Selection of different endothelialization modes and different seed cells for tissue-engineered vascular graft

Qingjin Cai a,1, Wanshan Liao a,1, Fangchao Xue a,1, Xiaochen Wang a, Weiming Zhou a, Yanzhao Li b, Wen Zeng a,b,c,d,

a Department of Cell Biology, Third Military Medical University, Chongqing, 400038, China
b State Key Laboratory of Trauma, Burn and Combined Injury, Chongqing, China
c Departments of Neurology, Southwest Hospital, Third Military Medical University, Chongqing, China

A R T I C L E   I N F O
Keywords:
Tissue-engineered vascular grafts
scaffold materials
seed cells
vascular endothelialization
endothelial heterogeneity

A B S T R A C T
Tissue-engineered vascular grafts (TEVGs) have enormous potential for vascular replacement therapy. However, thrombosis and intimal hyperplasia are important problems associated with TEVGs especially small diameter TEVGs (<6 mm) after transplantation. Endothelialization of TEVGs is a key point to prevent thrombosis. Here, we discuss different types of endothelialization and different seed cells of tissue-engineered vascular grafts. Meanwhile, endothelial heterogeneity is also discussed. Based on it, we provide a new perspective for selecting suitable types of endothelialization and suitable seed cells to improve the long-term patency rate of tissue-engineered vascular grafts with different diameters and lengths.

Cardiovascular disease (CVD) is the most common cause of death worldwide, accounting for 31% of all deaths globally and being the cause of 40% of deaths in the Chinese population [1,2]. The number of deaths is expected to grow to more than 23.6 million by 2030 [3]. Autologous or allogeneic vascular transplantation is a common and effective method for the treatment of cardiovascular diseases. For example, the internal thoracic artery and the great saphenous vein are commonly used by surgeons as autologous vascular substitutes [4]. Besides, blood vessels are also needed for hemodialysis that cures diseases such as kidney failure [5]. The data show that more than 500,000 vascular bypass grafts are implanted in patients in the United States every year to replace damaged blood vessels [6]. However, autologous or allogeneic vessels couldn’t meet the clinical demands constantly because of vascular availability or donor shortage [7]. There is an increasing demand for vascular grafts that could be used for coronary artery bypass grafting and peripheral artery bypass grafting. Therefore, constructing tissue-engineered vascular grafts (TEVGs) is accepted as a promising and acceptable alternative solution. While autologous veins or arterial grafts with a diameter of 3–5 mm are used for aorta-coronary artery anastomosis constantly [8]. In other words, most of the patients could benefit from constructing small-diameter TEVGs (<6 mm internal diameter; ID).

1. Common types and materials of TEVGs

TEVG is a kind of vascular substitute with good biocompatibility and mechanical properties constructed by the tissue engineering methods. It contains three elements: seed cells, scaffold materials and signals [9]. In general, scaffolds are used as supporting structures to make seed cells adhere and proliferate, reaching functional maturity [10]. However, different scaffold materials have variant properties, the common scaffold materials of TEVGs (Fig. 1) will be discussed to select proper materials for producing TEVGs. Among them, suitable vascular scaffold materials should imitate the natural extracellular environment, provide appropriate mechanical and biological properties and possess good biocompatibility simultaneously [11].

Non-degradable synthetic materials such as expanded polytetrafluoroethylene (ePTFE), polyester (PET), and polyurethane (PU) have been used as substitutes for large blood vessels for decades due to their good mechanical properties, durability and convenient production [12].
In the treatment of superficial femoral artery occlusion diseases and renal diseases requiring hemodialysis, ePTFE stent grafts show satisfactory safety and short-term patency [13]. However, clinical studies found that the long-term patency rate of small-diameter ePTFE grafts were not encouraging [14]. Moreover, these materials are short of cellular communication signals and integrin-binding sites, which might decrease cell attachment and infiltration [15].

Shinoka et al. first to produce tubular scaffolds with regenerative and repair functions by using polyglycolic acid (PGA) [16]. Shum et al. constructed a PGA large-diameter scaffold with a diameter of 7 mm [17]. After the scaffold implanting, the percentages of collagen and DNA contents are close to those in the natural aorta, and the mechanical string-stress curve is close to that of natural blood vessels. PGA is also used to produce small-diameter TEVGs, which remain patent for 24 days [18]. However, PGA grafts degrade rapidly within 2–3 weeks and lose their mechanical integrity [19]. Compared with the PGA, poly-caprolactone (PCL) has a slower degradation rate and provides adequate mechanical properties effectively [20]. The porous scaffolds made of PCL have sufficient mechanical strength and porosity to satisfy the demand for clinical vascular transplantation [21]. However, poor regeneration of vascular walls, irregular cell infiltration and partial calcification are still obstacles to limiting PCL applications in the long run [22]. Take cell infiltration for example, pore sizes of PCL scaffolds play an important role in cell processes: the nanopore size membranes are helpful to acquire the collagen fibers and ECM, whereas macropores are significant in cell seeding and neo-vascularization in vivo [23]. Therefore, the macropores PCL grafts could enhance cell infiltration and extracellular matrix (ECM) secretion [24]. However, the irregular cell infiltration restricted the re-construction of vessels’ structure.

Natural polymer materials such as collagen, gelatin, and chitosan are nontoxic and have good biocompatibility, and that promote cell adhesion and retain differentiation function [25–27]. For example, Badhe et al. prepared a double-layer tissue-engineered scaffold with a mixture of chitosan and gelatin, which supports the growth and spreading of cells [28]. Collagen can be used for producing vascular grafts alone [29]. While by combining Hyaluronic acid (HA) and human-like collagen, the vascular scaffolds improve biophysical and mechanical properties that are close to those of the natural ECM [30]. It shows that collagen combined with other materials could demonstrate better prospects for future researches. However, as the activation signals of the coagulation pathway, collagen is easy to form thrombus, which limits collagen to being used in the clinical therapy [31–33]. Hyaluronic acid (HA) can promote adhesion and proliferation of ECs [34]. Moreover, HA is shown to be able to inhibit fibroblast proliferation, which plays a crucial role in vascular thrombosis and anastomotic hyperplasia [35]. When coating a HA hydrogel layer, the decellularized TEVGs have the benefits of inhibition of thrombus formation and promotion of endothelium attachment [36]. These studies indicate that HA has better application prospects for TEVGs.

