 Angiogenic sprouting gets initiated by the permeabilisation of the parent vessel wall (**a**) by various proteases (**b**) like matrix metalloproteinases or serine proteases in the direction of hypoxic tissue. This gives the migrating and proliferating ECs access to the connective tissue and paves the way for the second and most significant phase of angiogenic sprouting. It results in a weakening of EC-EC contacts, EC migration through the basement membrane, and proteolysis of the stromal ECM accompanied by proliferation and migration. The activation of the VEGF pathway through VEGFR2 finally induces the tip-cell phenotype in the leading ECs which then migrates up the VEGF gradient (**c**)

migration through the breach in the basement membrane, and proteolysis of stromal ECM accompanied by their proliferation and migration [113]. All of these processes involve vascular endothelial growth factor (VEGF) signalling [122]. Its pro-angiogenic effect is primarily mediated via binding to the tyrosine kinase receptor vascular endothelial growth factor receptor 2 (VEGFR2) [110]. VEGFs are essential regulators of both, vasculogenesis and angiogenesis. VEGF ligands can bind to VEGFR1 to 3 and to co-receptors like heparin sulphate proteoglycans (SPGs) and neuropilins [123]. VEGF binding signals the disassembly of the VE-cadherin/beta-catenin complex at adherens junctions leading to disruption of the endothelial cell-cell contacts [124]. Activation of the VEGF pathway through VEGFR2 binding induces the tip-cell phenotype in leading endothelial cells [125]. These tip cells migrate up the VEGF gradient generated by hypoxic tissues [126]. VEGFR1 is associated with negative regulations of the developing tube but is not required for their development. Furthermore, VEGFR1 has been reported to negatively influence VEGFR2 signaling, while others suggest VEGFR1 promotes VEGFR2 activity in pathological angiogenesis [127, 128]. Stalk cells respond to the local VEGF concentration, elongating and proliferating behind the migrating tip-cells to form a solid tube of cells that extends from the parent vessel [129].

The netrin, semaphorin, ephrin, and slit families of molecules regulate sprout navigation as well [130]. Signalling from binding of the guidance molecule netrin-1 to the endothelial transmembrane protein CD146 (also known as melanoma cell adhesion molecule, MCAM) promotes angiogenesis and vascular development in vertebrates [131]. Plexin-semaphorin interactions have been shown to mediate sprouting angiogenesis [132]. To activate their receptor, the Eph receptor tyrosine kinase, ephrins typically have to be membrane-bound [122]. Therefore the transmembrane protein Ephrin receptor ligand Ephrin-B2 is a cell contact-dependent sprout guidance molecule [133]. It drives further remodelling and maturation of the vascular endothelium [122]. There is emerging evidence that interactions of the slit family of secreted proteins with the roundabout (Robo) receptors also play a role in angiogenesis [134]. However, whether Robo/Slit signalling has a pro-migratory or repulsive effect on EC migration remains debated [111].

Another growth factor important for the regulation of angiogenic vessel formation is angiopoetin 1 (Ang-1) [135]. Through binding to the associated receptor tyrosine kinase Tie-2, Ang-1 stimulates EC migration and cytoskeletal reorganization in the absence of cell-cell contacts [136]. On the other hand, Ang-1/Tie-2 signalling promotes the recruitment of pericytes and tightening of the interactions between ECs and the ECM in the presence of cell contacts, whereby the vascular integrity is maintained [112].

Notch signalling plays a central role in regulating sprout formation. Notch signaling in angiogenesis is conducted primarily through Notch1-Dll4, and Notch1-Jagged 1 signalling [137]. Cross-talk between Notch1-Dll4 and VEGF establishes an adequate tip cell – stalk cell ratio for organized vascular patterning [138]. Dll4 is upregulated in response to increased VEGF concentration and tip cell activation, then binds to Notch1, which decreases VEGFR2 expression, decreasing sensitivity to VEGF gradients and thereby promoting a stalk-cell phenotype [110, 139]. Notch1-Dll4 signaling helps inhibit excessive tip-cell activation, and thus bars rampant sprouting in the same area from the parent vessel as well as branching of a sprout [110, 140]. Notch1-Jagged 1 signalling inhibits Notch1—Dll4 signalling and thus promotes sprouting and branching [141].

In developing vessels, transforming growth factor beta (TGF beta) signalling can either induce a pro-angiogenic response or an inhibitory one [142]. This is imputed to interactions between TGF beta and the receptors activin receptor-like kinases 1 and 5 (ALK 1 and 5), where each type elicits an opposing response [143]. It has been proposed that high, prolonged amounts of TGF beta stimulation results in ALK 5 signalling dominating, fostering ECM production, quiescence, and differentiation along mural-cell lineages such as pericytes and SMCs. On the other hand, low or moderate amounts of TGF beta exposure allow ALK 1 signalling to dominate, which impedes maturation and differentiation engages migration and proliferation [144]. Bone morphogenetic proteins (BMPs) are also agonists to ALK receptors and have similarly been reported to exert pro- as well as anti-angiogenic effects [145].

Eventually, the sprout will come into contact with a sprout from another vessel and the two will anasmatose via tip-cell filopodia interactions [146]. Stalk cells will proliferate further to form a complete lumen, ECs will secrete a basement membrane about the vessel, and pericytes will begin to associate with the vessel [113]. During vessel maturation, endothelial cells express a variety of cell adhesion proteins on their cell surface in order to restore a tight vessel wall that functions as selective barrier, the retentiveness of which has given these mature endothelial cells the title of phalanx cells [122]. In addition to the aforementioned TGF-beta, BMP, and Ang1-Tie signalling, interaction of ECs with interstitial matrix proteins during lumen formation and tubular morphogenesis is mediated through several integrins differing in their ligand binding specificity [117, 147]. Adherens junctions mediating cell-cell adhesion between ECs are predominantly formed by vascular endothelial (VE)-cadherin [148]. Cadherin-based adherens junctions are connected to the actin-microfilament system of adjacent cells via catenins [122]. It is suggested that VE-Cadherin, alpha-catenin, and beta-catenin form a complex at early stages of intercellular adhesion between endothelial cells [149].

Taken together signalling in angiogenic sprouting is a complex assembly of well orchestrated events that are thightly controlled through a variety of factors. Therefore, the main challenge is to fully understand these complex biological systems and to mimick it in detail in order to develop a functional vascular graft.

Assays to Study Angiogenesis

Despite the immense progress in understanding blood vessel development there still exists no 'gold standard' assay that adequately fulfills all important demands such as rapid assessment, quantification and measurability, reproducibility, and comparability with the *in vivo* situation at once. Therefore a proper selection or combination of multiple assays is required in order to answer one particular scientific question [150]. The following part summarizes the most common models that are currently used to study angiogenesis.

In vitro *Angiogenesis Assays*

In vitro angiogenesis assays are typically performed to study EC behavior within a well-defined microenvironment and to observe interactions of ECs with non-endothelial cell types which are thought to exert important paracrine regulatory effects during sprouting [151]. Figure 2 depicts an overview about the different types of assays.

In general, *in vitro* assays offer good reproducibility, precision, and a tight control of the components participating in the angiogenic process [150]. Unfortunately, most assays recapitulate only a few stages of angiogenesis and do neither resemble vessel morphology nor physiological aspects *in vivo* [152]. Cell proliferation assays aim to determine the ability of ECs to respond to chemical, biological, and mechanical stimuli by quantifying the rate of cell growth e.g. *via* cell counting and diverse viability assays. Migration assays provide information regarding the ability of intrinsic or extrinsic regulators to promote or inhibit EC migration. Frequently used are scratch assays (Fig. 2a) and transmembrane/Boyden chamber assays (Fig. 2b). For performing a tube formation assay (Fig. 2c and d) ECs are seeded on 2D or within 3D basement membrane compounds (fibrin, collagen, Matrigel™) and the formation of capillary-like structures is monitored over time [153]. Vessel formation is quantifiable by counting length and number of formed tubes and branches. Since they are easy to perform, rapid, reliable, and sensitive to composition and mechanical properties of a specific ECM structure, tube formation assays are the most widely used *in vitro* assays to examine sprouting angiogenesis.

Ex vivo *and* in vivo *Angiogenesis Assays*

Studying angiogenesis requires a reliable, physiologically relevant, and technically straightforward assay [154]. *Ex vivo* assays bridge the gap from cell-based assays that can not reflect the whole complex process of angiogenesis, to time-consuming and expensive *in vivo* assays. The latter recapitulate the entire spatiotemporally controlled angiogenic program to establish the physiological organization of the vascular network or vessels characteristic for certain vascular diseases. Figure 3 gives an overview of commonly performed *ex vivo* and *in vivo* assays.

Ex vivo models such as the retina-based angiogenesis assays (Fig. 3a) are mimicking appropriately aspects of retinopathy of prematurity, proliferative diabetic retinopathy, and age-related macular degeneration [148, 155]. Like vascular explants such as the aortic rings assay (Fig. 3b) also retinal explants mimic the natural heterotypic microenvironment of sprouting vessels more precise than isolated ECs alone [155, 156]. The native spatial organization of ECs, supporting cell types, and ECM molecules as well as endogenously generated growth factor gradients are represented in those models [150, 157]. Eye and aortic explant assays are matrix invasion assays investigating tube formation. Polymeric scaffolds or Matrigel™ plugs containing pro- or anti-angiogenic factors are subcutaneously implanted into rodents and analysed *ex vivo* [158, 159]. Immunohistochemical analysis of explants provides beneficial information about host tissue response feedback mechanisms. Nevertheless, the influence of blood flow, shear forces, and blood pressure cannot be studied [148]. Moreover, they are usually not derived from the organ that is actually targeted [159]. Therefore, *ex vivo* assays should rather be considered as link between *in vitro* and *in vivo* assays that combines the benefits of both, accuracy and physiological relevance.

An *in vivo* model is the hindbrain assay in rodents (Fig. 3c). Due to the ease of access to the femoral artery and the low mortality rate, the murine hindlimb ischemia is considered as a powerful tool for preclinical testing of new therapies for peripheral artery disease [160]. Due to their small size, optical transparency, and high number of offspring, zebrafish are a favored *in vivo* model used in vascular biology (Fig. 3d) [148, 150]. Live cell imaging using time-lapse microscopy enables easy, rapid, and quantitative analysis of angiogenic processes. The chick chorioallantoic membrane (CAM) assay (Fig. 3e) is another well-established *in vivo* angiogenesis assay typically used for the validation of *in vitro* observations about molecular mechanisms of tumor-associated neovascularization and preclinical screening of novel anti-angiogenic agents [161].

To complete this chapter, vasculogenic *in vivo* assays should be mention here. The most common ones in this category are Matrigel®-based plug assays. Matrigel® provides a

