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Review

A review of the evidence to support electrical stimulation-induced vascularization in engineered tissue

Ying-tong Wang [a, b], Xiao-ting Meng [a, *]

[a] Department of Histology & Embryology, College of Basic Medical Sciences, Jilin University, Changchun, PR China
[b] The Undergraduate Center of Hospital of Stomatology, Jilin University, Changchun 130021, China

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ABSTRACT

Tissue engineering presents a promising solution for regenerative medicine and the success depends on the supply of oxygen/nutrients to the cells by rapid vascularization. More and more technologies are being developed to facilitate vascularization of engineered tissues. In this review, we indicated that a regulatory system which influences all angiogenesis associated cells to achieve their desired functional state is ideal for the construction of vascularized engineered tissues *in vitro*. We presented the evidence that electrical stimulation (ES) enhances the synergistic promotion of co-cultured angiogenesis associated cells and its potential regulatory mechanisms, highlighted the potential advantages of a combination of mesenchymal stem cells (MSCs), endothelial cells (ECs) and ES to achieve tissue vascularization, with particular emphasis on the different biological pathways of ES-regulated ECs. Finally, we proposed the future direction of using ES to reconstruct engineered tissue blood vessels, pointed out the potential advantages and disadvantages of ES application on tissue vascularization.

Contents

1. Introduction

Based on cell self-assembly and engineering design *in vitro*, multi-potential stem cells can be used to construct 3D engineered tissue models which can simulate natural tissues structurally and

* Corresponding author.
E-mail address: mengxt@jlu.edu.cn (X.-t. Meng).
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functionally. However, these 3D engineered tissue models have some limitations, including the reconstruction of natural tissue architecture, complex cell composition, and functional vascular systems, among others. Physiologically, 3D thick engineered tissue requires a network of microvessels to provide oxygen and nutrients, as well as to deal with the discharge of metabolic waste. Studies have shown that the success of *in vitro* tissue engineering for constructing vital engineered tissues depend on the supply of oxygen and nutrients to cells in the tissue, with the abundant capillary network serving as the structural basis for its nutritional function [1].

Therefore, the survival and maturation of engineered tissue are affected by nutrient supply limitations deep in the tissue [2]. On the other hand, blood supply is also time-efficient and space-distance efficient. Cells can obtain large amounts of nutrients and oxygen, thus promoting and maintaining their survival, only when they are close to capillaries (distance <200 μm) [3]. If the blood supply cannot be quickly established, tissue necrosis occurs [4]. For these reasons, the manufacture of mature and functional vasculature systems has been a major focus in the field, which involves the rapid formation of vascular networks within tissues and the increasing complexity of engineered tissues' vascular cells to improve overall tissue function.

The ideal vascularized engineered tissues should mimic the hierarchical order of the vascular tree *in vivo*, containing arterioles, venules, and capillaries. Although several studies have shown success in developing microvascular network in engineered tissues, it is far from highly organized, branched, dense and stable mature vascular networks [5–7].

Recent studies have shown that electrical stimulation (ES) holds promise for vascular regeneration. It has been well documented that the endogenous electric fields (EFs) generated by the stream of blood flow influences the arterial diameter by increasing the secretion of NO from endothelial cells [8,9]. Moreover, Clover et al. [10] noted an increase in capillary density by 25% when treated with ES for 6 weeks. Experiments have proven that the direct response of different vascular wall elements to ES was dose and frequency-dependent [11]. It not only affected the directed migration, proliferation, re-orientation, and elongation of endothelial cells (ECs) but also augmented the lengths of the tube-like structures and increased the expression of vascular endothelial growth factor (VEGF), thus improving the rate of blood vessel formation [12]. Most importantly, ES promoted the differentiation and vasculogenesis/angiogenesis of mesenchymal stem cells (MSCs), which may further promote vascularization of engineered tissue [13–15].

This review provided a brief introduction to current vascularization strategies from a tissue engineering standpoint. We then discussed recent research on ES-promoted vascularization, with special emphasis on different biological pathways of ES-regulated ECs. Furthermore, we reviewed the evidence that ES enhances the synergistic promotion of co-cultured vasculogenesis-related cells and their potential regulatory mechanisms. Finally, we presented future directions for using ES to reestablish engineered tissue vasculature with desirable functionality, highlighting a successful path for clinical translation in regenerative therapy.