The decellularized native blood vessels scaffolds retain the structure and properties of ECM. Abundant growth factors and cell adhesion signals are also left behind [37]. So decellularized scaffolds can serve as cell adhesion sites and provide mechanical strength support for TEVGs [38]. Moreover, decellularized scaffolds have many advantages, such as strong affinity, good biocompatibility, and extremely low immunogenicity, etc. However, there are also defects such as low accessibility, fast degradation rate, and inability to alter the content and structure of an ECM [39]. Besides, the implantation of large amounts of complex ECM into the vascular system may promote thrombosis [40].

To solve the problem of intimal hyperplasia and thrombosis of TEVGs, the coating modified on TEVGs is a feasible method. Studies have shown that the use of fluorosurfactant (FSP) or heparin coating can inhibit the intimal hyperplasia and thrombosis of ePTFE vascular grafts effectively [41]. The patency rate of polyurethane (PU) vascular grafts modified by heparin and cell-adhesive peptides was significantly higher than that of unmodified PU vascular grafts after 9 weeks of implantation [42]. Decellularized scaffolds coated with vascular endothelial growth factor (VEGF) and peptide also reduce intima formation and thrombus formation [43–45]. These studies suggest that coating on grafts can reduce intimal hyperplasia and thrombosis, which is important for the application of TEVGs.

Overall, non-degradable synthetic materials are mainly used to produce large-diameter TEVGs, while these materials are not recommended for small-diameter TEVGs. Due to the defects of polymer degradable materials and natural polymer materials, there is a trend of producing composite materials by mixing several materials to give play to complementary advantages. Many studies have shown that composite materials improve the grafts properties and provide positive results.
From a wide range of sources, decellularized scaffolds have good biological properties and low immunogenicity. Besides, decellularized scaffolds can promote the growth, proliferation, and differentiation of inoculated cells. These good properties make the decellularized scaffolds become a promising material in TEVGs and an active research field. Here, a more complete summary of the advantages and disadvantages of different scaffold materials is shown as follows (Table 1), which helps to provide a reference for choosing proper materials to construct TEVGs.

2. Selection of endothelialization forms for TEVGs with different lengths and diameters

Due to the environment, pressure and blood flow of TEVGs with different diameters and lengths are different in vivo, the patency of vascular grafts is significantly affected by cell composition, length, diameter, internal surface modification and pretreatment of TEVGs [60]. For example, with the same scaffold and diameter, the vascular patency time will decrease when the length of TEVGs increases (Table 2). Similarly, with the same scaffold and length, TEVGs of smaller diameters will shorten the vascular patency time (Table 3). That is, the patency time of TEVGs is positively correlated with the diameter and negatively correlated with length. Classifying the TEVGs and giving different solutions respectively might be a possible method to obtain a satisfactory long-term patency rate. In other words, TEVGs of different lengths and diameters should have different ways to achieve endothelialization.

2.1. Vascular grafts larger than 6 mm in diameter

With stable biological properties, PET and ePTFE are commonly used as substitutes for large-diameter (ID ≥ 6 mm) vascular grafts. The 5-year patency rate of large-diameter PET graft (7–9 mm) used in aortic bypass grafts is 93% [71,72]. These large vascular grafts usually do not need endothelialization actively to obtain satisfactory results. However, when these materials are applied to smaller-diameter vessels (ID < 6 mm), it is commonly difficult to reach expected targets due to occlusive thrombosis [73]. Besides, biomaterials are prone to chronic foreign body reactions, and fibrosis capsules are formed around the grafts, which is not favorable to the long-term patency of TEVGs [74,75].

Table 1
Advantages and disadvantages of different scaffold materials for TEVGs.

| Scaffold type               | Materials                  | Advantage                                                                 | Disadvantage                                                                 | References |
|----------------------------|----------------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------|------------|
| Non-degradable            | ePTFE, PET, PU, etc.       | *Have successfully been employed for decades to bypass and reconstruct medium to large diameter vessels | *Short of cellular communication signals and integrin-binding sites            | [7,12,15,51] |
| Biodegradable             | PGA, PCL, etc.             | *Can be tailored with specific physical properties to suit particular applications | *Tight degradation products and loss of mechanical properties during degradation | [19,20,52-54] |
| Natural polymer scaffold  | Collagen, gelatin, chitosan, etc. | *Promote adhesion and proliferation of ECs                                 | *Material sourced from an animal could lead to potential disease transmission  | [35,55-57]  |
| Decellularized scaffold    | Umbilical artery, umbilical vein, animal artery, etc. | *Keep the structure and properties of ECM                                 | *Fast degradation rate of scaffolds                                           | [9,39,58,59] |

Table 2
Comparison of patency of TEVGs with different length.

| Number | Scaffold                      | Inner diameter (ID: mm) | Length (cm) | Graft patency time | Date and References |
|--------|-------------------------------|--------------------------|-------------|--------------------|---------------------|
| 1      | Decellularized vascular scaffold | <4                       | 1           | 100% at 8 weeks    | 2014 [61]           |
| 2      |                               | <4                       | 3           | 100% at 2 weeks    | 2015 [62]           |
| 3      |                               | 2                        | 4           | 50% at 3 months    | 2013 [63]           |
|        |                               | 2                        | 7           | 100% at 4 weeks    | 2019 [64]           |
| 4      |                               | 4                        | 12          | 100% at 30 days    | 2011 [65]           |
|        |                               | 4                        | 6           | 80% at 6 months    | 2017 [66]           |

Table 3
Comparison of patency of TEVGs with different inner diameter.

| Number | Scaffold                      | Length (cm) | Inner diameter (ID: mm) | Graft patency time | Date and References |
|--------|-------------------------------|-------------|-------------------------|--------------------|---------------------|
| 1      | Decellularized vascular scaffold | 15          | 3.5-4.5                 | 100% at 6 weeks    | 2017 [67]           |
| 2      |                               | 15          | 6                       | 60% at 6 months    | 2017 [68]           |
|        |                               | 1           | <4                      | 100% at 8 weeks    | 2014 [61]           |
|        |                               | 1           | 1.3                     | 100% at 4 weeks    | 2014 [69]           |
|        |                               | 1           | 1                       | 100% at 3 weeks    | 2020 [70]           |
2.2. Vascular grafts with a diameter of 4–6 mm and a length less than 5 cm

Small-diameter vascular grafts are prone to inducing obstruction because of thrombosis, so these vascular grafts should possess some properties to avoid thrombosis [76]. Under normal conditions, ECs regulate blood coagulation and platelet functions, which reduces platelet aggregation and inhibits thrombosis. Once vascular injury (EC injury or detachment) occurs, thrombi will form at the site of the injury to prevent excessive blood loss (Fig. 2). So endothelialization is viewed as one of the most important factors in inhibiting thrombosis [77]. Therefore, vascular grafts with endothelium are considered as a key to inhibiting intimal hyperplasia and thrombosis [78,79].