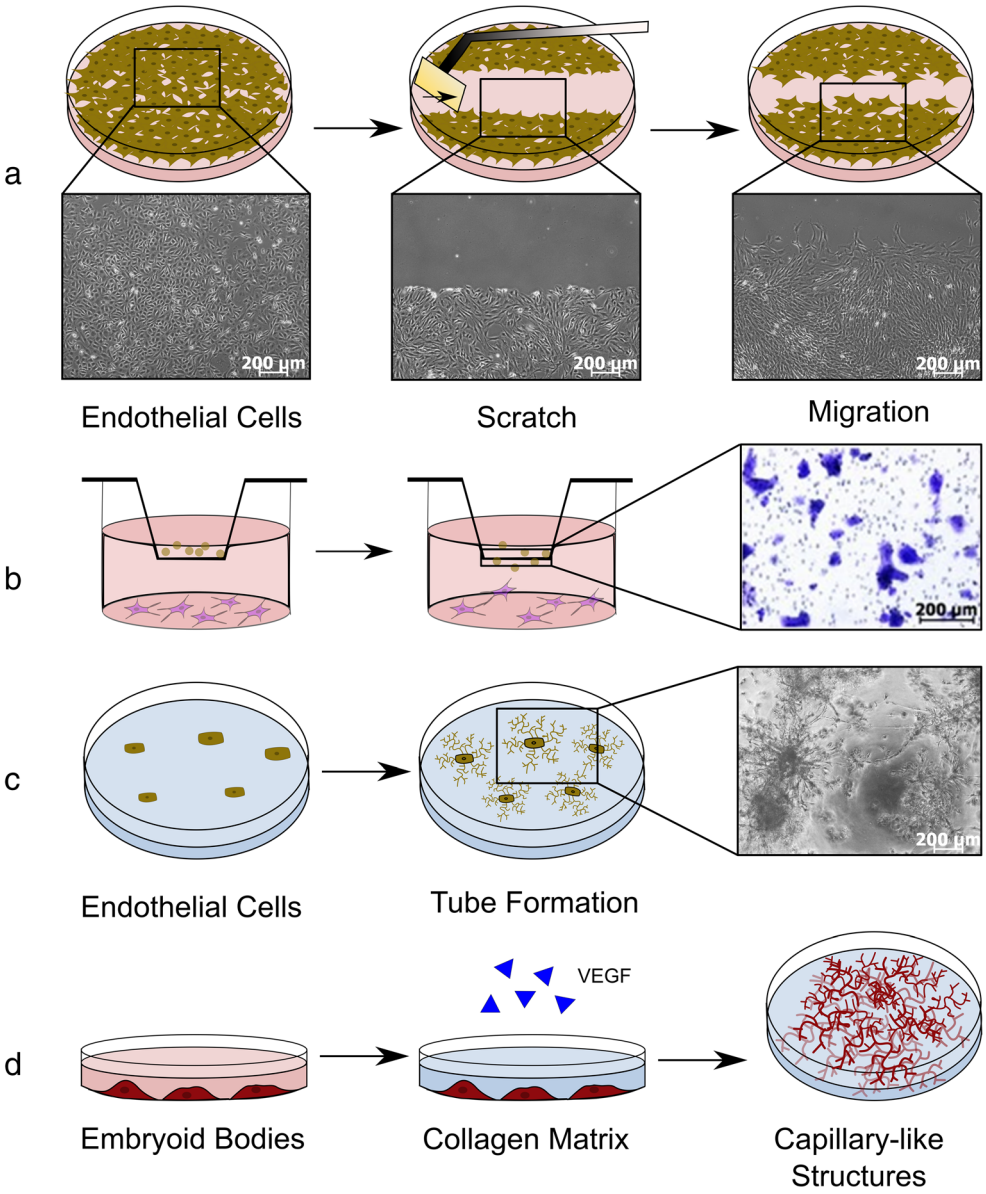
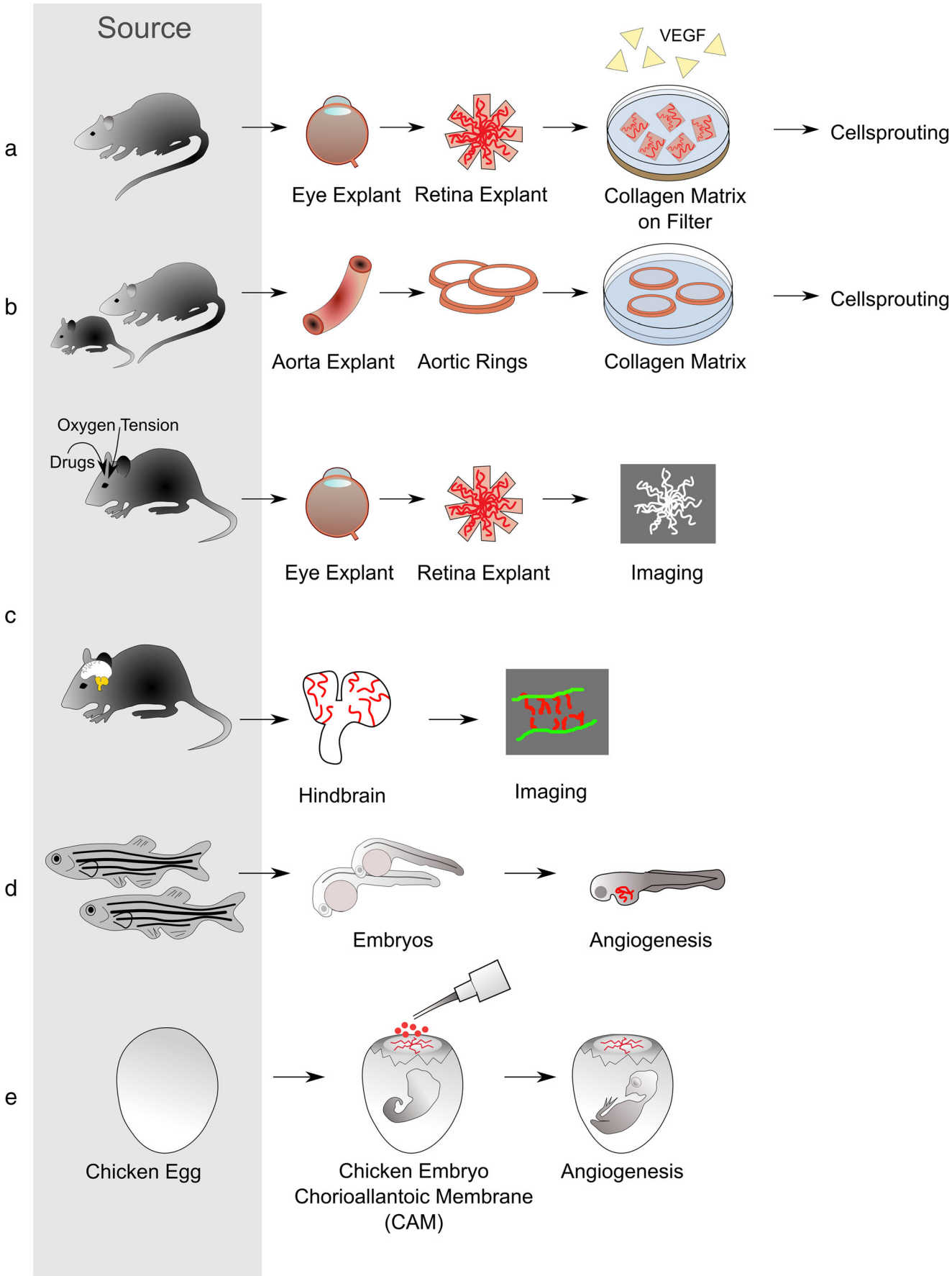


Fig. 2 Frequently used *in vitro* angiogenic sprouting assays to study EC behavior during sprouting angiogenesis. Common are wound or scratch assays (**a**) as well as a Boyden chamber (**b**) to investigate migration of the ECs. In a tube formation assay (**c**, **d**) ECs are seeded on a 2D or 3D matrix (e.g. Matrigel™) and the formation of capillary-like structures can be monitored. *Micrographs of wound assay and boyden chamber membrane kindly provided by Patrick Babczyk*

natural environment for endothelial cells introduced into the matrix. Following subcutaneous injection the liquid solidifies and permits penetration by host cells that induce vascularization. Evaluation of a Matrigel® plug assay is achieved by determination of the hemoglobin content or by histological assessment *via* blood vessel staining and subsequent determination of vascular density [158]. Matrigel® plug assays are reproducible and easy to implement and thus are routinely performed [162]. Malero-Martin and Bischoff reported an adapted version of the Matrigel® plug assay which utilized the matrix to deliver human blood-derived EPCs and mature SMCs subcutaneously into mice. One week after implantation a microvascular network was formed that contained host erythorcytes indicating that a *de novo* vascular network was formed and functional anastomoses with the host circulatory system developed [163].

Computational Angiogenesis Assays

For the clinical applicability of vascular grafts it is important to understand how sprouting angiogenesis within a porous scaffold can be controlled [164]. Since *in vivo* sprouting experiments are not only time-consuming but also expensive, *in silico* models have become more and more a powerful predictive tool that allows rapid screening of potential biomaterial designs for vascular tissue engineering [110, 165]. For example for the description of cell migration, cell-matrix and cell-cell interactions many simulations combine discrete modeling of single cells with continuous modeling of the ECM and soluble factors resulting in hybrid models that include mechanical laws [109]. Van Oers and colleagues proposed a hybrid computational model based on biomechanical interactions between ECs and the ECM [166]. They could show that


Source
a
VEGF
Eye Explant
Retina Explant
Collagen Matrix on Filter
Cellsprouting
b
Aorta Explant
Aortic Rings
Collagen Matrix
Cellsprouting
Oxygen Tension
Drugs
c
Eye Explant
Retina Explant
Imaging
Hindbrain
Imaging
d
Embryos
Angiogenesis
e
Chicken Egg
Chicken Embryo Chorioallantoic Membrane (CAM)
Angiogenesis

◀ **Fig. 3** *Ex vivo* and *in vivo* assays to study angiogenesis. *Ex vivo* vascular explants such as retinal explant cultures (**a**) and aortic rings (**b**, upper row) mimic the natural heterotypic microenvironment of sprouting vessels more precise than isolated ECs. Polymeric scaffolds or Matrigel™ plugs containing pro- or anti-angiogenic factors are subcutaneously implanted into mice and immunohistochemical analysis of the explants (**b**, lower row) provide beneficial information about host tissue response feedback mechanisms. A powerful tool for preclinical testing of new therapies for peripheral artery disease is murine hindlimb ischemia (**c**). Another favored model for vascular biology is the zebrafish (**d**) because of its small size, transparency, and its high numbers of offspring. Chick chorioallantoic membrane (CAM) assay (**e**) is a well-established *in vivo* angiogenesis assay which is typically used for the validation of *in vitro* observed molecular mechanisms of tumor-associated neovascularizations and preclinical screening of novel anti-angiogenic agents

a set of assumptions mimicking mechanical cell-cell communication *via* ECM is sufficient to recapitulate the collective EC behavior during sprouting and network formation. In summary, computational models should be regarded as complementary concept to experimental approaches that very likely accelerates scientific progress in particular in the field of vascular biology.

Graft Materials for Vascular Tissue Engineering

Today a large variety of advanced materials for diverse blood vessel engineering strategies exists and is still increasing [167]. Vascular grafts aim to replicate the functionality of the natural endothelium [168]. To do so, graft material design has to meet some fundamental requirements which constitute a biocompatible chemical composition, an adequate architecture, and mechanical and degradation properties according to the application as well as an appropriate surface topography [169]. A lot of progress has been made in the development of advanced biomaterials to provide a tailored microenvironment that not only promotes the ingrowth of vascular cells, but also stimulates ECM production and prevents thrombus formation [170]. However, despite rapid progress in the field of engineering native vessel-like grafts, several of the currently applied biomaterials face diverse drawbacks especially with respect to insufficient cell seeding and distribution, immune-rejection, and inflammatory reactions, as well as the threat of mechanical failure under physiological conditions [171, 172]. Generally, vascular graft materials can be classified as either natural, derived from a biological source, or biosynthetic, incorporating biological and man-made elements, or pureley synthetic matrices [173]. The ultimate graft material does not exist, it needs to be chosen according to its application. While natural graft materials might mimic their natural model the best, decellularization can have a negative impact on (bio)mechanical behavior, biocompatibility, and immunogenic potential. Their longer leadtime makes them not readily available, which needs to be considered as well. Synthetic graft materials are highly flexible by adopting their physical properties. However, their low patency rates, a restricted biocompatibility, and a time-consuming population need to be considered. If synthetic grafts are used without cells, they are available on demand because of their long shelflife. Biosynthetic grafts combine both advantages but also disadvantages of natural and synthetic materials. However, the materials within the different classes can still differ a lot in terms of their origin and fabrication. Prominent examples of each class are discussed henceforth and are summarized in Fig. 4. Important facts (such as the used grafts and cells or the results) of the cited *in vivo* studies in the following sections are provided at a glance in Table 1.

Natural Vascular Grafts

Tissue-derived vascular grafts such as decellularized ECM originating from different species, collagen, silk fibroin, and fibrin are widely used materials to promote vascular healing (Fig. 4). Compared to fresh allografts, decellularized vein allografts displayed satisfactory mechanical stability, minimal evidence of antigenicity and a recolonization with smooth muscle alpha-actin positive stained cells [174]. The decellularized bovine ureter SynerGraft® model 100 was reported to be an appropriate vascular graft alternative when autologous vein is not available [175]. However, residual cells and xenoantigens *e.g.* galactose-alpha-1,3-galactose in the decellularized bovine ureter were described to be responsible for inflammatory reactions that might lead to xenograft failure [176]. The decellularization process itself was furthermore shown to have an impact on cellular behavior. An aggressive decellularization for example was shown to induce a shift in the macrophage phenotype from M1 to M2 *in vitro*. In fact this shift was not evident *in vivo* but remarkable differences were found with regard to the spatial distribution of M1 versus M2 macrophages within various decellularized scaffolds of porcine origin [177]. For repopulating decellularized scaffolds stem cells are of great interest. In one instance, decellularized human arteries were repopulated by cells expressing the stem cell markers CD34 and SSEA. Following implantation in a murine graft model the transplanted stem cells subsequently expressed mature vascular cell markers such as CD31, calponin, and myosin heavy chain [178]. Besides stem cells, primary cells can be used for repopulating decellularized scaffolds. When creating a tissue-engineered blood vessel, repopulation of endothelium-denuded human umbilical veins (HUVs) appears to be superior to decellularized HUVs since the nuclease treatment during decellularization resulted in partial removal of ECM components and substantially altered surface properties [179].

Collagen is the main structural component of various connective tissues in mammals and is composed of triple-helical