2. Current strategies of engineered tissue vascularization

The functional vascular system consists of arteries, veins and capillaries, and these tubules share some structural features. Their inner membrane is composed of ECs, which regulate coagulation, imparts selective permeability, and is involved in immune cell transport [16]. Capillaries are the smallest vessels, and their pericytes are adjacent to the monolayer EC and basement membrane to maintain their stability. Arterioles and venules are surrounded by several layers of smooth muscle cells (SMCs) outside the intimal layer.

Natural *in vivo* vascularization involves two processes (Fig. 1). The first is “vasculogenesis,” which involves the formation of new blood vessels from a single cell. This occurs mainly during embryonic development. The second process of blood vessel formation is “angiogenesis,” which creates new blood vessels from existing ones by sprouting or dividing them to increase tissue blood vessels. In adults, angiogenesis is the main process of blood vessel formation [17].

In this case, engineered tissue vascularization methods can be divided into two categories: *in vitro* vascularization and *in vivo* vascularization. *In vitro* vascularization may be a simulation of vasculogenesis and can be obtained through the structural design of biological scaffold, bio-printing or by co-culturing ECs with supporting cells in a self-organizing method under the induction of angiogenic promoted factors [13,18–20]. *In vivo* vascularization is achieved by transplanting engineered tissues constructed *in vitro* into the host. When engineered tissues are transplanted into the host, the host-derived blood vessel network invades the engineered tissues, and the angiogenesis process mimics the natural angiogenesis of tissues in the human body [21]. However, the invasion rate of host-derived blood vessels is difficult to ensure the supply of blood nutrients in deep part of engineered tissues to avoid tissue necrosis. Host-mediated angiogenesis is a slow process, with the rate of invasion of host blood vessels limited to a few tenths of a micron per day after *in vivo* transplantation. Studies have shown that complete vascularization of 1 mm graft tissue takes about 2~3 weeks [22,23]. Therefore, tissue-engineered grafts with diameters greater than the gas and nutrient diffusion limits (200 μm) require a pre-formed network of blood vessels [24].

Pre-vascularized tissue grafts require only inoculation rather than complete neovascularization, consequently face shorter periods of hypoxia and nutrient deprivation after transplantation, and therefore achieve higher survival rate *in vivo*. It has been identified that pre-vascularized tissue grafts were perfused only 4 days after transplantation, while non-vascularized grafts showed similar results 2 weeks later [25,26]. Moreover, the *in vitro* vascular network established by the self-organization approach more closely resembles the *in vivo* angiogenesis, morphology, and permeability [27].

As a result, the most common method for vascularizing engineered tissue is to incorporate ECs into the artificial tissue, forming a series of tubular structures [28]. It is worth noting that these tubular structures are primitive endothelial tubes rather than mature vasculatures, it does not include complete vascular lineages, such as SMCs and pericytes, which are critical for stabilizing the tubular structure, initiating vascular development and their functions [29]. Therefore, to increase the complexity and stability of micro-vessels, the adjacent mural cells, including pericytes or MSCs and vascular smooth muscle cells (vSMCs), also need to be added into the engineered tissue construction procedure.

Some groups have recently extracted vasculature from stem cell aggregates. With the proper stimulus, stem cells can differentiate into a range of cell types that mimic the cellular complexity of natural vasculature, leading to a more mature neovascularization phenotype. For example, hiPSC-derived ECs formed an authentic vascular plexus when cocultured with hiPSC-derived pericytes. This coculture system recapitulated (1) major steps of vascular development including EC proliferation and primary plexus remodeling, and (2) EC-mediated maturation and acquisition of contractile vSMC phenotype by pericytes. In addition, hiPSC-derived ECs integrated into developing vasculature as xenografts in zebrafish [30].