Compared with decellularized vascular grafts of non-endothelialization, re-endothelialized decellularized vascular grafts have an excellent anti-thrombus property and ability to inhibit the abnormal proliferation of smooth muscle cells (SMCs). That avoids the occurrence of intimal hyperplasia and maintains patency for 6 weeks after implantation [67]. In an endothelialization study of porcine coronary arteries using adipose stem cells, there is integration between host tissue and implanted tissue, and the graft patency rate reaches 100% [81]. One study found that ECs on implanted vascular grafts originated entirely from the host, while seed cells were lost after vascular transplantation [82]. Therefore, the in situ endothelialization of TEVGs with a diameter of 4–6 mm is a feasible option.

2.3. Vascular grafts less than 4 mm in diameter and less than 5 cm in length

With an inner diameter of less than 4 mm, the vascular grafts usually form thrombus after implantation in the early stage, resulting in vascular occlusion and failure to maintain long-term patency. In one study about the decellularized vascular grafts (length: 1 cm; ID: ~1–1.5 mm), which was constructed by the static seeding method in 5 days. After the 14-day follow-up, only one of six rats was alive in the non-endothelialization group, the vascular grafts were short of ECs and there was endothelial damage and thrombotic plaque formation on the luminal side. In contrast, four of six rats were alive in the re-endothelialization groups, ECs are observed in the implanted vascular grafts and there were fewer thrombotic plaques or fibrin clots that were noted in the lumen [83]. Besides, compared with the control group, the decellularized vessels of re-endothelialization in vitro retained >95% monolayer EC coverage in 1 h after implantation, endothelial coverage increased. 9 weeks later, intimal hyperplasia decreased, and growth of the basement membrane was observed [84]. Two patients were treated by the grafts with endothelialization, there was no myocardial infarction occurred, and the grafted vessels remained patent in the six-month follow-up [85]. At six months after the implantation of the reendothelialized decellularized porcine aorta, the vascular graft had acquired a complete layer of ECs, and the fibroblasts had grown uniformly into the medial membrane, showing a structure similar to that of normal arteries, with good patency and no apparent thrombosis [66].

In a word, TEVGs (ID: >6 mm, length: >5 cm) do not need to facilitate endothelialization since the faster blood flow speed does not easily form thrombus; TEVGs (ID: 4–6 mm, length: <5 cm) are prone to thrombosis and graft occlusion due to the decreased blood flow velocity and smaller blood vessel diameter, so this type of TEVGs requires vascular endothelialization. Compared with endothelialization in vitro, relying on seed cells to realize endothelialization in vivo is a safer and more convenient option. For TEVGs (ID < 4 mm, length: <5 cm), acute thrombus occlusion may occur after implantation in the early stage, leading to a major cause of graft failure. Therefore, it is necessary to implant TEVGs after endothelialization in vitro.

3. Selection of different seed cells for endothelialization of TEVGs

The vascular endothelial layer has antithrombotic functions and prevents platelet adhesion, which is very important to increase the patency of vessels and reduce the incidence of vascular stenosis or occlusion [93]. Studies (Table 4) showed that even though the inner diameter and length of TEVGs are different, re-endothelialized TEVGs get better results in increasing the patency rates of grafts than TEVGs without endothelialization. Therefore, a complete endothelial layer is crucial to the TEVGs, and selecting the ideal seed cell source of the vascular endothelial layer is considered a key factor. The sources of common endothelial seed cells in studies are shown in Fig. 3. To meet clinical needs, seed cells should have the following characteristics: (1) sufficient number of seed cells to meet the requirements, and the sources of acquisition should be diverse; (2) the processes of isolating and acquiring seed cells should be relatively easy; and (3) trauma for both patients and donors should be minimized.

3.1. Umbilical vein endothelial cells (HUVECs)

To date, HUVEC is still the preferred seed cell for TEVGs. An analysis of all studies from 2013 to 2018 found that 59% of studies still used HUVECs as a source of human ECs [94]. The use of umbilical cord cells

Fig. 2. The role of endothelial cells in regulating thrombosis. Under normal physiological conditions, ECs secrete Prostacyclin (PGI2) and nitric oxide (NO), which are important for the regulation of blood coagulation and platelet functions by synergistically increasing cAMP content in platelets. Ecto-nucleotidase derived from ECs hydrolyze ATP and ADP to AMP and adenosine, which also reduces platelet aggregation. In addition to the factors above, ECs also inhibit thrombosis by inactivating coagulation factors and inhibiting thrombin activity [80]. When endothelium is damaged, negatively charged extracellular matrix (ECM) such collagen exposed to blood, result in accumulation of platelets, von Willebrand factor (vWF) and red blood cells (RBCs), eventually lead to the formation of thrombus [15].
has the advantage of harvesting the patient’s complete vascular structure without invasive procedure and obtaining approximately 20–30 cm of vascular tissue. Meanwhile, the umbilical cord can separate a large number of immature and fast-growing cells, so a sufficient number of cells can be obtained in a short time for scaffold seeding in a short time. Experimental studies have shown that the human umbilical cord artery (UCA) and umbilical cord vein (UCV) have similar cell growth characteristics to the saphenous vein, and no difference has been found in the mechanical properties of tissue engineering constructs [95]. Schechner et al. demonstrated that TEVGs with HUVECs could connect with the host circulatory system and be remodeled into complex capillaries after surgical implantation into severe combined immunodeficiency (SCID) mice [96]. However, it is difficult to purify umbilical cord-derived endothelial cells, which are easily mixed with a large number of fibroblasts and SMCs without antithrombotic ability. Moreover, HUVECs are unable to adhere to the scaffold materials firmly and easily washed away by blood flow, resulting in the formation of thrombus.