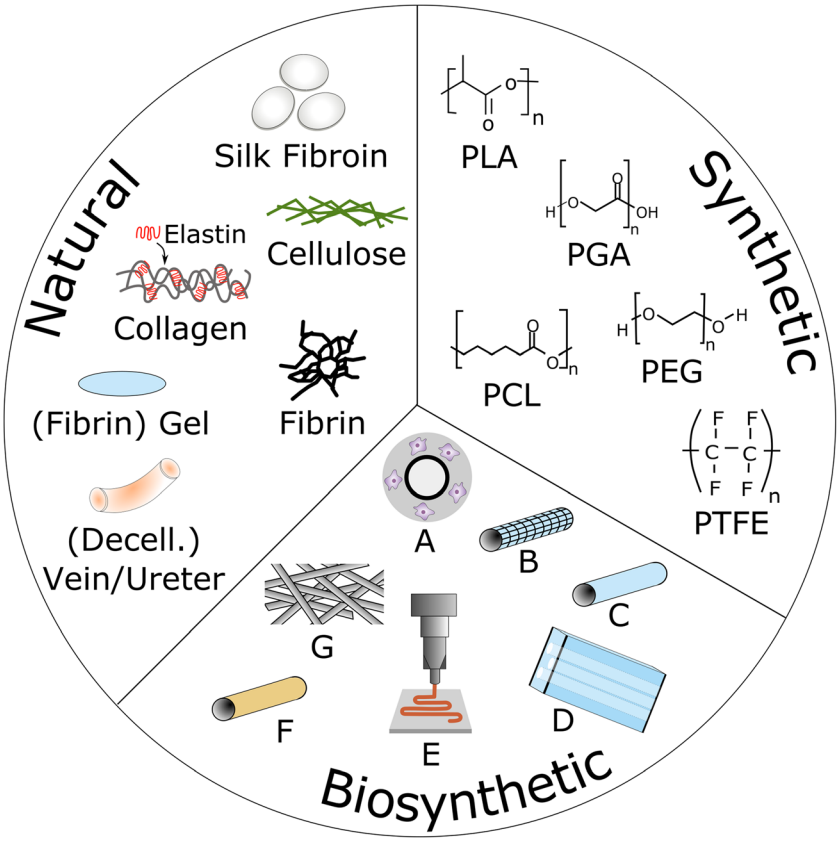


Fig. 4 Graft material used in vascular tissue engineering. Natural materials used for vascular grafts contain decelluarized ECM such as collagen, silk fibroin or fibrin. Furthermore, decellularized vein allografts display satisfactory mechanical stability, minimal evidence of antigenicity and a recolonization with smooth muscle cells. Besides natural, synthethic materials are an attractive alternative due to their flexibility, mechanical strength and stiffness. Combining natural and synthetic materials (biosynthetic) opens more possibilities for tailoring the grafts properties to suit the needs of a particular application. Possible materials are collagen layers containing cells and an acellular support sleeve (A), fibrin-based scaffolds (B), nanostructured polyurethane blended with gelatin (C), microchannels in methacrylated gelatin (D), 3D printed scaffolds (E), hybrid-meshes (F) or heparin-functionalized polymers (F)

fibrils. It is also found in the ECM of blood vessels [180]. Due to their good cell adherence properties, low immunogenicity and robust tensile strength, collagen-based scaffolds are ideally suited for the fabrication of vascular grafts [181]. Huynh and colleagues reported that an acellular collagen graft material derived from porcine submucosa of the small intestine and bovine type I collagen successfully integrated into the host tissue and served as scaffold for restoring a functional blood vessel in a rabbit arterial bypass model [182]. Another hybrid construct made of collagen with incorporated elastin displayed an improved solid elastic mechanical behavior compared to control constructs composed of collagen only [183]. Despite these promising studies collagen-based grafts are not convenient for every clinical application. Since collagens are often applied in gel-like condition which lacks mechanically stability, they are contraindicated for applications such as in the coronary system. Furthermore, prion proteins can be an issue if xenogenous collagen is used. Next to others, a study performed by Manduz and coworkers revealed that the bovine mesenteric vein graft ProCol® should not be recommended for infrainguinal arterial reconstruction due to its low primary patency rates and high risk for graft failure in this context [184, 185].

Silk fibroin, a semicrystalline fibrous biopolymer, offers the necessary mechanical properties along with controllable biodegradation and biocompatibility [186]. Its processing by electrospinning provides an attractive opportunity for producing nanofibrillar matrices for small calibre vessel regeneration. Electrospun silk fibroin nanofibres displayed good cytocompatibility and a mean compliance value higher than the values reported for both Goretex® and Dacron® which makes them a suitable graft material [187]. The mechanical fortitude facilitates the application of mechanical stimuli. In another study, tubular electrospun silk fibroin scaffolds were sequentially seeded with human coronary artery SMCs and human aortic endothelial cells and cultured under dynamic flow conditions. With respect to cell proliferation and alignment, ECM production and cell phenotype, tissue engineered vascular grafts under physiological flow had a better outcome relative to those cultivated under static conditions [188]. Blending and drying of silk fibroin with noncrystallizable tropoelastin resulted in a protein-based biomaterial that

Table 1 Summary of graft types, cells, model systems, and results used in the cited in vivo studies

Graft	Cells	Model system	Results	Reference
small diameter graft; jugular veins as -fresh allograft -fresh allograft -decell. Allograft (SDS)	none	canine	-no ruptures or aneurysm in any group -luminal narrowing in both allografts -fresh allografts: significant mononuclear cell infiltrate, intimal hyperplasia, intramural hemorrhage consistent with rejection -decell. Allografts: minimal evidence of rejection, but compact fibrin layer along lumen -repopulation (α-SMA+) of decell. Allografts by 8 weeks -decell. Allografts exhibit satisfactory strength, reduced antigenicity compared to fresh allografts, support of cellular repopulation	[173]
haemodialysis access graft; -decellarized bovine urether (SynerGraft Model 100)	none	human	-mean time of occlusion (19 events): 215± 141 days with patency re-established in 14 of 18 surgical interventions −30 angioplasties performed on 14 grafts for luminal/anastomotic stenosis −2 grafts: areas of dilatation, but still usable after 930 and 602 days with no further changes in graft size -primary patency, assisted primary patency, secondary patency, and freedom from infection: 29, 45, 81, and 95% at 1 year	[174]
complex venous access graft; -decellularized bovin ureter (SynerGraft)	none	human	-aneurysmal dilatation (3 grafts) -thrombosis (2 grafts) --> acute and chronic transmural inflammation (residual xenooantigens?)	[175]
decellularized porcine small intestinal ECM	none	glirine	-aggressive decellularization associated with shift from M1 to M2 macrophages (in vitro, but not quantitatively in vivo) -different distribution of M1 vs. M2 phenotype within scaffolds (in vivo)	[176]
decellularized human left internal mammary artery branches as grafts	none	murine (mouse)	-repopulation by cells expressing stem cell markers (CD34 and SSEA); markers of mature ECs and SMCs (CD31, calponin, MHC) -migratory capacity of cultured cells was sign. Higher than mouse SMCs	[177]
small diameter grafts; collagen derived from submucosa of small intestine and type I bovine collagen	none	leoprine	-excellent hemostasis and patency -within 3 month post implantations grafts were remodeled into cellularized vessels exhibiting physiological activity in response to vasoactive agents	[181]
infrainguinal reconstruction with a bovine mesenteric vein graft (ProCol)	none	human	-primary patency rate 0% at 3 month, with graft failing between 4 and 113 days -aneurysmal dilatation (2 grafts)	[183]
infrainguinal reconstruction with a bovine mesenteric vein graft (ProCol)	none	human	-graft thrombosis (2 patients) on postoperative day 1 (28.5%) -shortest patency 6 month, longest 18 month -aneurysmal dilation in 2 grafts (28.5%) after 12 and 18 month, respectively	[184]
microvascular grafts; silk fibroin tubes	none	murine (rat)	−4-week time period: graft patency and endothelial lining of lumen surfaces	[185]
fibrin gel with entrapped cells	human dermal fibroblasts	murine (rat), ovine	-after 7–9 weeks bioreactor culture grafts were extensively remodeled by the fibroblasts into circumferentially-aligned tubes of collagen and other ECM with burst pressures of 1400–1600 mmHg and compliances comparable to native arteries	[189]

Table 1 (continued)

Graft	Cells	Model system	Results	Reference
			-tissue suture retention force was suitable for implantation in rat model and (with PLA sewing rings attrapped at both ends) also in ovine model	
fibrin matrix out of thrombin from a single donor	none	human	-sufficient amount of thrombin can be obtained from a single donor to create a fibrin matrix of high efficiency without the risk of immunological and infectious side effects	[190]
decellularized engineered allografts; fibrin gel tubes cultured with cells (5 weeks) and decellularized afterwards	ovine dermal fibroblasts	ovine	-at 8 ($n = 5$) and 24 ($n = 4$) weeks all grafts were patent showing no evidence of dilatation or mineralization -mid-graft lummen diameter was unchanged -extensive recellularization occured (most cells expressing alphaSMA) -endothelialization was complete by 24 weeks (elastin deposition evident)	[192]
small-diameter grafts as carotid artery grafts; tubular hydrogels from bacterially synthesized cellulose	none	ovine	-bursting strength postoperative approx. 800 mmHg, suture retention strength 4–5 N -patency rate of 50% (n = 5), physiological performance of patent grafts at 4, 8, and 12 weeks postoperative comparable to native arteries -neoformation of vascular wall-like sturcture along the BC scaffold (immigrated vascular SMCs), homogenous endothelialization of inner graft surface without signs of proothrrombogenic or inflammatory potential	[193]
TE vessels grown from allogenic cells in biomimetic perfusion system; afterwards decellularization + seeding with recipient cells	-allogenic porcine SMCs -endothelial progenitor cells or endothelial cells from recipient	porcine	-all EPC and EC seeded grafts remained patent for 30 days whereas controls were patent in only 3/8 implants -EPC and EC seeded grafts showed less neointimal hyperplasia and fewer proliferating cells -proteins in mammalian target of rapamycin signaling pathway tended to be decreased in TEV	[196]
sheet created from iPS cell-derived vascular cells	male mouse iPSCs differentiated into embroid bodies by hanging drop method (SSEA-1+), EC and SMC marker positive	murine (mouse)	-all mice survived without thrombosis, aneurysm formation, graft rupture, or calicification -endothelialization (von Willlebrand+) and inner layer of SMCs (actin+, calponin+) at 10 weeks -number of seeded differentiated iPSCs decreased over time (42.2% week 1; 10.4% week 4; 9.8% week 10) -fraction of iPSCs Y-chromosome fluorescent positive at 1 week -no iPSCs co-localized with von Willebrand+ or SMC-actin+ cells at 10 weeks	[205]
macroporous electrospun PCL scaffolds as rat abdominal aorta graft	none	murine (rat)	-enhanced cell infiltration and ECM secretion -all grafts showed satisfactory patency for up to 100 days -complete endothelium coverage at day 100 and correctly organized SMC layer with abundant ECM similar to native arteries -arteries demonstrated contractile response to adrenaline and acetylcholine-induced relaxation -thicker-fiber scaffolds induced a large number of M2 macrophage infiltration into graft wall which further promoted cellular infiltration and vascularization	[206]

Table 1 (continued)

Graft	Cells	Model system	Results	Reference
large pore grafts from PLA coated with PLCL small pore grafts from electrospun PLA as infra-renal aortic interposition conduit	none	murine (mouse)	-large pore grafts induced well-organized neointima after 12 month -small pore grafts showed neointimmal calicification in thin neointima -macrophage infiltration and few vascular SMCs were observed in the thin neointima of the small pore grafts at 12 month -neointima of large pore grafts was composed of abundant vascular SMCs and a lower density of macrophages -large pore graft SMCs expressed transcription factors of both osteoblasts and osteoclasts	[207]
microporous PCL scaffold modified with collagen and MAP-RGD as rabbit carotid artery graft	none	leoprine	-MAP-RGD coating reduced possibility of early graft failure and enhanced re-endothelialization by in situ recuritment of EC/EPC (patency rate: 2/3) -endothelialization prior to implantation aggravated the formation of thrombosis and/or ICH (patency rate: 0/3)	[208]
PVDF-based textile fibres with incorporated superparamagnetic iron oxide nanoparticles molded with a mixture of fibrin and cells, then endothelialized in vitro before implanting as arteriovenous shunt between carotid artery and jugular vein	fibroblasts, SMCs	ovine	-grafts were biocompatible and functional -efficient endothelialization and endogenous neo-vascularization within the vessel wall	[218]
small caliber vessel grafts; fibrin scaffold supported by P(L/D)LA 96/4 mesh, seeded with cells as carotid artery graft	autologous arterial-derived cells	ovine	-significant stenosis in one explant after 3 month -complete absence of thrombus formation on luminal surface, with no evidence for aneurysm formation or calcification after 6 month -remodelling of fibrin scaffold with mature autologous proteins and excellent cell distribution within graft wall -confluent monolayer of endothelial cells lining luminal surface (vWf and eNOS+)	[222]
small diameter graft; electrospun synthetic PCL and chitosan, heparin immobilization of graft	none	murine (rat)	-heparin was released from the graft for up to 1 month and improved the hemocompatibility (reduced platelet adhesion and prolonged coagulation time) (in vitro) -sustained release of heparin in vivo provided optimal antithrombogenic effect by reduced in thrombus formation and maintaining patency -heparin functionalization enhanced in situ endothelialization, preventing occurrence of restenosis	[225]

showed some mechanical properties comparable to those of native aortic elastin or elastin-like polypeptides and supported the attachment and proliferation of mesenchymal stem cells [189]. An alternative strategy to generate polymeric scaffolds that mimic native arterial walls is the use of fibrillary fibrin gels which are formed when thrombin is added to fibrinogen [170]. Since it can be produced from the patients' own blood, fibrin has a compatability advantage over other biopolymers [190]. The amount of autologous thrombin obtained from a single donor is sufficient to create an efficient fibrin matrix without causing immunological and infectious reactions that might occur if bovine thrombin is used [191]. Syedain and colleagues investigated human dermal fibroblasts entrapped in fibrin gel and cultured under pulsatile flow in a bioreactor. Their concept resulted in vascular grafts with circumferential alignment of cell-produced collagen along with burst pressures and compliance comparable to native arteries [190]. Similar effectiveness was achieved using cyclic mechanical stretching [192]. In another study ovine dermal fibroblasts were used for converting a fibrin gel into an aligned tissue tube following decellularization and implantation into the femoral artery of sheep. After implantation, comprehensive ingrowth of αSMA-positive cells occurred and complete endothelialization together with elastin fiber accumulation throughout the construct could be detected [193].

Recently other natural biomaterials have also been investigated. Gluconacetobacter-synthesized cellulose was used to fabricate small arterial substitutes in sheep. Postexplantation analysis revealed newly formed vascular wall-like structures composed of immigrated SMCs and a homogeneous endothelial cell layer lining the inner graft surface [194]. Norotte and coworkers successfully engineered vascular tubes of desired shapes and hierarchical tree-like structure by printing SMCs and fibroblast aggregates with distinct diameter (300–500 μm) in layers together with agarose rods, followed by fusion of the discrete units [195]. In a recently published study it has been reported that the sustained release of vascular endothelial growth factor from gelatin microparticles incorporated in human endothelial progenitor cells-laden bioprinted Matrigel™/alginate scaffolds (ratio of 3:1) resulted in an increased *in vivo* vessel formation [196]. Natural material scaffolds can even be fabricated by cells alone. Engineered connective tissues were grown from banked porcine SMCs using a biomimetic perfusion system and the tissues were subsequently decellularized. The remaining ECM was seeded with either endothelial progenitor or endothelial cells and implanted in the porcine carotid artery to provide a vascular graft that resisted both, clotting and intimal hyperplasia [197]. Culturing of iPSC-derived mesenchymal progenitor cells under pulsatile conditions caused layer formation of calponin-positive smooth muscle cells which were embedded in a collagenous matrix [198].