In general, the formation, maturation, and stabilization of microvascular systems require endothelium-pericyte-smooth

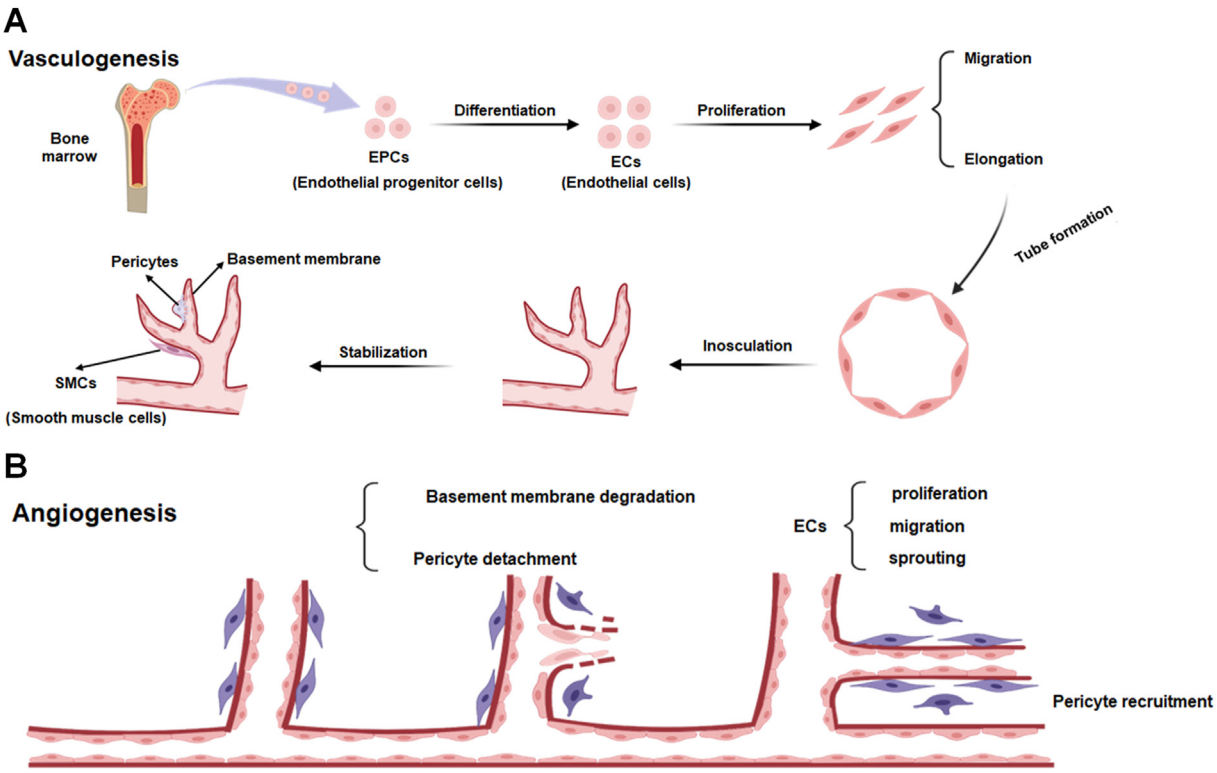

Fig. 1. Natural *in vivo* vascularization involves two processes. A: "vasculogenesis" which involves the formation of new blood vessels from a single cell. This occurs mainly during embryonic development. B: "angiogenesis" which creates new blood vessels from existing ones by sprouting or dividing them to increase tissue blood vessels.

muscle cell interactions. Therefore, a regulatory system that influences all these angiogenic cells to achieve their desired functional state is ideal for the construction of vascularized engineered tissues *in vitro*.

3. ES-regulated vascularization

3.1. ES-regulated tissue development and repair

It is well known that endogenous electric fields (EFs) play a key role in many important physiological processes, including embryogenesis, wound healing, tissue repair, and normal growth of organisms [31,32].

For example, during embryonic development, endogenous EFs appear on the medial side of the neural folds and on the lateral walls of the neural tubes. Blocking endogenous EFs can cause developmental defects in neural tube formation [31]. Regeneration of damaged tissues is also closely related to the intensity of endogenous EFs [33]. Furthermore, ES can reduce pain, help restore damaged nerve function, accelerate the healing of bone, cartilage, and other tissues [34,35]. As a result, ES is widely used in the treatment of tissue regeneration [36].

In vitro studies further demonstrate that the EFs can drive cell migration, proliferation, differentiation, and morphology changes in different cell types, including ECs. Each of these cellular behaviors is necessary to promote tissue development and regeneration [37].