### 3.2. Mesenchymal stem cells (MSCs)

Mesenchymal stem cells (MSCs) exist in a variety of tissues and can be isolated, grown and differentiated into many cell lineages in vitro. After MSCs are implanted into the transplant recipient, there is no obvious immune rejection response [97]. Under natural conditions, some kinds of MSCs have biological properties that promote angiogenesis [98]. Among them, umbilical cord mesenchymal stem cells (UC-MSCs) are easy to obtain and not limited by ethical concerns. UC-MSCs also have a high proliferation rate and amplification potential in vitro, as well as the ability to downregulate the immune response and promote tissue repair. Moreover, UC-MSCs could reduce the risk of contamination and damage during amplification in vitro [99]. In recent years, MSC products derived from UCs have been increasingly introduced into clinical research, accounting for approximately 27% of current clinical trial products [100]. With the support of UC-MSC and cytokine supplementation, fibrin scaffolds can achieve efficient expansion of umbilical cord blood endothelial cells [101]. That provides a solution to the problem of limited and insufficient expansion in vitro. A recent report records cases of forearm venous thrombosis after getting UC-MSC treatment [102]. These cases may be associated with a higher level of the pro-coagulant factor TF/CD142 expressed by UC-MSCs and a stronger inflammatory response [103]. This puts forward new requirements for the potential safety issues of UC-MSCs and indicates the clinical application of UC-MSCs would be cautious. However, a retrospective study indicated that 93% of published studies had safe or positive results, and an additional 18% of published studies indicated that the applications of UC-MSC were safe and that any patient’s death in these studies was usually attributed to their underlying disease [104].

### Table 4
Comparison of patency rate of reendothelialized or non-reendothelialized small-diameter TEVGs.

| Number | Inner diameter (mm) | Length (cm) | Experimental group | Control group | Observing time | Date and References |
|--------|--------------------|-------------|-------------------|---------------|----------------|-------------------|
| 1      | 4                  | 5-6         | 66.7%             | 37.5%         | 24 weeks       | 2013 [86]        |
| 2      | 3                  | 5           | 83.3% (5/6)       | 16.7% (1/6)   | 3 months       | 2014 [87]        |
| 3      | 1                  | 0.6         | 89%               | 29%           | 4 weeks        | 2005 [88]        |
| 4      | 4                  | 4-5         | 100% (4/4)        | 0 (0/9)       | 6 months       | 2008 [89]        |
| 5      | 3-4                | 4           | 100% (2/2)        | 0 (0/3)       | 5 months       | 2010 [90]        |
| 6      | 1                  | 0.5         | 90% (9/10)        | 10% (1/10)    | 6 months       | 2018 [91]        |
| 7      | 4                  | 12          | 100% (5/5)        | 37.5% (3/8)   | 30 days        | 2011 [65]        |
| 8      | 3                  | 4-5         | 95% (19/20)       | 60% (12/20)   | 3 months       | 2012 [92]        |

Note: Experimental group: reendothelialized small-diameter TEVGs; Control group: non-reendothelialized small-diameter TEVGs.

**Fig. 3. Sources of common endothelial seed cells.** The most commonly used cells in tissue engineering blood vessels (TEBV) include human umbilical vein endothelial cells (HUVECs), mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), embryonic stem cells (ESCs), and pluripotent stem cells (iPSCs).
3.3. Endothelial progenitor cells (EPCs)

Endothelial progenitor cells (EPCs) are usually derived from bone marrow or peripheral blood, and EPCs have the potential to be induced to differentiate into ECs of various tissues [104,105]. EPCs can form vascular structures both in vitro and in vivo, which have permeability that is similar to the vascular endothelium, and EPCs may be well superior to vascular-derived endothelium in the formation of vascular networks [105]. There is a good prospect to obtain ECs from EPCs of peripheral blood with the noninvasive operation, which is beneficial to clinical promotion and application [106]. Moreover, endothelial progenitor cell-induced endothelial cells that are from porcine peripheral blood were similar to the normal aortic ECs of pigs in growth structures, phenotypes and functions [107]. Kaushal et al. use EPCs obtained from peripheral blood to construct TEVGs, which maintain patency for 130 days and show similar contractility and NO-mediated vasodilation to those of the natural carotid artery [108]. In addition, EPCs can be rapidly covered on implanted artificial vascular scaffolds [109], which provides important support for the in situ induction of vascular endothelialization.

Compared with EPCs derived from peripheral blood, cord blood endothelial progenitor cells (CB-EPCs) are more stable and have better physiological functions in TEVGs [110]. CB-EPCs proliferated in vitro and cultured on a three-dimensional PGA-PLLA scaffold, which retained an endothelial phenotype [111]. Martin et al. demonstrated the feasibility that cord blood and adult peripheral blood are used as sources of EPCs [112]. However, the TEVGs formed by EPCs in adult peripheral blood were unstable and gradually disappeared within 3 weeks. In contrast, the vascular function formed by CB-EPCs is normal and can be maintained for more than 4 months [110]. Therefore, CB-EPCs may be more suitable as a source of seed cells for TEVGs.

At present, the major limitations to the widespread of EPCs are isolating difficulty and few quantities in peripheral blood or bone marrow. In addition, the characteristics and mechanism of EPCs differentiation are also unclear, possibly due to the lack of standardized methods for isolation and identification [113]. Moreover, EPCs in different periods and subsets of EPCs are heterogeneous in angiogenic potential and tissue specificity [113,114]. CB-EPCs also have limitations in autologous cell therapy because most patients are unable to obtain their own cord blood. Establishing a cell bank is a good strategy to solve this problem [94].

3.4. Embryonic stem cells (ESCs)

Embryonic stem cells (ESCs) are unique cells population isolated from early embryos (before the gastrula stage) or primary gonads, which have the ability of unlimited proliferation, self-renewal and multipotent differentiation [115]. Moreover, the differentiation of ESCs into ECs is feasible in theory [116,117]. The differentiation of ESCs depends on their microenvironment, including biomechanical stress, cytokines or growth factors, ECM, and communication with neighboring cells [118]. Therefore, regulation of the extracellular environment can promote the differentiation of ESCs into ECs. The addition of exogenous ET versus can promote the directional differentiation of primitive endothelial cells derived from ESCs, and the differentiated endothelial cells show no differences from arteriovenous endothelial cells [119]. Leptin also regulates the differentiation of ESCs into ECs [120]. VEGF plays an important role in improving survival and migration of ECs induced by ESCs and promoting vascular remodeling [121,122]. In addition, regulating signaling pathways is an effective mechanism to promote directional differentiation of ESCs. Upregulating the Notch1 signaling pathway can promote the differentiation of ESC into ECs [123], while the downregulating TGF-β signaling pathway also has similar effects [124]. However, TGF-β signaling also plays a role in ESCs differentiating into SMCs [125], and the release of TGF-β stimulates extracellular matrix production by SMCs [126], which are the primary cell type in the pre-atherosclerotic intima [127]. Therefore, the specific mechanisms of between TGF-β and embryonic stem cell differentiation need to be further clarified. To accomplish efficient differentiation of ECs from ESCs, it is necessary to regulate the MAPK and PI3K signaling pathways, and this approach does not require cell sorting or magnetic purification to acquire a very pure endothelial cell population [128].