In summary, natural grafts can be divided into scaffolds composed of one or more natural material components of the ECM and decellularized scaffolds. About one third of the studies used natural ECM components, whereas the remaining two thirds took decellularized tissues for scaffolding. For the latter most commonly veins, arteries, urether, or intestine of bovine, porcine, and human origin were used. As natural scaffold materials the majority of the studies used collagen, followed by fibrin and fibroin manufactured either by electrospinning or in form of gels. Gelatin is the third most commonly used ECM component used for generating vascular scaffolds. A small minority of studies used agarose or cellulose.

Grafts that utilize single ECM molecules or combinations thereof mimick the extracellular matrix but are limited in mechanical strength and show a high degree of compaction [199, 200]. Those scaffolds can be produced in large quantities and could be stored which makes those scaffolds readily available in urgent cases. This is in contrast to decellularized grafts, which evolved as promising approach in tissue engineering since the native tissue architecture is preserved within those scaffolds. Especially in combination with a recellularization using patient-derived cells, particularly stem cells, decellularized scaffolds show a high potential in vascular tissue engineering since major drawbacks that can be found for scaffolds using donor cells, such as immune reactions, are low. Nevertheless, before considering the clinical use of decellularized vascular grafts, some difficulties need to be overcome: The decellularization process needs to be optimized, as for example detergents used for the process can have a substantial effect on the grafts mechanical and biochemical behavior and therefore may have an impact on the grafts biocompatibility or the immunogenic potential [200, 201]. Furthermore, in comparison to scaffolds made up of natural ECM components, decellularized tissues are not readily available and thus need a longer leadtime.

No matter if decellularized tissue or natural ECM component scaffolds, data on studies where acellular scaffolds has been used are rare. A vast majority utilized endothelial cells or their progenitors to populate the scaffolds, followed by smooth muscle and stem cells and their progenitors. Rarely, fibroblasts are used for (re-)populating scaffolds.

Recently, there has been considerable increase in research on decellularized pericardial tissue [202] used for bioprosthetic valve and total artificial hearts. By glutaraldehyde treatment, which effect on pig valves was discovered in 1968 by Alain Carpentier, the collagen gets prevented from denaturation and the immunological responses become reduced by masking the host antigens [203]. However, since glutaraldehyde-fixed biophrosthetic heart valves underwent calcification over time [204], calcium-mitigating adjuncts were added later [205] which resulted in near doubling of

the average graft durability [203]. In very young patients and children calcification remained an issue, so that heat treatment was successfully introduced to alleviate calcification [203]. Today, glutaraldehyde-treated tissue of several autologous or xenogenic origins are used for numerous applications, for example in valve repair and replacement, conduit, or patches [206, 207]. For detailed in-depth reading on heart valve replacements we recommend the review of Dijkman *et al*. [208].

Next to natural materials, the market is offering a wide range of synthetic polymers which are discussed in the next paragraph.

Synthetic Vascular Grafts

Synthetic materials are an attractive alternative to tissue-derived materials mainly due to the flexibility of adapting their physical properties such as mechanical strength and stiffness or elasticity to the specific need. This is why vascular grafts made of synthethic material find a wide clinical application in the treatment of cardiovascular diseases [209]. However, one major challenge with synthetic polymer-based vascular grafts is the low patency rate compared to natural or biosynthetic graft materials [170]. Therefore, advanced strategies focus on the modification of the luminal surface with protein coatings such as adhesion or signalling molecules in order to enhance the integration of vascular grafts within the host's tissue [180]. Another point that has to be addressed is the biocompatibility of these materials. Acidic or cytotoxic degradation products that are released during the tissue remodeling process at the implantation site may cause inflammation, cell apoptosis, or tissue necrosis [169].

A broad spectrum of synthetic polymers and co-polymers has been investigated for vascular tissue engineering applications. Materials such as expanded polytetrafluoroethylene (ePTFE), polyglycolic acid (PGA), polyethylene glycol (PEG), polycaprolactone, polylactid acid (PLA), and poly(ester urethane) urea are often used to fabricate synthetic vascular grafts.

Biodegradable polymers as vascular grafts degrade gradually to secretion of degradation factors and the synthesis of ECM by host cells during the tissue remodeling process [210]. PGA is commonly applied in vascular tissue engineering approaches as it displays a sufficient biodegradation rate without toxic breakdown product release [170]. Vessel walls engineered from human bone marrow-derived mesenchymal stem cells which were seeded in PGA mesh scaffolds and cultured in a pulsatile perfusion system under optimized conditions were found to be histologically and molecularly similar to native vessels [92]. Seeding of induced pluripotent stem cell-derived vascular cells onto a polyglycolic acid-poly-l-lactide and poly(l-lactide-co-ε-caprolactone) scaffold has been performed to create a biodegradable sheet which was implanted in the *inferior vena cava* of mice. In the acute phase the differentiated iPSCs exerted a paracrine effect to induce neotissue formation, although the number of seeded iPSCs decreased over time by apoptosis [211]. In another study venous valves were fabricated based on polyglycolic acid-poly-4-hydroxybutyrate composites seeded with *in vitro* conditioned autologous ovine bone marrow-derived MSC. This generated graft can overcome immunologic and thromboembolic complications reported for xeno- and allogeneic transplants and thus provide the potential to be used for the replacement of diseased venous valves [212].

Polycaprolactone is another synthetic polymer that has been extensively studied for its potential use as vascular graft material. Small pore size of electrospun vascular grafts often limits cell infiltration and thereby restricts the regeneration and the remodeling process of neoartery formation. Large-pore PCL scaffolds were prepared to overcome this problem. The macroporous grafts enhanced cell ingrowth and the secretion of ECM components. Furthermore, the neoarteries displayed layer formation similar to those of a native vessel wall as well as a contractile phenotype in response to adrenaline and acetylcholine-induced relaxation [213]. Large-pore PLA fibers coated with poly(L-lactide-co-ε-caprolactone) (PLCL) (PLA-PLCL) are more suitable vascular grafts compared to small-pore PLA nanofibers (PLA-nano) since they promote the formation of a well-organized neointima and prevent neointimal calcification [214]. Coating of microporous PCL scaffolds with recombinant mussel adhesive protein fused with arginine-glycine-aspartic acid peptide (MAP-RGD) improved the *in situ* recruitment of endothelial and endothelial-progenitor cells after implantation in rabbit carotid arteries whereas pre-endothelialization of the constructs increased the risk of thrombus formation and intimal hyperplasia [215]. Electrospinning of synthetic polymers into nanofibers enables the formation of graft materials with high porosity and surface area-to-volume ratios mimicking naturally occurring structures such as collagen and elastin fibrils [170]. An electrospun synthetic copolymer from poly(L-lactid-co- ε -caprolactone) [P(LLA-CL)] (75:25) with aligned nanofibers significantly improved the adhesion and proliferation rate of human coronary artery SMCs compared to plane polymer films [216].

A multiple of studies on synthetic vascular grafts focus on PEG due its resistance to protein adsorption, an important event with regard to platelet adhesion and occlusion of vessel implants [72, 210]. To adapt PEG-based polymers to a specific application they are often modified with functional groups or degradable peptides and proteins. Pfeiffer and coworkers showed that fibronectin-coated PEG electrospun grafts promoted endothelial cell attachment and viability better than equivalently pretreated ePTFE grafts [217]. ePTFE (GORE-TEX®) is, besides polyester (Dacron®), the most commonly used material for vascular grafts. Roll and colleagues reported that one study reported significant differences in primary

patency for Dacron® and one favouring PFTE grafts. However, in seven trials found no significant differences were identified between the two materials [218].

Co-electrospun polyethylene glycol and poly-ε-caprolactone created a highly porous vascular graft that has been modified by anchoring heparin to surface exposed lysine groups (PCL-LYS-H). Dynamic co-culture of ECs on these PCL-LYS-H scaffolds and SMCs on PCL resulted in a three-dimensional tissue engineered graft with mechanical properties that showed promise towards meeting today's clinical demands [219].

Due to its three-dimensional structure consisting of a diisocyanate hard domain, a chain extender, and a diol soft domain polyurethane-based scaffold, these materials can be combined with numerous additives to take various shapes, modify surface characteristics, and yield hybrid composites having mechanical properties that range from elastomeric to stiff [170]. ECs cultured on biomaterials of polyurethane that incorporate gold nanoparticles displayed an increased proliferation and migration rate which was associated with induced expression levels of both, endothelial nitric oxide synthase and phosphorylated-Akt (p-Akt) [220]. Rapid incorporation of muscle-derived stem cells within tubular poly(ester urethane) urea (PEUU) scaffolds by using a rotational vacuum seeding device resulted in an even distribution of the cells while maintaining their viability, high proliferation rates, and stem cell antigen-1 expression [221].

While the afforementioned polymers are perhaps the most widely explored of synthetic graft materials, many other artificial substances have been investigated for their potential medical application as tissue-engineered blood vessel substitutes. Rat bone marrow MSCs, genetically modified with the endothelial nitric oxide synthase gene, integrated within the microfibers of an electrospun poly(propylene carbonate) scaffold to build up a vascular graft produced a nitric oxide rate that was comparable to that of mature vessels [222]. Another biocompatible nanomaterial with improved hemocompatibility, antithrombogenicity, enhanced mechanical and surface properties, calcification resistance, and reduced inflammatory response was developed by incorporating polyhedral oligomeric silsesquioxane (POSS) into polymers [223]. Poly-(sulfobetaine methacrylate [SBMA]) hydrogels modified with the peptide RGD and the vascular endothelial growth factor-mimicking peptide KLTWQELYQLKYKG provided a homogeneous vascular graft with an interconnected pore structure which supported endothelial cell adhesion and proliferation [224]. Incorporation of ultrasmall superparamagnetic iron oxide nanoparticles into polyvinylidene fluoride (PVDF)-based textile fibers is suitable for the fabrication of tissue-engineered vascular grafts that can be traced *in vivo* in a non-invasive manner using magnet resonance imaging [225].

Leferink and coworkers produced micro-objects made of a photocurable epoxy-resin and used them as elements in cellular building blocks for bottom-up tissue engineering approaches. The researchers suggest applying their stacked, tube-like scaffolding elements to develop vessel-like constructs by using vascular endothelial cells with their micro-objects [226].

Summing up, ePTFE, PGA, PLA, PEG, and poly (ester urethane) urea are the most commonly used synthetic polymers to fabricate vascular grafts. Synthetic graft materials have the advantage that various properties can be tuned, resulting in a good reproducibility, and a wide-ranging control over material properties such as porosity, degradation time, and mechanical characteristics. But even if they can be synthesized with physicochemical and mechanical properties comparable to those of biological tissues they still allow only little control over cell behavior and show less biological activity [199, 201]. For example the cellular integration is often low for which reason there are strategies to modify the luminal surface with protein coatings, peptides, or functional groups to enhance this process. Futhermore the toxic and allergic potential must be evaluated and excluded when using synthetic grafting material, because oftentimes acidic or cytoxic degradation products are released by synthetic polymers.

The majority of the studies utilize endothelial cells to populate the scaffolds, followed by smooth muscle cells and stem cells. The need to seed the scaffolds with cells is, if applied, a major drawback of synthetic grafts: *in vitro* culture steps [200] are time-consuming and limit the application in situations where a fast solution is essential. However, if synthetic scaffolds could be applied without cells, their usage can be an advantage since the scaffolds can be stored due to their long shelflife and would be available on demand.

Biosynthetic Vascular Grafts

The combination of natural and synthetic materials to establish so-called hybrid constructs opens more possibilities for tailoring the grafts properties to suit the needs of a particular application. Controlling cell adherence and biological activity can be directed by the nature-derived components whereas the physical and chemical properties can be designed by choosing a specific synthetic compound [72]. A construct-sleeve hybrid graft created from a collagen layer containing cells and an acellular, uncrosslinked, and glutaraldehyde-treated support sleeve fabricated from type I collagen gels possessed an increased mechanical strength compared to unstiffened control constructs (Fig. 4A) [227]. Another group has shown that nanostructured polyurethane Tecophilic (TP) blended with gelatin (gel) at a weight ratio of 70:30 maintained the contractile phenotype of seeded vascular SMCs without signs of prothrombogenic potential [228]. In another work, autologous artery-derived cells were seeded onto a fibrin-based scaffold

reinforced by a poly(L/D)lactide 96/4 mesh (Fig. 4B) and cultivated under dynamic flow conditions prior to implantation in the carotid artery of sheep. The explanted vascular composite grafts revealed a good mid-term patency in the arterial circulation of this large animal model [229]. Bertassoni and coworkers successfully embedded microchannels inside methacrylated gelatin (Fig. 4D). The created vascular network was shown to improve cell viability and differentiation within the hydrogel constructs. In addition, the formation of endothelial monolayers within these microchannels could be demonstrated [230].

A variety of bioactive factors have been investigated for their beneficial effect on vascular cell adhesion, migration, growth, and angiogenic sprouting. The melding of natural biomaterials with synthetic ones enables the design of scaffold-based ligand-anchoring and controlled release systems for these factors. One example is a hybrid mesh of poly(ε-caprolactone)-collagen blend (PCL/Col) and hyaluronic acid (HA) hydrogel (Fig. 4F) which has been loaded with the angiogenic growth factors VEGF [196] and PDGF-BB. Co-culturing of human umbilical vein endothelial cells and lung fibroblasts within this bio-functionalized construct resulted in primitive capillary network formation [231]. A similar outcome has been observed for the heparin-functionalized poly(ε-caprolactone) (PCL) / chitosan (CS) hybrid small-diameter vascular grafts (Fig. 4G). These constructs promoted *in situ* endothelialization and thereby could prevent the appearance of restenosis - one of the main reasons for graft failure [232].