Although ESCs have good prospects for the future studies, there are still some problems to be solved. First, there is still no conclusion on the ethical controversy about ESCs [129]. Second, the serious risk of teratoma formation hinders the use of ESCs in TEVGs [130]. Therefore, how to isolate and identify ESCs that can’t form teratomas but can differentiate into ECs is a great challenge for researchers. In fact, solving the immune rejection of transplantation is also a huge challenge for the application of ESCs [131,132]. While establishing a cell bank through immune matching is a good solution strategy [133].

3.5. Induced pluripotent stem cells (iPSCs)

Since Shinya Yamanaka et al. succeeded in inducing somatic cells to pluripotent stem cells [134], induced pluripotent stem cells (iPSCs) have become the focus of research. The vascular grafts constructed by iPSC-induced ECs morphology and inflammatory response are similar to normal blood vessels [135]. The scaffold material or the decellularized vascular scaffold is similar to the basement membrane of normal blood vessels, which can provide good adhesion to ECs [136,137]. This promotes TEVGs to achieve endothelialization in vitro. Besides, the smooth muscle layer planted in vitro can provide ECs with microenvironment and biomechanical support close to normal blood vessels, which can better help TEVGs endothelialization in vitro [138]. ECs from iPSCs have the same angiogenic effect as those derived from ESCs [139], and the endothelialization potential is very similar [140]. Besides, iPSCs provide an unlimited cell bank that can be used to obtain a large number of SMCs and ECs with good biological characteristics [141].

Margariti et al. found that the PIPS-ECs displayed good attachment, stabilization, patency, and typical vascular structure when seeded on decellularized vessel scaffolds [142]. Samuel et al. successfully obtained ECs and multipotent progenitor cells (MPCs) from type 1 diabetic iPSC lines and used them to construct functional and durable blood vessels in vivo [143]. It shows that iPSCs from patients can also construct normal, patient-specific TEVGs. Li et al. constructed TEVGs by using vascular SMCs induced by iPSCs and found that the new type of TEVG can withstand surgical operation and arterial pressure. But there appeared to be dilatation in the vascular grafts [144]. That is because the hiPSC-derived TEVGs have low mechanical strength that leads to significant radial dilation after implantation. Luo et al. produced the hiPSC-derived TEVGs, which have good patency without luminal dilatation, effectively maintaining mechanical and contractile function [145]. This is a beneficial attempt at exploration for the clinical study of TEVGs seeded with iPSCs. In addition, iPSCs derived from peripheral blood are successfully used to construct TEVGs, providing a new basis to obtain somatic cells as seed cells conveniently and rapidly [146]. Some studies suggest that some kinds of iPSC-derived cells show immunogenicity [147], but iPSC-derived ECs could tolerate the immune response and survive for a long time [148]. These low-immunogenicity iPSCs still retain their pluripotent stem cell potential and differentiation ability [149]. Li et al. achieved the allotransplantation of TEVGs based on iPSCs [144], which provides experimental evidence for the application of iPSCs in allograft transplantation.

However, pluripotent stem cells may cause malformations or tumors [150], and the cell differentiation of iPSCs is a complex and not fully defined process. Besides, the heterogeneity of cell subsets produced by iPSCs is also a major problem that researchers need to solve [151]. What’s more, the long time and high cost of reprogramming and differentiation process also limit the application of iPSCs [152].

In various types of seed cells, the most commonly used seed cells are HUVECs, while iPSCs are considered to be a better seed cell source.
iPSCs could come from the patients, and the iPSCs are differentiated to the same type of ECs to get the function similar to the endothelium of the transplanted site, which makes the TEVGs adapt to the nearby vascular endothelium and reduces the occurrence of complications. In addition, for patients with vascular abnormalities caused by gene mutations, the patient-specific iPSCs can be genetically edited to differentiate into normal vascular cells and regain vascular function. Based on the above conclusions, we proposed a tissue-engineered vascular therapy method based on patient-specific iPSC (Fig. 4). A more complete summary of these common seed cells is as follows (Table 5), which helps to know the advantages and disadvantages of the seed cells and to select proper seed cells for TEVGs.

Currently, research on the endothelialization of vascular grafts by using seed cells is in full swing. While some studies have found that seeded cells are not incorporated into the developing TEVGs, they are lost in the early stages after the implant, and TEVGs are composed of recruited host cells which mainly come from the adjacent vessel walls [82,177,178]. This may be a departure from the traditional concept that seed cells are viewed as part of neovessels. But with seed cells, vascular grafts have more satisfactory results in patency rate, intimal hyperplasia, and tissue regeneration [65,179]. Therefore, seed cells may only play a temporary role in the development of blood vessels at the early stages, and likely act in a paracrine manner to inhibit thrombosis and maintain long-term patency [157].

Besides, there is a problem with how the density of seed cells affects the endothelialization of TEVGs. In the process of planting in TEVGs, the density of ECs varies from $5 \times 10^5$ (decellularized scaffold) to $1 \times 10^8$/ml (PGA scaffold) [142,144]. When using bone marrow as a cell source, higher seeding densities may mitigate graft stenosis [180]. And a cell density of more than 400 cells per cm$^3$ of the scaffold material is needed because the cell density is able to prevent acute thrombosis and bring beneficial scaffold remodeling after implantation [181]. Therefore, for vascular scaffolds of different materials and diameters, appropriate cell density plays a very important role in achieving endothelialization in vitro.

### Table 5
Advantages and disadvantages of different seed cells.

| Seed cell | Advantages | Disadvantages | References |
|-----------|------------|---------------|------------|
| HUVEC | *Non-invasive harvesting method from ‘medical waste’*<br> *Can be easily isolated in high numbers*<br> *A large number of published studies – comparable results* | *Lack of organ-specificity*<br> *Heterogeneity of ECs*<br> *Limited replicative capacity in culture* | [94,153] |
| MSC | *Can be isolated from virtually all tissues*<br> *Multilineage differentiation potential*<br> *Paracrine and immunomodulatory properties*<br> *Antithrombogenic property* | *Heterogeneity of the MSC population*<br> *Harvested from elderly or diabetic patients have diminished regenerative potential in vascular tissue engineering*<br> *Difficulties in identifying and isolating native MSCs* | [154-160] |
| EPC | *Non-invasive means*<br> *Robust proliferative and blood vessel-forming abilities*<br> *Can create a non-thrombogenic surface* | *Standardization of detection and cultivation procedures is essential*<br> *Heterogeneity of EPC populations from different origins*<br> *Few quantity and isolating difficulty* | [161-166] |
| ESC | *Potential to differentiate into every cell type of the body*<br> *Consistency with physiological development* | *Ethical controversy*<br> *Risk of teratocarcinoma formation*<br> *Inefficiency of Induction differentiation* | [167-170] |
| iPSC | *Obtain large numbers of clinically relevant cells*<br> *A robust source of patient-specific cells*<br> *Avoid allergenic immune rejection* | *Potential genetic and epigenetic alterations*<br> *Tumorigenicity*<br> *Lack of robust and highly reproducible*<br> *Differentiation protocols*<br> *High costs* | [167,171-176] |

**Fig. 4.** A tissue-engineered vascular therapy strategy based on the patient’s iPSCs. Blood vessels from animals were decellularised preserving microarchitecture, and extracellular matrix. Then we acquired the decellularized scaffolds, which provide good adhesion to ECs. Meanwhile, somatic cells from the patient are reprogrammed to iPSCs. Next, the iPSCs differentiated to ECs and seeded on the decellularized scaffolds to achieve endothelialization of scaffold in vitro, finally implanted into patients.
4. Heterogeneity of vascular endothelium

4.1. Morphological and functional differences of arteriovenous endothelium

There are significant differences in morphology between arterial and venous endothelium [182,183]. Arterial ECs are long and narrow or oval, while venous ECs are short and wide, which is related to the fact that the velocity of blood flow in the venous circulation is significantly lower than that in the arterial circulation. There are also differences in intercellular junctions between the two endothelia, as well as in mediated intercellular adhesion and communication. For example, the intercellular junctions between arteries of all diameters were tighter than those in veins, especially postcapillary venules, which showed loose tight junctions [184].

Besides, the function between arterial endothelium and venous endothelium is also obviously different, which may be because of the different types of blood vessels and the sites within the vasculature [185]. Arteries have greater vascular tension and vasoconstriction than veins to maintain blood pressure and maintain blood perfusion. However, veins, especially postcapillary venules, are the main sites of leukocyte chemotaxis during inflammation [184,186]. In the oxidative stress response, the venous endothelium showed a significant increase in inflammation and a decrease in proliferative phenotype [187].

4.2. Difference of gene expression of the vascular endothelium in different parts

In the process of angiogenesis, progenitor cells gradually acquire markers of ECs phenotype and then are directionally differentiated into arterial, venous and capillary ECs [188,189]. ECs in different areas are heterogeneous cell populations [190,191], and each of them has unique molecular markers [192], but this molecular marker can be altered [193]. While tissue microenvironment may play an important role in regulating endothelial heterogeneity [194,195].

Mike et al. found six genes encoding brain endothelium—specific transcription factors [196]. Among them, Foxf2, Foxl2, Foxq1 and Lef1 play a role in the differentiation of the central nervous system ECs in early development; Pparg and Zip551 may contribute to the phases of late development or maturity. Besides, genes such as Ptgds and Atp1a2, which are involved in classical neurological expression processes such as neurotransmitter transport, axonal development and regulation of ion transmembrane transport, are significantly enriched in the brain ECs [197].

Renomedullary ECs upregulate OXPHOS and other genes involved in hypoxia reaction, glycolysis and oxidative phosphorylation to cope with hypertonic dehydration response [198]. Genes such as Myl2, Myl3 and Aqp7 that are specifically upregulated by cardiac ECs, and these genes are involved in processes such as myocardial tissue development, myofibril assembly and cardiac contraction [197]. Although the aorta is in the neighborhood of the heart, the genes such as Ehd3 and Fam167b are specifically expressed in aortic ECs and are not expressed in cardiomyocytes [199].

Compared with lymphatic ECs, pulmonary vascular ECs were highly enriched for the genes Epsa1, Klf4, Gata2, Klf2 and Sox17 [200]. Moreover, genes involved in immune function were significantly upregulated [197]. In newborn alveoli (AT1/AT2), the tissue factor of ECs located in different tissues and organs show functional, transcriptomic and metabolomic heterogeneity. That may match with the functions of relative tissues and organs. Besides, the differentiation of ECs relies on both epigenetic mechanisms and the interaction with the microenvironment [201]. Therefore, if we do not pay sufficient attention to endothelial cell heterogeneity at the transplantation site, the seed cells on TEVGs may not match the host endothelium in function.

4.3. Seed cells should be homogeneous with host endothelium

Due to the microenvironmental differences between arteries and veins, saphenous veins often used in coronary artery bypass surgery confront a problem of graft adaptation [202]. If there is no adaptability, the probability of transplant failure increased 13 times [203]. Kudo et al. found that ECs from veins are not matched with the artery when implanted in the arteries, which means that the heterogenous cell population is restricted in clinical application [204,205]. However, there is heterogeneity in seed cells from different sources. For example, ESC differentiates into the venous EC by default [206], and iPSC-derived arterial ECs exhibit functional differences from iPSC-derived venous ECs [207]. Due to the existence of endothelial heterogeneity, attention should be focused on the heterogeneity between seed cells and host endothelium, which may be a significant factor influencing vascular endothelialization. To maintain the long-term patency of TEVGs after implantation and make seed cells easy to adapt to the transplantation environment, reducing the heterogeneity between seed cells and host endothelium may be a solution. Besides, identifying endothelial heterogeneity is helpful for the construction of organ-specific TEVGs.

5. Summary and future

Compared with autologous or allogeneic vessels, TEVG is a good choice for the treatment of cardiovascular diseases because of decreased trauma, low immunogenicity, easy to preserve and so on. However, the scaffold materials, inner diameter, and length of TEVGs affect the application of TEVGs. So different endothelialization strategies should adapt to the TEVGs with different inner diameters and lengths. Further, 1) TEVGs (ID > 6 mm) do not require endothelialization; 2) achieving endothelialization in vivo is a safer and more convenient option for TEVGs (ID:4–6 mm, length:<5 cm); 3) for the TEVGs (ID < 4 mm, length:<5 cm), it is necessary to achieve endothelialization in vitro before implantation. Besides, cell heterogeneity and tissue heterogeneity should be considered in the selection of seed cells. Suitable seed cells may need to remain homogenous with the ECs of the host at the implantation site.

In future studies, the surface coating of the grafts and the preparation of composite materials can both improve the material properties, which are feasible and promising research directions. To reduce thrombosis and maintain long-term vascular patency, suitable seed cells should be selected in different environments for vascular endothelialization. However, which kind of seed cells are used in a special environment remains unknown. After vascular graft implantation, defining the function of seed cells is critical to promote further research. Besides, it is also meaningful and necessary for TEVGs to study the effects of between the density of seed cells and vascular endothelialization. From the perspective of clinical needs, TEVGs should be prepared faster, more efficiently and safely. At the same time, TEVGs should be inexpensive and convenient to store. In conclusion, the results of scientific research should be combined with clinical practice so that the research results benefit patients and society.