To resume, biosynthetic grafts try to make use of the best properties of both, synthetic and natural polymer scaffolds. They feature the strength, tunability, and manufacturing control of synthetic materials and the biocompatibility and biochemical cues of natural materials to a high degree. In the absence of appropriate autologous graft material biosynthetic scaffolds seem to be a promising alternative. However biosynthetic grafts not only combine the best properties of each class, but also show the disadvantages of both. Furthermore, as for synthetic grafts, a major drawback is the long period of *in vitro* culture to generate robust constructs seeded with cells [200] which limits their usage in emergencies where this kind of scaffolds are needed.

Conclusion and Future Perspective

Coronary vascular disease constitutes an imminent and expanding challenge that medicine must address in the near future. Vascular grafting offers a wide range of therapeutic options comprising different material of natural, synthetic, or biosynthetic origin that can is used alone or in combination with various (stem) cells to create a bioactive vascular graft. Strategic design of graft materials can enable researchers to more closely regulate biological processes crucial to the formation of native-esque tissues, such as differentiation, ECM synthesis, matrix remodeling, and more complicated, albeit crucial processes like angiogenic sprouting. Ultimately, such advanced materials will enable engineered vascular grafts to overcome the insufficient biocompatibility, immunogenicity, and mechanical stability that currently bars their widespread use. Especially the use of 3D printing is likely to develop to a promising innovative system for graft design the next years.

Tissue-engineered vascular constructs are beneficial over conventional grafts since they adapt to the body by allowing remodeling, self-repair, and growth of the tissue. The latter is especially important when thinking of pediatrics. As cell source for tissue engineered vascular grafts, stem cells and stem cell-derived vascular cells are likely to further increase in use. Especially iPSCs will pave the way for personalized medicine. Patient-specific vascular grafts and regeneration offer completely new therapeutic options that meet the requirements for each individual. However, this will also bring along difficulties: Grafts that are readily available to patients are more attractive to clinicians, patients, and finally are easier to bring together with various regulatory environments. One main drawback that hampers clinical implementation is the waiting time for graft production. The use of autologous cells requires waiting times up to a month. Although there are studies that report using low-pressure systems to harvest bone marrow, isolate cells, and seed them on the same day [211] other studies need to follow in order to improve fabrication time, costs, and cell preparation (including differentiation efficiency and -time).

Compliance with Ethical Standards

Disclosures The authors declare no potential conflicts of interest.

References

1. World Health Organization. Cardiovascular diseases (CVDs): World Health Organization. Accessed March 31, 2018, at http://www.who.int/mediacentre/factsheets/fs317/en/.
2. Dahl, S. L. M., Kypson, A. P., Lawson, J. H., et al. (2011). Readily available tissue-engineered vascular grafts. *Science Translational Medicine, 3*(68), 68ra9.
3. Leeper, N. J., Hunter, A. L., & Cooke, J. P. (2010). Stem cell therapy for vascular regeneration: adult, embryonic, and induced pluripotent stem cells. *Circulation, 122*(5), 517–526.
4. Kaebisch, C., Schipper, D., Babczyk, P., Tobiasch, E. (2015). The role of purinergic receptors in stem cell differentiation. *Computational and Structural Biotechnology Journal,* 1375–1384.
5. Takahashi, K., & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell, 126*(4), 663–676.

6. Yu, J., Vodyanik, M. A., Smuga-Otto, K., et al. (2007). Induced pluripotent stem cell lines derived from human somatic cells. *Science, 318*(5858), 1917–1920.
7. Salem, H. K., & Thiemermann, C. (2010). Mesenchymal stromal cells: current understanding and clinical status. *Stem Cells, 28*(3), 585–596.
8. Melchiorri, A. J., Hibino, N., & Fisher, J. P. (2013). Strategies and techniques to enhance the in situ endothelialization of small-diameter biodegradable polymeric vascular grafts. *Tissue Engineering. Part B, Reviews, 19*(4), 292–307.
9. Liu, R. H., Ong, C. S., Fukunishi, T., Ong, K., & Hibino, N. (2018). Review of vascular graft studies in large animal models. *Tissue Engineering. Part B, Reviews, 24*(2), 133–143.
10. Knight, D. K., Gillies, E. R., & Mequanint, K. (2014). Vascular grafting strategies in coronary intervention. *Frontiers in Materials, 1*, 1–16.
11. Seifu, D. G., Purnama, A., Mequanint, K., & Mantovani, D. (2013). Small-diameter vascular tissue engineering. *Nature Reviews. Cardiology, 10*(7), 410–421.
12. Potente, M., Gerhardt, H., & Carmeliet, P. (2011). Basic and therapeutic aspects of angiogenesis. *Cell, 146*(6), 873–887.
13. Crisan, M., Corselli, M., Chen, W. C. W., & Péault, B. (2012). Perivascular cells for regenerative medicine. *Journal of Cellular and Molecular Medicine, 16*(12), 2851–2860.
14. Pacilli, A., & Pasquinelli, G. (2009). Vascular wall resident progenitor cells: a review. *Experimental Cell Research, 315*(6), 901–914.
15. Pucovský, V. (2010). Interstitial cells of blood vessels. *ScientificWorldJournal, 10*, 1152–1168.
16. Wagenseil, J. E., & Mecham, R. P. (2009). Vascular extracellular matrix and arterial mechanics. *Physiological Reviews, 89*(3), 957–989.
17. Chantler, P. D., & Frisbee, J. C. (2015). Arterial function in cardiometabolic diseases: from the microcirculation to the large conduits. *Progress in Cardiovascular Diseases, 57*(5), 489–496.
18. Wanjare, M., Kusuma, S., & Gerecht, S. (2013). Perivascular cells in blood vessel regeneration. *Biotechnology Journal, 8*(4), 434–447.
19. Brouard, S., Otterbein, L. E., Anrather, J., et al. (2000). Carbon monoxide generated by heme oxygenase 1 suppresses endothelial cell apoptosis. *The Journal of Experimental Medicine, 192*(7), 1015–1026.
20. Mecham, R. P., Madaras, J., McDonald, J. A., & Ryan, U. (1983). Elastin production by cultured calf pulmonary artery endothelial cells. *Journal of Cellular Physiology, 116*(3), 282–288.
21. Rajendran, P., Rengarajan, T., Thangavel, J., et al. (2013). The vascular endothelium and human diseases. *International Journal of Biological Sciences, 9*(10), 1057–1069.
22. Brouard, S., Berberat, P. O., Tobiasch, E., Seldon, M. P., Bach, F. H., & Soares, M. P. (2002). Heme oxygenase-1-derived carbon monoxide requires the activation of transcription factor NF-kappa B to protect endothelial cells from tumor necrosis factor-alpha-mediated apoptosis. *The Journal of Biological Chemistry, 277*(20), 17950–17961.
23. Iivanainen, E., Nelimarkka, L., Elenius, V., et al. (2003). Angiopoietin-regulated recruitment of vascular smooth muscle cells by endothelial-derived heparin binding EGF-like growth factor. *The FASEB Journal, 17*(12), 1609–1621.
24. Schwartz, S. M., Majesky, M. W., & Murry, C. E. (1995). The intima: development and monoclonal responses to injury. *Atherosclerosis, 118*(Suppl), S125–S140.
25. Khurana, R., Simons, M., Martin, J. F., & Zachary, I. C. (2005). Role of angiogenesis in cardiovascular disease: a critical appraisal. *Circulation, 112*(12), 1813–1824.
26. Sims, D. E. (1986). The pericyte—A review. *Tissue and Cell, 18*(2), 153–174.
27. Armulik, A., Abramsson, A., & Betsholtz, C. (2005). Endothelial/pericyte interactions. *Circulation Research, 97*(6), 512–523.
28. Kusumbe, A. P., Ramasamy, S. K., & Adams, R. H. (2014). Coupling of angiogenesis and osteogenesis by a specific vessel subtype in bone. *Nature, 507*(7492), 323–328.
29. Ramasamy, S. K., Kusumbe, A. P., Wang, L., & Adams, R. H. (2014). Endothelial Notch activity promotes angiogenesis and osteogenesis in bone. *Nature, 507*(7492), 376–380.
30. Margariti, A., Zeng, L., & Xu, Q. (2006). Stem cells, vascular smooth muscle cells and atherosclerosis. *Histology and Histopathology, 21*(9), 979–985.
31. Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., et al. (1998). Embryonic stem cell lines derived from human blastocysts. *Science, 282*(5391), 1145–1147.
32. Baldwin, J., Antille, M., Bonda, U., et al. (2014). In vitro pre-vascularisation of tissue-engineered constructs A co-culture perspective. *Vascular Cell, 6*, 13.
33. Kimmelman, J., Hyun, I., Benvenisty, N., et al. (2016). Policy: global standards for stem-cell research. *Nature, 533*(7603), 311–313.
34. Hentze, H., Soong, P. L., Wang, S. T., Phillips, B. W., Putti, T. C., & Dunn, N. R. (2009). Teratoma formation by human embryonic stem cells: evaluation of essential parameters for future safety studies. *Stem Cell Research, 2*(3), 198–210.
35. Riha, G. M., Lin, P. H., Lumsden, A. B., Yao, Q., & Chen, C. (2005). Review: application of stem cells for vascular tissue engineering. *Tissue Engineering, 11*(9–10), 1535–1552.
36. Calloni, R., Cordero, E. A. A., Henriques, J. A. P., & Bonatto, D. (2013). Reviewing and updating the major molecular markers for stem cells. *Stem Cells and Development, 22*(9), 1455–1476.
37. Adewumi, O., Aflatoonian, B., Ahrlund-Richter, L., et al. (2007). Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. *Nature Biotechnology, 25*(7), 803–816.
38. Reubinoff, B. E., Pera, M. F., Fong, C. Y., Trounson, A., & Bongso, A. (2000). Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. *Nature Biotechnology, 18*(4), 399–404.
39. Kehat, I., Kenyagin-Karsenti, D., Snir, M., et al. (2001). Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *The Journal of Clinical Investigation, 108*(3), 407–414.
40. Levenberg, S., Golub, J. S., Amit, M., Itskovitz-Eldor, J., & Langer, R. (2002). Endothelial cells derived from human embryonic stem cells. *Proceedings of the National Academy of Sciences of the United States of America, 99*(7), 4391–4396.
41. Adams, B., Xiao, Q., & Xu, Q. (2007). Stem cell therapy for vascular disease. *Trends in Cardiovascular Medicine, 17*(7), 246–251.
42. Mauritz, C., Schwanke, K., Reppel, M., et al. (2008). Generation of functional murine cardiac myocytes from induced pluripotent stem cells. *Circulation, 118*(5), 507–517.
43. Tateishi, K., Takehara, N., Matsubara, H., & Oh, H. (2008). Stemming heart failure with cardiac- or reprogrammed-stem cells. *Journal of Cellular and Molecular Medicine, 12*(6a), 2217–2232.
44. Kane, N. M., Meloni, M., Spencer, H. L., et al. (2010). Derivation of endothelial cells from human embryonic stem cells by directed differentiation: analysis of microRNA and angiogenesis in vitro and in vivo. *Arteriosclerosis, Thrombosis, and Vascular Biology, 30*(7), 1389–1397.
45. Lee, E. J., Lee, H.-N., Kang, H.-J., et al. (2010). Novel embryoid body-based method to derive mesenchymal stem cells from human embryonic stem cells. *Tissue Engineering. Part A, 16*(2), 705–715.
46. Moon, S.-H., Kim, J. M., Hong, K.-S., Shin, J. M., Kim, J., & Chung, H.-M. (2011). Differentiation of hESCs into mesodermal