Declaration of competing interest

Authors do not have any conflicts of interest to declare.

Acknowledgements

This work was supported by The National Science Fund for Outstanding Young Scholars (No:31822021); The Key Research and Development Plan Young Scientists Program (No:2017YFA0106000); The National Key Research and Development Plan
References

[1] M.A. Evans, et al., Cardiovascular disease, aging, and clonal hematopoiesis, J. Am. Coll. Cardiol. 15 (2010) 419–438, https://doi.org/10.1016/j.jacc.2010.01.016.

[2] D. Zhao, et al., Epidemiology of cardiovascular disease in China: current features and implications, J. Nat Rev Cardiol. 16 (2019) 203–212, https://doi.org/10.1038/s41569-018-0125-x.

[3] S.C. Smith Jr., et al., Our time: a call to save preventable death from cardiovascular disease (heart disease and stroke), J. Glob. Health. 7 (2017) 297–305, https://doi.org/10.7189/jogh.07-02.02.

[4] C.C. Canver, et al., The effect of thick fibers and large pores of electrospun poly(lactic acid) scaffolds for vascular tissue engineering, J. Mater. Sci. Eng C Mater. Biol. Appl. 34 (2014) 393–401, https://doi.org/10.1016/j.msec.2013.09.044.

[5] H. C. Wu, et al., Cytoculture of endothelial and smooth muscle cells on a collagen membrane in the development of a small-diameter vascular graft, J. Biomat. Sci. Pol. 28 (2007) 1385–1392, https://doi.org/10.1165/rri.2006.11.012.

[6] C. van der Hauwaert, Human vascular tissue engineered blood vessels (TEBVs), J. Sci. Adv. Mater. Eng. 7 (2011) 71–77, https://doi.org/10.1002/adfm.201908963.

[7] D. Zhao, et al., Biphasic and different cell effects, J. Cytotechnology. 68 (2016) 355–369, https://doi.org/10.1007/s10875-016-9725-3.

[8] A. Zhang, et al., Microstructural responses in the polyurethane scaffolds that were fabricated by coimmobilization of heparin and cell-adhesive peptide, J. Adv. Funct. Mater. 26 (2016) 837–844, https://doi.org/10.1002/adfm.201503023.

[9] M. J. Bastijanic, et al., Dual biofunctional polymer modifications to address endothelialization and smooth muscle cell integration of ePTFE vascular grafts, J. Vasc. Surg. 60 (2014) 71–81, https://doi.org/10.1016/j.jvs.2014.01.029.

[10] L. X. Qiu, et al., Decellularization of fibroblast cell sheets for natural extracellular matrix scaffold preparation, J. Tissue Eng. Part A 26 (2020) 26–45, https://doi.org/10.1177/1945998019898599.

[11] W. S. Choi, et al., Enhanced patency and endothelialization of small-caliber vascular grafts fabricated by coimmobilization of heparin and cell-adhesive peptides, J. J. Sci. Adv. Mater. Eng. 8 (2016) 436–446, https://doi.org/10.1051/jasme/2016051.

[12] K. H. Sipilä, Tuning the biomimetic behavior of scaffolds for vascular applications, J. Tissue Eng. Part B Rev. 26 (2018) 90–97, https://doi.org/10.1089/ten.TEB.2019.0229.

[13] N. R. Richbourg, et al., Thrombin and collagen induce a feedback inhibitory signaling pathway in platelets involving dysregulated protein kinase A from an Ncappel-kappal complex, J. Biol. Chem. 285 (2010) 18352–18363, https://doi.org/10.1074/jbc.M110.077602.

[14] M. F. Kee, et al., Platelet mechanosensing of collagen matrices, J. Plast. Reconstr. Aesthet. Surg. 10 (2015), e012664, https://doi.org/10.1177/026982281562664.

[15] S. M. D. Clapp, et al., Uncovering RHAMM and CD44 receptor signaling pathways in vascular disease: uncovering RHAMM and CD44 receptor signaling pathways, J. Matrix Biol. 26 (2007) 58–68, https://doi.org/10.1089/jmb.2006.08.261.

[16] A. Ruiz, et al., Differentiation of cell adhesion and growth by copolymers of polyurethane with hyaluronic acid, J. Polym. Sci. 101 (2015) 2870–2882, https://doi.org/10.1002/jpol.34597.

[17] S. D. Dimirovskya, et al., Glycolycas-like hydrogel coatings for small diameter vascular grafts, J. Mater. Chem. B 30 (2020), 190963, https://doi.org/10.1039/d0mb00963a.

[18] M. L. F. Hiquigna, L.G. Griffiths, Small diameter xenogenic extracellular matrix scaffolds for vascular applications, J. Tissue Eng. Part B 26 (2020) 26–45, https://doi.org/10.1177/1945998019898599.

[19] Q. X. Qiu, et al., Decellularization of fibroblast cell sheets for natural extracellular matrix scaffold preparation, J. Tissue Eng. Part A 26 (2020) 71–81, https://doi.org/10.1177/1945998019898599.

[20] H. F. Mohamed, et al., Accelerated endothelialization and suppressed thrombosis formation of acellular vascular grafts by modifying with neointima-inducing peptide: a time-dependent analysis of graft patency in rat-aboralional transplantation model, Cells 8 (2019) 806–813, https://doi.org/10.3390/cells8060890.

[21] H. Yamanaka, et al., Cell-free submillimeter diameter vascular grafts for free flap survival in rat model, J. Biomater. Sci. Pol. 179 (2018) 156–163, https://doi.org/10.1080/09205063.2018.602232.

[22] S. H. Bajkov, et al., In vitro hemocompatibility and cytocompatibility of a three-layered vascular scaffold fabricated by sequential electrospinning of: PCL, collagen, and PLLA nanofibers, J. Biomat. Sci. 31 (2016) 438–449, https://doi.org/10.1080/09299071.2016.1198863.

[23] Y. C. Jiang, et al., Electrospun polycaprolactone/gelatin composites with two distinct mechanisms, J. J. Biomed. Mat. Res. A 108 (2020) 426–435, https://doi.org/10.1002/jbm.a.36825.