subtypes: vascular-, hematopoietic- and mesenchymal-lineage cells. *International Journal of Stem Cells, 4*(1), 24–34.
47. Wang, Z. Z., Au, P., Chen, T., et al. (2007). Endothelial cells derived from human embryonic stem cells form durable blood vessels in vivo. *Nature Biotechnology, 25*(3), 317–318.
48. Guo, X., Stice, S. L., Boyd, N. L., & Chen, S.-Y. (2013). A novel in vitro model system for smooth muscle differentiation from human embryonic stem cell-derived mesenchymal cells. *American Journal of Physiology. Cell Physiology, 304*(4), C289–C298.
49. Xiao, Q., Pepe, A. E., Wang, G., et al. (2012). Nrf3-Pla2g7 interaction plays an essential role in smooth muscle differentiation from stem cells. *Arteriosclerosis, Thrombosis, and Vascular Biology, 32*(3), 730–744.
50. Kim, J. B., Zaehres, H., Wu, G., et al. (2008). Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. *Nature, 454*(7204), 646–650.
51. Kim, J. B., Sebastiano, V., Wu, G., et al. (2009). Oct4-induced pluripotency in adult neural stem cells. *Cell, 136*(3), 411–419.
52. Takahashi, K., Ichisaka, T., & Yamanaka, S. (2006). Identification of genes involved in tumor-like properties of embryonic stem cells. *Methods in Molecular Biology, 329*, 449–458.
53. Zomer, H. D., Vidane, A. S., Gonçalves, N. N., & Ambrósio, C. E. (2015). Mesenchymal and induced pluripotent stem cells: general insights and clinical perspectives. *Stem Cells Cloning, 8*, 125–134.
54. Singh, V. K., Kalsan, M., Kumar, N., Saini, A., & Chandra, R. (2015). Induced pluripotent stem cells: applications in regenerative medicine, disease modeling, and drug discovery. *Frontiers in Cell and Development Biology, 3*, 2.
55. Lin, T., & Lin, Y. (2017). p53 switches off pluripotency on differentiation. *Stem Cell Research & Therapy, 8*(1), 44.
56. Yu, J., Hu, K., Smuga-Otto, K., et al. (2009). Human induced pluripotent stem cells free of vector and transgene sequences. *Science, 324*(5928), 797–801.
57. Xie, C.-Q., Huang, H., Wei, S., et al. (2009). A comparison of murine smooth muscle cells generated from embryonic versus induced pluripotent stem cells. *Stem Cells and Development, 18*(5), 741–748.
58. Gui, L., Dash, B. C., Luo, J., et al. (2016). Implantable tissue-engineered blood vessels from human induced pluripotent stem cells. *Biomaterials, 102*, 120–129.
59. Narazaki, G., Uosaki, H., Teranishi, M., et al. (2008). Directed and systematic differentiation of cardiovascular cells from mouse induced pluripotent stem cells. *Circulation, 118*(5), 498–506.
60. Park, S.-W., Jun Koh, Y., Jeon, J., et al. (2010). Efficient differentiation of human pluripotent stem cells into functional CD34+ progenitor cells by combined modulation of the MEK/ERK and BMP4 signaling pathways. *Blood, 116*(25), 5762–5772.
61. Choi, K.-D., Yu, J., Smuga-Otto, K., et al. (2009). Hematopoietic and endothelial differentiation of human induced pluripotent stem cells. *Stem Cells, 27*(3), 559–567.
62. Patsch, C., Challet-Meylan, L., Thoma, E. C., et al. (2015). Generation of vascular endothelial and smooth muscle cells from human pluripotent stem cells. *Nature Cell Biology, 17*(8), 994–1003.
63. Sahara, M., Hansson, E. M., Wernet, O., Lui, K. O., Später, D., & Chien, K. R. (2015). Manipulation of a VEGF-Notch signaling circuit drives formation of functional vascular endothelial progenitors from human pluripotent stem cells. *Cell Research, 25*(1), 148.
64. Lu, S.-J., Feng, Q., Caballero, S., et al. (2007). Generation of functional hemangioblasts from human embryonic stem cells. *Nature Methods, 4*(6), 501–509.
65. Goldman, O., Feraud, O., Boyer-Di Ponio, J., et al. (2009). A boost of BMP4 accelerates the commitment of human embryonic stem cells to the endothelial lineage. *Stem Cells, 27*(8), 1750–1759.
66. Bai, H., Gao, Y., Arzigian, M., Wojchowski, D. M., Wu, W.-S., & Wang, Z. Z. (2010). BMP4 regulates vascular progenitor development in human embryonic stem cells through a Smad-dependent pathway. *Journal of Cellular Biochemistry, 109*(2), 363–374.
67. Tatsumi, R., Suzuki, Y., Sumi, T., Sone, M., Suemori, H., & Nakatsuji, N. (2011). Simple and highly efficient method for production of endothelial cells from human embryonic stem cells. *Cell Transplantation, 20*(9), 1423–1430.
68. James, D., Nam, H.-s., Seandel, M., et al. (2010). Expansion and maintenance of human embryonic stem cell-derived endothelial cells by TGFbeta inhibition is Id1 dependent. *Nature Biotechnology, 28*(2), 161–166.
69. Friedenstein, A. J., Piatetzky-Shapiro, I. I., & Petrakova, K. V. (1966). Osteogenesis in transplants of bone marrow cells. *Journal of Embryology and Experimental Morphology, 16*(3), 381–390.
70. Pansky, A., Roitzheim, B., & Tobiasch, E. (2007). Differentiation potential of adult human mesenchymal stem cells. *Clinical Laboratory, 53*(1–2), 81–84.
71. Zhang, Y., & Tobiasch, E. (2011). The role of purinergic receptors in stem cells in their derived consecutive tissues. In P. Di Nardo (Ed.), *Adult stem cell standardization* (pp. 73–98). Aalborg: River Publishers.
72. Babczyk, P., Conzendorf, C., Klose, J., Schulze, M., Harre, K., & Tobiasch, E. (2014). Stem cells on biomaterials for synthetic grafts to promote vascular healing. *Journal of Clinical Medicine, 3*(1), 39–87.
73. Tobiasch, E. (2009). Adult Human mesenchymal stem cells as source for future tissue engineering. In C. Zacharias, K. W. ter Horst, K.-U. Witt, V. Sommer, M. Ant, U. Essmann, & L. Mülheims (Eds.), *Forschungsspitzen und Spitzenforschung, Innovationen an der Fachhochschule Bonn-Rhein-Sieg: Festschrift für Wulf Fischer* (pp. 329–338). Heidelberg: Physica-Verlag.
74. Tobiasch, E. (2011). Differentiation potential of adult human mesenchymal stem cells. In G. M. Artmann, S. Minger, & J. Hescheler (Eds.), *Stem cell engineering, principles and applications* (pp. 61–77). Berlin, Heidelberg: Springer-Verlag Berlin Heidelberg.
75. Zhang, Y., Khan, D., Delling, J., & Tobiasch, E. (2012). Mechanisms underlying the osteo- and adipo-differentiation of human mesenchymal stem cells. *The Scientific World Journal, 2012* http://downloads.hindawi.com/journals/tswj/2012/793823.pdf.
76. Phinney, D. G. (2012). Functional heterogeneity of mesenchymal stem cells: implications for cell therapy. *Journal of Cellular Biochemistry, 113*(9), 2806–2812.
77. Dominici, M., Le Blanc, K., Mueller, I., et al. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy, 8*(4), 315–317.
78. Roberts, I. (2004). Mesenchymal stem cells. *Vox Sanguinis, 87*(Suppl), 238–241.
79. Romanov, Y. A., Svintsitskaya, V. A., & Smirnov, V. N. (2003). Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. *Stem Cells, 21*(1), 105–110.
80. Zuk, P. A., Zhu, M., Ashjian, P., et al. (2002). Human adipose tissue is a source of multipotent stem cells. *Molecular Biology of the Cell, 13*(12), 4279–4295.
81. Oswald, J., Boxberger, S., Jorgensen, B., et al. (2004). Mesenchymal stem cells can be differentiated into endothelial cells in vitro. *Stem Cells, 22*(3), 377–384.
82. Portalska, K. J., Leferink, A., Groen, N., et al. (2012). Endothelial differentiation of mesenchymal stromal cells. *PLoS One, 7*(10), e46842 http://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0046842&type=printable.

83. Crisan, M. (2013). Transition of mesenchymal stem/stromal cells to endothelial cells. *Stem Cell Research & Therapy, 4*(4), 95.
84. Hsiao, S. T.-F., Asgari, A., Lokmic, Z., et al. (2012). Comparative analysis of paracrine factor expression in human adult mesenchymal stem cells derived from bone marrow, adipose, and dermal tissue. *Stem Cells and Development, 21*(12), 2189–2203.
85. Amable, P. R., Teixeira, M. V. T., Carias, R. B. V., Granjeiro, J. M., & Borojevic, R. (2014). Protein synthesis and secretion in human mesenchymal cells derived from bone marrow, adipose tissue and Wharton's jelly. *Stem Cell Research & Therapy, 5*(2), 53.
86. Ge, Q., Zhang, H., Hou, J., et al. (2018). VEGF secreted by mesenchymal stem cells mediates the differentiation of endothelial progenitor cells into endothelial cells via paracrine mechanisms. *Molecular Medicine Reports, 17*(1), 1667–1675.
87. Wojakowski, W., Kucia, M., Zuba-Surma, E., et al. (2011). Very small embryonic-like stem cells in cardiovascular repair. *Pharmacology & Therapeutics, 129*(1), 21–28.
88. Ratajczak, M. Z., Zuba-Surma, E. K., Wysoczynski, M., Ratajczak, J., & Kucia, M. (2008). Very small embryonic-like stem cells: characterization, developmental origin, and biological significance. *Experimental Hematology, 36*(6), 742–751.
89. Guerin, C. L., Loyer, X., Vilar, J., et al. (2015). Bone-marrow-derived very small embryonic-like stem cells in patients with critical leg ischaemia: evidence of vasculogenic potential. *Thrombosis and Haemostasis, 113*(5), 1084–1094.
90. Ratajczak, M. Z., Ratajczak, J., Suszynska, M., Miller, D. M., Kucia, M., & Shin, D.-M. (2017). A novel view of the adult stem cell compartment from the perspective of a quiescent population of very small embryonic-like stem cells. *Circulation Research, 120*(1), 166–178.
91. Ross, J. J., Hong, Z., Willenbring, B., et al. (2006). Cytokine-induced differentiation of multipotent adult progenitor cells into functional smooth muscle cells. *The Journal of Clinical Investigation, 116*(12), 3139–3149.
92. Gong, Z., & Niklason, L. E. (2008). Small-diameter human vessel wall engineered from bone marrow-derived mesenchymal stem cells (hMSCs). *The FASEB Journal, 22*(6), 1635–1648.
93. Liu, J. Y., Swartz, D. D., Peng, H. F., Gugino, S. F., Russell, J. A., & Andreadis, S. T. (2007). Functional tissue-engineered blood vessels from bone marrow progenitor cells. *Cardiovascular Research, 75*(3), 618–628.
94. Bajpai, V. K., & Andreadis, S. T. (2012). Stem cell sources for vascular tissue engineering and regeneration. *Tissue Engineering. Part B, Reviews, 18*(5), 405–425.
95. Cao, Y., Sun, Z., Liao, L., Meng, Y., Han, Q., & Zhao, R. C. (2005). Human adipose tissue-derived stem cells differentiate into endothelial cells in vitro and improve postnatal neovascularization in vivo. *Biochemical and Biophysical Research Communications, 332*(2), 370–379.
96. Colazzo, F., Chester, A. H., Taylor, P. M., & Yacoub, M. H. (2010). Induction of mesenchymal to endothelial transformation of adipose-derived stem cells. *The Journal of Heart Valve Disease, 19*(6), 736–744.
97. Harris, L. J., Abdollahi, H., Zhang, P., McIlhenny, S., Tulenko, T. N., & DiMuzio, P. J. (2011). Differentiation of adult stem cells into smooth muscle for vascular tissue engineering. *The Journal of Surgical Research, 168*(2), 306–314.
98. Heydarkhan-Hagvall, S., Schenke-Layland, K., Yang, J. Q., et al. (2008). Human adipose stem cells: a potential cell source for cardiovascular tissue engineering. *Cells Tissues Organs (Print), 187*(4), 263–274.
99. Peters, E. B. (2018). Endothelial progenitor cells for the vascularization of engineered tissues. *Tissue Engineering. Part B, Reviews, 24*(1), 1–24.
100. Medina, R. J., Barber, C. L., Sabatier, F., et al. (2017). Endothelial progenitors: a consensus statement on nomenclature. *Stem Cells Translational Medicine, 6*(5), 1316–1320.
101. Peichev, M., Naiyer, A. J., Pereira, D., et al. (2000). Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. *Blood, 95*(3), 952–958.
102. Medina, R. J., O'Neill, C. L., O'Doherty, T. M., et al. (2011). Myeloid angiogenic cells act as alternative M2 macrophages and modulate angiogenesis through interleukin-8. *Molecular Medicine, 17*(9–10), 1045–1055.
103. Medina, R. J., O'Neill, C. L., O'Doherty, T. M., Wilson, S. E. J., & Stitt, A. W. (2012). Endothelial progenitors as tools to study vascular disease. *Stem Cells International, 2012*, 346–735.
104. Tasev, D., Koolwijk, P., & van Hinsbergh, V. W. M. (2016). Therapeutic potential of human-derived endothelial colony-forming cells in animal models. *Tissue Engineering. Part B, Reviews, 22*(5), 371–382.
105. Kaushal, S., Amiel, G. E., Guleserian, K. J., et al. (2001). Functional small-diameter neovessels created using endothelial progenitor cells expanded ex vivo. *Nature Medicine, 7*(9), 1035–1040.
106. Patel, J., Donovan, P., & Khosrotehrani, K. (2016). Concise review: functional definition of endothelial progenitor cells: a molecular perspective. *Stem Cells Translational Medicine, 5*(10), 1302–1306.
107. Basile, D. P., & Yoder, M. C. (2014). Circulating and tissue resident endothelial progenitor cells. *Journal of Cellular Physiology, 229*(1), 10–16.
108. Leiendecker, A., Witzleben, S., Schulze, M., & Tobiasch, E. (2017). Template-mediated biomineralization for bone tissue engineering. *Current Stem Cell Research & Therapy, 12*(2), 103–123.
109. Heck, T. A. M., Vaeyens, M. M., & van Oosterwyck, H. (2015). Computational models of sprouting angiogenesis and cell migration: towards multiscale mechanochemical models of angiogenesis. *Mathematical Modelling of Natural Phenomena, 10*(1), 108–141.
110. Ribatti, D., & Crivellato, E. (2012). "Sprouting angiogenesis", a reappraisal. *Developmental Biology, 372*(2), 157–165.
111. Smet, F., de Segura, I., Bock, K., de Hohensinner, P. J., & Carmeliet, P. (2009). Mechanisms of vessel branching: filopodia on endothelial tip cells lead the way. *Arteriosclerosis, Thrombosis, and Vascular Biology, 29*(5), 639–649.
112. Dew, L., MacNeil, S., & Chong, C. K. (2015). Vascularization strategies for tissue engineers. *Regenerative Medicine, 10*(2), 211–224.
113. Ausprunk, D. H., & Folkman, J. (1977). Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during tumor angiogenesis. *Microvascular Research, 14*(1), 53–65.
114. van Hinsbergh, V. W. M., & Koolwijk, P. (2008). Endothelial sprouting and angiogenesis: matrix metalloproteinases in the lead. *Cardiovascular Research, 78*(2), 203–212.
115. Lu, P., Takai, K., Weaver, V. M., Werb, Z. 2011. Extracellular matrix degradation and remodeling in development and disease. *Cold Spring Harbor Perspectives in Biology, 3*(12).
116. Chen, Q., Jin, M., Yang, F., Zhu, J., Xiao, Q., & Zhang, L. (2013). Matrix metalloproteinases: inflammatory regulators of cell behaviors in vascular formation and remodeling. *Mediators of Inflammation, 2013*, 928315.
117. Davis, G. E., & Senger, D. R. (2005). Endothelial extracellular matrix: biosynthesis, remodeling, and functions during vascular morphogenesis and neovessel stabilization. *Circulation Research, 97*(11), 1093–1107.

118. Gonzalo, P., Moreno, V., Gálvez, B. G., & Arroyo, A. G. (2010). MT1-MMP and integrins: Hand-to-hand in cell communication. *Biofactors, 36*(4), 248–254.
119. Montuori, N., & Ragno, P. (2014). Role of uPA/uPAR in the modulation of angiogenesis. *Chemical Immunology and Allergy, 99*, 105–122.
120. Stojkovic, S., Kaun, C., Heinz, M., et al. (2014). Interleukin-33 induces urokinase in human endothelial cells—possible impact on angiogenesis. *Journal of Thrombosis and Haemostasis, 12*(6), 948–957.
121. Sahni, A., & Francis, C. W. (2004). Stimulation of endothelial cell proliferation by FGF-2 in the presence of fibrinogen requires alphavbeta3. *Blood, 104*(12), 3635–3641.
122. Michiels, C. (2003). Endothelial cell functions. *Journal of Cellular Physiology, 196*(3), 430–443.
123. Olsson, A.-K., Dimberg, A., Kreuger, J., & Claesson-Welsh, L. (2006). VEGF receptor signalling - in control of vascular function. *Nature Reviews. Molecular Cell Biology, 7*(5), 359–371.
124. Lamalice, L., Le Boeuf, F., & Huot, J. (2007). Endothelial cell migration during angiogenesis. *Circulation Research, 100*(6), 782–794.
125. Chappell, J. C., & Bautch, V. L. (2010). Vascular development. In P. Koopman (Ed.), *Organogenesis in development* (pp. 43–72). San Diego: Academic Press.
126. Ruhrberg, C., Gerhardt, H., Golding, M., et al. (2002). Spatially restricted patterning cues provided by heparin-binding VEGF-A control blood vessel branching morphogenesis. *Genes & Development, 16*(20), 2684–2698.
127. Carmeliet, P., Moons, L., Luttun, A., et al. (2001). Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. *Nature Medicine, 7*(5), 575–583.
128. Matsumoto, T., & Claesson-Welsh, L. (2001). VEGF receptor signal transduction. *Science's STKE, 2001*(112), re21.
129. Gerhardt, H., Golding, M., Fruttiger, M., et al. (2003). VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *The Journal of Cell Biology, 161*(6), 1163–1177.
130. Pircher, A., Wellbrock, J., Fiedler, W., Heidegger, I., Gunsilius, E., & Hilbe, W. (2014). New antiangiogenic strategies beyond inhibition of vascular endothelial growth factor with special focus on axon guidance molecules. *Oncology, 86*(1), 46–52 https://www.karger.com/Article/FullText/356871.
131. Tu, T., Zhang, C., Yan, H., et al. (2015). CD146 acts as a novel receptor for netrin-1 in promoting angiogenesis and vascular development. *Cell Research, 25*(3), 275–287.
132. Basile, J. R., Barac, A., Zhu, T., Guan, K.-L., & Gutkind, J. S. (2004). Class IV semaphorins promote angiogenesis by stimulating Rho-initiated pathways through plexin-B. *Cancer Research, 64*(15), 5212–5224.
133. Nakayama, M., Nakayama, A., van Lessen, M., et al. (2013). Spatial regulation of VEGF receptor endocytosis in angiogenesis. *Nature Cell Biology, 15*(3), 249–260.
134. Guijarro-Muñoz, I., Cuesta, A. M., Alvarez-Cienfuegos, A., Geng, J. G., Alvarez-Vallina, L., & Sanz, L. (2012). The axonal repellent Slit2 inhibits pericyte migration: potential implications in angiogenesis. *Experimental Cell Research, 318*(4), 371–378.
135. Singh, H., Tahir, T. A., Alawo, D. O. A., Issa, E., & Brindle, N. P. J. (2011). Molecular control of angiopoietin signalling. *Biochemical Society Transactions, 39*(6), 1592–1596.
136. Fukuhara, S., Sako, K., Noda, K., Nagao, K., Miura, K., & Mochizuki, N. (2009). Tie2 is tied at the cell-cell contacts and to extracellular matrix by angiopoietin-1. *Experimental & Molecular Medicine, 41*(3), 133–139.
137. Krebs, L. T., Xue, Y., Norton, C. R., et al. (2000). Notch signaling is essential for vascular morphogenesis in mice. *Genes & Development, 14*(11), 1343–1352 http://genesdev.cshlp.org/content/14/11/1343.full.
138. Jakobsson, L., Franco, C. A., Bentley, K., et al. (2010). Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting. *Nature Cell Biology, 12*(10), 943–953.
139. Hellström, M., Phng, L.-K., Hofmann, J. J., et al. (2007). Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. *Nature, 445*(7129), 776–780.
140. Lobov, I. B., Renard, R. A., Papadopoulos, N., et al. (2007). Delta-like ligand 4 (Dll4) is induced by VEGF as a negative regulator of angiogenic sprouting. *Proceedings of the National Academy of Sciences of the United States of America, 104*(9), 3219–3224.
141. Benedito, R., Roca, C., Sörensen, I., et al. (2009). The notch ligands Dll4 and Jagged1 have opposing effects on angiogenesis. *Cell, 137*(6), 1124–1135.
142. Carmeliet, P. (2003). Angiogenesis in health and disease. *Nature Medicine, 9*(6), 653–660.
143. Goumans, M.-J., Valdimarsdottir, G., Itoh, S., Rosendahl, A., Sideras, P., & ten Dijke, P. (2002). Balancing the activation state of the endothelium via two distinct TGF-beta type I receptors. *The EMBO Journal, 21*(7), 1743–1753.
144. Gaengel, K., Genové, G., Armulik, A., & Betsholtz, C. (2009). Endothelial-mural cell signaling in vascular development and angiogenesis. *Arteriosclerosis, Thrombosis, and Vascular Biology, 29*(5), 630–638.
145. Moreno-Miralles, I., Schisler, J. C., & Patterson, C. (2009). New insights into bone morphogenetic protein signaling: focus on angiogenesis. *Current Opinion in Hematology, 16*(3), 195–201.
146. Bentley, K., Mariggi, G., Gerhardt, H., & Bates, P. A. (2009). Tipping the balance: robustness of tip cell selection, migration and fusion in angiogenesis. *PLoS Computational Biology, 5*(10), e1000549.
147. Prowse, A. B. J., Chong, F., Gray, P. P., & Munro, T. P. (2011). Stem cell integrins: implications for ex-vivo culture and cellular therapies. *Stem Cell Research, 6*(1), 1–12.
148. Geudens, I., & Gerhardt, H. (2011). Coordinating cell behaviour during blood vessel formation. *Development, 138*(21), 4569–4583.
149. Lampugnani, M. G. (1995). The molecular organization of endothelial cell to cell junctions: differential association of plakoglobin, beta-catenin, and alpha- catenin with vascular endothelial cadherin (VE-cadherin). *The Journal of Cell Biology, 129*(1), 203–217.
150. Irvin, M. W., Zijlstra, A., Wikswo, J. P., & Pozzi, A. (2014). Techniques and assays for the study of angiogenesis. *Experimental Biology and Medicine (Maywood, N.J.), 239*(11), 1476–1488.
151. Zhang, B.-H., Guo, C.-X., Wang, H.-X., et al. (2014). Cardioprotective effects of adipokine apelin on myocardial infarction. *Heart and Vessels, 29*(5), 679–689.
152. Nakatsu, M. N., Davis, J., Hughes, C. C. W. (2007). Optimized fibrin gel bead assay for the study of angiogenesis. *Journal of Visualized Experiments,* (3). 186.
153. Arnaoutova, I., George, J., Kleinman, H. K., & Benton, G. (2009). The endothelial cell tube formation assay on basement membrane turns 20: state of the science and the art. *Angiogenesis, 12*(3), 267–274.
154. Stiffey-Wilusz, J., Boice, J. A., Ronan, J., Fletcher, A. M., & Anderson, M. S. (2001). An ex vivo angiogenesis assay utilizing commercial porcine carotidartery: Modification of the rat aortic ring assay. *Angiogenesis, 4*(1), 3–9.
155. Rezzola, S., Belleri, M., Gariano, G., et al. (2014). In vitro and ex vivo retina angiogenesis assays. *Angiogenesis, 17*(3), 429–442.
156. Arima, S., Nishiyama, K., Ko, T., et al. (2011). Angiogenic morphogenesis driven by dynamic and heterogeneous collective endothelial cell movement. *Development, 138*(21), 4763–4776.

157. Auerbach, R. (2003). Angiogenesis assays: a critical overview. *Clinical Chemistry, 49*(1), 32–40.
158. Akhtar, N., Dickerson, E. B., & Auerbach, R. (2002). The sponge/Matrigel angiogenesis assay. *Angiogenesis, 5*(1), 75–80.
159. Staton, C. A., Stribbling, S. M., Tazzyman, S., Hughes, R., Brown, N. J., & Lewis, C. E. (2004). Current methods for assaying angiogenesis in vitro and in vivo. *International Journal of Experimental Pathology, 85*(5), 233–248. https://onlinelibrary.wiley.com/doi/full/10.1111/j.0959-9673.2004.00396.x .
160. Niiyama, H., Huang, N. F., Rollins, M. D., Cooke, J. P. (2009). Murine model of hindlimb ischemia. *Journal of Visualized Experiments* (23).
161. Tufan, A., & Satiroglu-Tufan, N. (2005). The chick embryo chorioallantoic membrane as a model system for the study of tumor angiogenesis, invasion and development of anti-angiogenic agents. *Current Cancer Drug Targets, 5*(4), 249–266.
162. Kisucka, J., Butterfield, C. E., Duda, D. G., et al. (2006). Platelets and platelet adhesion support angiogenesis while preventing excessive hemorrhage. *Proceedings of the National Academy of Sciences of the United States of America, 103*(4), 855–860.
163. Melero-Martin, J. M., & Bischoff, J. (2008). Chapter 13 an in vivo experimental model for postnatal vasculogenesis. In D. A. Cheresh (Ed.), *Angiogenesis, in vivo systems* (pp. 303–329). San Diego: Academic Press/Elsevier.
164. Mehdizadeh, H., Sumo, S., Bayrak, E. S., Brey, E. M., & Cinar, A. (2013). Three-dimensional modeling of angiogenesis in porous biomaterial scaffolds. *Biomaterials, 34*(12), 2875–2887.
165. Peirce, S. M., Mac Gabhann, F., & Bautch, V. L. (2012). Integration of experimental and computational approaches to sprouting angiogenesis. *Current Opinion in Hematology, 19*(3), 184–191.
166. van Oers, R. F. M., Rens, E. G., LaValley, D. J., Reinhart-King, C. A., & Merks, R. M. H. (2014). Mechanical cell-matrix feedback explains pairwise and collective endothelial cell behavior in vitro. *PLoS Computational Biology, 10*(8), e1003774.
167. Langer, R., & Vacanti, J. (2016). Advances in tissue engineering. *Journal of Pediatric Surgery, 51*(1), 8–12.
168. Goh, E. T., Wong, E., Farhatnia, Y., Tan, A., & Seifalian, A. M. (2014). Accelerating in situ endothelialisation of cardiovascular bypass grafts. *International Journal of Molecular Sciences, 16*(1), 597–627.
169. Zippel, N., Schulze, M., & Tobiasch, E. (2010). Biomaterials and mesenchymal stem cells for regenerative medicine. *Recent Patents on Biotechnology, 4*(1), 1–22.
170. Ravi, S., & Chaikof, E. L. (2010). Biomaterials for vascular tissue engineering. *Regenerative Medicine, 5*(1), 107–120.
171. Kucukgul, C., Ozler, S. B., Inci, I., et al. (2015). 3D bioprinting of biomimetic aortic vascular constructs with self-supporting cells. *Biotechnology and Bioengineering, 112*(4), 811–821.
172. Iordache, F., Grumezescu, A. M., Maniu, H., & Curutiu, C. (2017). Development of scaffolds for vascular tissue engineering: biomaterial mediated neovascularization. *Current Stem Cell Research & Therapy, 12*(2), 155–164.
173. Berardinelli, L. (2006). Grafts and graft materials as vascular substitutes for haemodialysis access construction. *European Journal of Vascular and Endovascular Surgery, 32*(2), 203–211.
174. Martin, N. D., Schaner, P. J., Tulenko, T. N., et al. (2005). In vivo behavior of decellularized vein allograft. *The Journal of Surgical Research, 129*(1), 17–23.
175. Darby, C. R., Roy, D., Deardon, D., & Cornall, A. (2006). Depopulated bovine ureteric xenograft for complex haemodialysis vascular access. *European Journal of Vascular and Endovascular Surgery, 31*(2), 181–186.
176. Spark, J. I., Yeluri, S., Derham, C., Wong, Y. T., & Leitch, D. (2008). Incomplete cellular depopulation may explain the high failure rate of bovine ureteric grafts. *The British Journal of Surgery, 95*(5), 582–585.
177. Keane, T. J., Londono, R., Turner, N. J., & Badylak, S. F. (2012). Consequences of ineffective decellularization of biologic scaffolds on the host response. *Biomaterials, 33*(6), 1771–1781.
178. Jones, S. G., Hu, Y., Xu, Q., & Jahangiri, M. (2014). Stem cells accumulate on a decellularized arterial xenograft in vivo. *The Annals of Thoracic Surgery, 97*(6), 2104–2110.
179. Mangold, S., Schrammel, S., Huber, G., et al. (2015). Evaluation of decellularized human umbilical vein (HUV) for vascular tissue engineering - comparison with endothelium-denuded HUV. *Journal of Tissue Engineering and Regenerative Medicine, 9*(1), 13–23.
180. Schulze, M., & Tobiasch, E. (2012). Artificial scaffolds and mesenchymal stem cells for hard tissues. In C. Kasper (Ed.), *Tissue engineering III: cell-surface interactions for tissue culture* (pp. 153–194). Berlin, Heidelberg: Springer.
181. Nicolas, F. L., & Gagnieu, C. H. (1997). Denatured thiolated collagen. *Biomaterials, 18*(11), 815–821.
182. Huynh, T., Abraham, G., Murray, J., Brockbank, K., Hagen, P. O., & Sullivan, S. (1999). Remodeling of an acellular collagen graft into a physiologically responsive neovessel. *Nature Biotechnology, 17*(11), 1083–1086.
183. Berglund, J. D., Nerem, R. M., & Sambanis, A. (2004). Incorporation of intact elastin scaffolds in tissue-engineered collagen-based vascular grafts. *Tissue Engineering, 10*(9–10), 1526–1535.
184. Kovalic, A. J., Beattie, D. K., & Davies, A. H. (2002). Outcome of ProCol, a bovine mesenteric vein graft, in infrainguinal reconstruction. *European Journal of Vascular and Endovascular Surgery, 24*(6), 533–534.
185. Manduz, S., Katrancioglu, N., Ozker, E., & Dogan, K. (2008). Early thrombosis in bovine mesenteric vein grafts after infrainguinal reconstruction. *International Journal of Angiology, 17*(1), 37–39.
186. Lovett, M., Eng, G., Kluge, J. A., Cannizzaro, C., Vunjak-Novakovic, G., & Kaplan, D. L. (2010). Tubular silk scaffolds for small diameter vascular grafts. *Organogenesis, 6*(4), 217–224.
187. Marelli, B., Alessandrino, A., Farè, S., Freddi, G., Mantovani, D., & Tanzi, M. C. (2010). Compliant electrospun silk fibroin tubes for small vessel bypass grafting. *Acta Biomaterialia, 6*(10), 4019–4026.
188. Zhang, X., Wang, X., Keshav, V., et al. (2009). Dynamic culture conditions to generate silk-based tissue-engineered vascular grafts. *Biomaterials, 30*(19), 3213–3223.
189. Hu, X., Wang, X., Rnjak, J., Weiss, A. S., & Kaplan, D. L. (2010). Biomaterials derived from silk-tropoelastin protein systems. *Biomaterials, 31*(32), 8121–8131.
190. Syedain, Z. H., Meier, L. A., Bjork, J. W., Lee, A., & Tranquillo, R. T. (2011). Implantable arterial grafts from human fibroblasts and fibrin using a multi-graft pulsed flow-stretch bioreactor with noninvasive strength monitoring. *Biomaterials, 32*(3), 714–722.
191. Haisch, A., Loch, A., David, J., Pruß, A., Hansen, R., & Sittinger, M. (2000). Preparation of a pure autologous biodegradable fibrin matrix for tissue engineering. *Medical and Biological Engineering and Computing, 38*(6), 686–689.
192. Gui, L., Boyle, M. J., Kamin, Y. M., et al. (2014). Construction of tissue-engineered small-diameter vascular grafts in fibrin scaffolds in 30 days. *Tissue Engineering. Part A, 20*(9–10), 1499–1507.
193. Syedain, Z. H., Meier, L. A., Lahti, M. T., Johnson, S. L., & Tranquillo, R. T. (2014). Implantation of completely biological engineered grafts following decellularization into the sheep femoral artery. *Tissue Engineering. Part A, 20*(11–12), 1726–1734.
194. Scherner, M., Reutter, S., Klemm, D., et al. (2014). In vivo application of tissue-engineered blood vessels of bacterial cellulose as