[24] Y. C. Jiang, et al., Electrospun polycaprolactone/gelatin composites with two distinct mechanisms, J. J. Biomed. Mat. Res. A 108 (2020) 426–435, https://doi.org/10.1002/jbm.a.36825.

[25] Y. C. Jiang, et al., Electrospun polycaprolactone/gelatin composites with two distinct mechanisms, J. J. Biomed. Mat. Res. A 108 (2020) 426–435, https://doi.org/10.1002/jbm.a.36825.

[26] Y. C. Jiang, et al., Electrospun polycaprolactone/gelatin composites with two distinct mechanisms, J. J. Biomed. Mat. Res. A 108 (2020) 426–435, https://doi.org/10.1002/jbm.a.36825.

[27] Y. C. Jiang, et al., Electrospun polycaprolactone/gelatin composites with two distinct mechanisms, J. J. Biomed. Mat. Res. A 108 (2020) 426–435, https://doi.org/10.1002/jbm.a.36825.

[28] Y. C. Jiang, et al., Electrospun polycaprolactone/gelatin composites with two distinct mechanisms, J. J. Biomed. Mat. Res. A 108 (2020) 426–435, https://doi.org/10.1002/jbm.a.36825.
I. L. Geenen, et al., Endothelial cells (ECs) for vascular tissue engineering: venous ECs are less thrombogenic than arterial ECs, J. J Tissue Eng Regen Med. 9 (2015) 7–8, https://doi.org/10.1016/j.jtider.2015.03.007.

N. G. Dela Paz, P. A. D. Cahill, E. M. Redmond, Vascular endothelium, J. Wiley Interdiscip Rev Dev Biol. 5 (2016) 186–209, https://doi.org/10.1002/2015-40417-9.

W. C. Aird, Phenotypic heterogeneity of the endothelium: I. Structure, function, and mechanisms, J. Cir Res. 100 (2007) 158–173, https://doi.org/10.1161/1. RES.0000255691.7641.4a.

P. A. Cahill, E. M. Redmond, Vascular endothelium – gatekeeper of vessel health, J. Atherosclerosis. 248 (2016) 97–109, https://doi.org/10.1016/j.atherosclerosis.2016.03.007.

B. Shrestha, et al., Differential arterial and venous endothelial redox responses to oxidative stress, J. Microcirculation 25 (2018), e12486, https://doi.org/10.1111/ micc.12486.

Q. Cai et al., Atherosclerosis.2016.03.007.

J. Fang, K. Hirschi, Molecular regulation of arteriovenous endothelial cell specification, J. F1000Res. 8 (2019), https://doi.org/10.21205/f100research.16791.1.

K. Red-Horse, A. F. Siekmann, Veins and arteries build hierarchical branching patterns differently: bottom-up versus top-down, J. Bioessays. 41 (2019), e1800198, https://doi.org/10.1002/bies.201800198.

H. Kaur, et al., Single-cell profiling reveals heterogeneity and functional patterning of GPCR expression in the vascular system, J. Nat Commun. 8 (2017) 15700, https://doi.org/10.1038/ncomms15700.

N. W. Charkin, K. K. Hirsch, Single cell analysis in vascular biology, J. Front Cardiovasc Med. 7 (2020) 42, https://doi.org/10.3389/fcmv.2020.00042.

K. L. Marcelo, et al., Regulation of endothelial cell differentiation and specification, J. Circ Res. 112 (2013) 1272–1287, https://doi.org/10.1161/j.ircirc.112.000506.

K. Wolf, et al., Molecular identity of arteries, veins, and lymphatics, J. J Vasc Surg. 69 (2019) 253–262, https://doi.org/10.1016/j.jvs.2018.06.195.

M. Potente, T. Makinen, Vascular heterogeneity and specialization in development and disease, J. Nat Rev Mol Cell Biol. 18 (2017) 477–494, https://doi.org/10.1038/nrm.2017.36.

F. Le Noble, et al., Flow regulates arterial-venous differentiation in the chick embryonic yolk sac, J. Development. 131 (2004) 361–375, https://doi.org/10.1242/dev.005929.

M. Hupe, et al., Gene expression profiles of brain endothelial cells during embryonic development at bulk and single-cell levels, J. Sci Signal. 10 (2017), https://doi.org/10.1126/scisignal.aag2476.

A. Jambusaria, et al., Endothelial heterogeneity across distinct vascular beds during homeostasis and inflammation, J. Elife. 9 (2020), https://doi.org/10.7554/elife.51413.

S. J. Dumas, et al., Single-cell RNA sequencing reveals renal endothelium specification, J. Circ Res. 112 (2013) 1272–1287, https://doi.org/10.1161/j.ircirc.112.000506.

W. Feng, et al., Single cell analysis of endothelial cells identified organ-specific molecular signatures and heart-specific cell populations and molecular features, J. Front Cardiovasc Med. 6 (2019) 165, https://doi.org/10.3389/fcmv.2019.00165.

M. Guo, et al., Single cell RNA analysis identifies cellular heterogeneity and adaptive responses of the lung at birth, J. Nat Commun. 10 (2019) 37, https://doi.org/10.1038/s41467-018-07770-1.

V. Mantrulio, et al., Angiogenesis in tissue engineering: as nature intended?, J. Front Bioeng Biotechnol. 8 (2020) 188, https://doi.org/10.3389/fbioe.2020.00188.

A. Muto, et al., Mechanisms of vein graft adaptation to the arterial circulation: insights into the neointimal algorithm and management strategies, Jpn. Cir J. 74 (2010) 1501–1512, https://doi.org/10.1253/circj.cj-10-0495.

C. D. Owens, et al., Vein graft failure, J. J Vasc Surg. 61 (2015) 203–216, https://doi.org/10.1016/j.jvs.2013.08.019.

F. A. Kudo, et al., Venous identity is lost but arterial identity is not gained during vein graft adaptation, J. Arterioscler Thromb Vasc Biol. 27 (2007) 1562–1571, https://doi.org/10.1161/jats.2006.27.11.1562.

A. Muto, et al., Eph-B4 prevents venous adaptive remodeling in the adult arterial environment, Jpn. J. Exp. Med. 208 (2011) 561–575, https://doi.org/10.1084/jjem.2011.2011854.

D. E. Glaser, et al., Functional characterization of embryonic stem cell-derived endothelial cells, J. J Vasc Surg. 48 (2011) 415–428, https://doi.org/10.1016/j.jvs.2011.05.1570.

S. Rosa, et al., Functional characterization of iPSC-derived arterial- and venous-like endothelial cells, J. Sci Rep. 9 (2019) 3826, https://doi.org/10.1038/s41598-019-40417-9.