small arterial substitutes: proof of concept? *The Journal of Surgical Research, 189*(2), 340–347.
195. Norotte, C., Marga, F. S., Niklason, L. E., & Forgacs, G. (2009). Scaffold-free vascular tissue engineering using bioprinting. *Biomaterials, 30*(30), 5910–5917.
196. Poldervaart, M. T., Gremmels, H., van Deventer, K., et al. (2014). Prolonged presence of VEGF promotes vascularization in 3D bioprinted scaffolds with defined architecture. *Journal of Controlled Release, 184*, 58–66.
197. Quint, C., Kondo, Y., Manson, R. J., Lawson, J. H., Dardik, A., & Niklason, L. E. (2011). Decellularized tissue-engineered blood vessel as an arterial conduit. *Proceedings of the National Academy of Sciences of the United States of America, 108*(22), 9214–9219.
198. Sundaram, S., One, J., Siewert, J., et al. (2014). Tissue-engineered vascular grafts created from human induced pluripotent stem cells. *Stem Cells Translational Medicine, 3*(12), 1535–1543.
199. Dhandayuthapani, B., Yoshida, Y., Maekawa, T., & Kumar, D. S. (2011). Polymeric scaffolds in tissue engineering application: a review. *International Journal of Polymer Science, 2011*(5110), 1–19.
200. Prewitz, M., Seib, F. P., Pompe, T., & Werner, C. (2012). Polymeric biomaterials for stem cell bioengineering. *Macromolecular Rapid Communications, 33*(17), 1420–1431.
201. Pashneh-Tala, S., MacNeil, S., & Claeyssens, F. (2015). The tissue-engineered vascular graft-past, present, and future. *Tissue Engineering. Part B, Reviews, 22*(1), 68–100.
202. Spina, M., Ortolani, F., El Messlemani, A., et al. (2003). Isolation of intact aortic valve scaffolds for heart-valve bioprostheses: extracellular matrix structure, prevention from calcification, and cell repopulation features. *Journal of Biomedical Materials Research. Part A, 67*(4), 1338–1350.
203. Carpentier, A. (2007). Lasker Clinical Research Award. The surprising rise of nonthrombogenic valvular surgery. *Nature Medicine, 13*(10), 1165–1168.
204. Magilligan, D. J., Lewis, J. W., Tilley, B., & Peterson, E. (1985). The porcine bioprosthetic valve. Twelve years later. *The Journal of Thoracic and Cardiovascular Surgery, 89*(4), 499–507.
205. Carpentier, A., Nashef, A., Carpentier, S., Ahmed, A., & Goussef, N. (1984). Techniques for prevention of calcification of valvular bioprostheses. *Circulation, 70*(3 Pt 2), I165–I168.
206. Sinha, P., Zurakowski, D., Kumar, T. K. S., He, D., Rossi, C., & Jonas, R. A. (2012). Effects of glutaraldehyde concentration, pretreatment time, and type of tissue (porcine versus bovine) on postimplantation calcification. *The Journal of Thoracic and Cardiovascular Surgery, 143*(1), 224–227.
207. Smadja, D. M., Saubaméa, B., Susen, S., et al. (2017). Bioprosthetic total artificial heart induces a profile of acquired hemocompatibility with membranes recellularization. *Journal of the American College of Cardiology, 70*(3), 404–406.
208. Dijkman, P. E., Fioretta, E. S., Frese, L., Pasqualini, F. S., & Hoerstrup, S. P. (2016). Heart valve replacements with regenerative capacity. *Transfusion Medicine and Hemotherapy, 43*(4), 282–290.
209. Adipurnama, I., Yang, M.-C., Ciach, T., & Butruk-Raszeja, B. (2017). Surface modification and endothelialization of polyurethane for vascular tissue engineering applications: a review. *Biomaterials Science, 5*(1), 22–37 http://pubs.rsc.org/en/Content/ArticlePDF/2017/BM/C6BM00618C.
210. Li, S., Sengupta, D., & Chien, S. (2014). Vascular tissue engineering: from in vitro to in situ. *Wiley Interdisciplinary Reviews. Systems Biology and Medicine, 6*(1), 61–76.
211. Hibino, N., Duncan, D. R., Nalbandian, A., et al. (2012). Evaluation of the use of an induced puripotent stem cell sheet for the construction of tissue-engineered vascular grafts. *The Journal of Thoracic and Cardiovascular Surgery, 143*(3), 696–703.
212. Weber, B., Robert, J., Ksiazek, A., et al. (2014). Living-engineered valves for transcatheter venous valve repair. *Tissue Engineering. Part C, Methods, 20*(6), 451–463.
213. Wang, Z., Cui, Y., Wang, J., et al. (2014). The effect of thick fibers and large pores of electrospun poly(ε-caprolactone) vascular grafts on macrophage polarization and arterial regeneration. *Biomaterials, 35*(22), 5700–5710.
214. Tara, S., Kurobe, H., Rocco, K. A., et al. (2014). Well-organized neointima of large-pore poly(L-lactic acid) vascular graft coated with poly(L-lactic-co-ε-caprolactone) prevents calcific deposition compared to small-pore electrospun poly(L-lactic acid) graft in a mouse aortic implantation model. *Atherosclerosis, 237*(2), 684–691.
215. Kang, T.-Y., Lee, J. H., Kim, B. J., et al. (2015). In vivo endothelization of tubular vascular grafts through in situ recruitment of endothelial and endothelial progenitor cells by RGD-fused mussel adhesive proteins. *Biofabrication, 7*(1), 15007. http://iopscience.iop.org/article/10.1088/1758-5090/7/1/015007/pdf .
216. Xu, C. (2004). Aligned biodegradable nanofibrous structure: a potential scaffold for blood vessel engineering. *Biomaterials, 25*(5), 877–886.
217. Pfeiffer, D., Stefanitsch, C., Wankhammer, K., et al. (2014). Endothelialization of electrospun polycaprolactone (PCL) small caliber vascular grafts spun from different polymer blends. *Journal of Biomedical Materials Research. Part A, 102*(12), 4500–4509.
218. Roll, S., Müller-Nordhorn, J., Keil, T., et al. (2008). Dacron vs. PTFE as bypass materials in peripheral vascular surgery—systematic review and meta-analysis. *BMC Surgery, 8*, 22.
219. Zhu, G.-C., Gu, Y.-Q., Geng, X., et al. (2015). Experimental study on the construction of small three-dimensional tissue engineered grafts of electrospun poly-ε-caprolactone. *Journal of Materials Science: Materials in Medicine, 26*(2), 112.
220. Hung, H.-S., Wu, C.-C., Chien, S., & Hsu, S.-h. (2009). The behavior of endothelial cells on polyurethane nanocomposites and the associated signaling pathways. *Biomaterials, 30*(8), 1502–1511.
221. Nieponice, A., Soletti, L., Guan, J., et al. (2008). Development of a tissue-engineered vascular graft combining a biodegradable scaffold, muscle-derived stem cells and a rotational vacuum seeding technique. *Biomaterials, 29*(7), 825–833.
222. Zhang, J., Qi, H., Wang, H., et al. (2006). Engineering of vascular grafts with genetically modified bone marrow mesenchymal stem cells on poly (propylene carbonate) graft. *Artificial Organs, 30*(12), 898–905.
223. Ghanbari, H., Mel, A., & de Seifalian, A. M. (2011). Cardiovascular application of polyhedral oligomeric silsesquioxane nanomaterials: a glimpse into prospective horizons. *International Journal of Nanomedicine, 6*, 775–786.
224. Lin, C.-Y., Wang, Y.-R., Lin, C.-W., et al. (2014). Peptide-modified zwitterionic porous hydrogels for endothelial cell and vascular engineering. *Biores Open Access, 3*(6), 297–310.
225. Mertens, M. E., Koch, S., Schuster, P., et al. (2015). USPIO-labeled textile materials for non-invasive MR imaging of tissue-engineered vascular grafts. *Biomaterials, 39*, 155–163.
226. Leferink, A., Schipper, D., Arts, E., et al. (2014). Engineered micro-objects as scaffolding elements in cellular building blocks for bottom-up tissue engineering approaches. *Advanced Materials (Weinheim, Germany), 26*(16), 2592–2599.
227. Berglund, J. D., Mohseni, M. M., Nerem, R. M., & Sambanis, A. (2003). A biological hybrid model for collagen-based tissue engineered vascular constructs. *Biomaterials, 24*(7), 1241–1254.

228. Vatankhah, E., Prabhakaran, M. P., Semnani, D., Razavi, S., Morshed, M., & Ramakrishna, S. (2014). Electrospun tecophilic/gelatin nanofibers with potential for small diameter blood vessel tissue engineering. *Biopolymers, 101*(12), 1165–1180.
229. Koch, S., Flanagan, T. C., Sachweh, J. S., et al. (2010). Fibrin-polylactide-based tissue-engineered vascular graft in the arterial circulation. *Biomaterials, 31*(17), 4731–4739.
230. Bertassoni, L. E., Cecconi, M., Manoharan, V., et al. (2014). Hydrogel bioprinted microchannel networks for vascularization of tissue engineering constructs. *Lab on a Chip, 14*(13), 2202–2211 http://pubs.rsc.org/en/content/articlepdf/2014/lc/c4lc00030g.
231. Ekaputra, A. K., Prestwich, G. D., Cool, S. M., & Hutmacher, D. W. (2011). The three-dimensional vascularization of growth factor-releasing hybrid scaffold of poly (epsilon-caprolactone)/collagen fibers and hyaluronic acid hydrogel. *Biomaterials, 32*(32), 8108–8117.
232. Yao, Y., Wang, J., Cui, Y., et al. (2014). Effect of sustained heparin release from PCL/chitosan hybrid small-diameter vascular grafts on anti-thrombogenic property and endothelialization. *Acta Biomaterialia, 10*(6), 2739–2749.