3.2. ES promotes vasculogenesis and angiogenesis

The formation and development of blood vessels depend on the dynamic balance of angiogenic promoters and inhibitors. Physical regulators such as flow shear stress and ES also important for the regulation of vasculogenesis/angiogenesis. Rumiana T et al. [38] identified that gelatin-based materials in combination with the application of EF induced an angiogenic response in ECs with enhanced cell adhesion, increased production of VEGF and membrane metalloproteinases (MMPs).

On the other hand, tube formation was significantly increased when ECs in 3D culture were stimulated by EF for 4 h. Continuous EF-exposure further increased the length of the tubular structure which is an important sign of maintaining vascular stability [39].

How is ES-induced vasculogenesis/angiogenesis achieved? ECs are the cellular basis of vasculogenesis/angiogenesis, the formation of microvascular networks still mainly depends on the self-assembly behavior of ECs, which is inefficient and demanding without appropriate stimulus [40]. Consequently, it can be inferred that ES promotes vasculogenesis/angiogenesis mainly by regulating the behavior of ECs.

3.3. ES regulates ECs in different biological ways

ECs, usually the single flattened epithelium lining the inner surface to form the inner wall of the blood vessels. In the process of vasculogenesis/angiogenesis, ECs they first proliferate, migrate, and then elongate to form cell cords and subsequently form lumens. Interestingly, ES can promote the directed migration of ECs, accelerate their proliferation rate, improve cell adhesion, change the shape of cells, and improve the expression and secretion of vasculogenesis/angiogenesis related factors [41,42]. These changes in cell behaviors are all essential for vasculogenesis/angiogenesis [12].

In vitro experiments showed that ES had regulatory effects on ECs from different sources, including human umbilical vein ECs (HUVECs) and microvascular ECs (Table 1).

Cunha et al. [12] stimulated HUVECs and human microvascular ECs with different intensities of direct current (DC) EFs

and found that the two kinds of cells migrated towards the cathode under ES. The migration speed was faster than that without ES. The ES-response threshold of HUVECs is 50 mV/mm, while that of human microvascular ECs is 100 mV/mm. Another research showed that 50 mV/mm~200 mV/mm DC EF could also increase the expression of VEGF in 3D environment and promote the lengthening of blood vessels [39]. When fibroblasts were exposed to ES, the expression of VEGF was significantly increased, thus promoting the proliferation of HUVECs [44]. ES also induced endothelium-dependent contraction of human umbilical cord vessels when human umbilical cord arteries and veins rings were submitted to EFs at 60 V for 30 s, at 8–16 Hz in square-wave pulses, 0.3 ms pulse width, and 0.1 ms delay [52].

Based on these results, the elongation and connection of ECs in engineered cardiac tissue (ECT) are sought to be promoted by ES to achieve vascularization [40]. Liu et al. encapsulated hiPSC-cardiomyocytes and HUVECs in the hydrogels and 3D bioprinted as ECT constructs. The ECT constructs were stimulated by EF which the voltage amplitude was set to 2.5 and 5 V/cm respectively, corresponding to the physiological level and high intensity level. They demonstrated that EF-promoted vascularization in ECT was achieved from the following aspects: (1) HUVECs in the ES groups had obvious elongation and connection compared with the control group; (2) ES increased the proliferation of HUVECs and the aspect ratio of ECs and this state can be maintained until day 24; (3) ES significantly increased the number of junctions and total branching length of the endothelial network by about 4 times; (4) ES also increased the secretion of signal factors interacting with cardiomyocytes and ECs, suggesting that it may promote the cross-talk between the two types of cells. This work provides enlightenment and experience for the vascularization of different types of engineered tissues.

Not only ECs, but other cells that make up blood vessels also respond to ES. Bai et al. [46] used EFs to stimulate different kinds of vascular cells (including microvascular ECs, vascular fibroblasts, SMCs and HUVECs), and proved that the EFs of 150~400 mV/mm could induce the directed migration of all kinds of vascular cells. S. Derhambakhsh et al. investigated that phenotype switching of SMCs in response to ES and indicated that ES can change the phenotype of SMCs, and can be an effective option to prevent the aberrant proliferation of SMCs and subsequent occlusion of arteries in vascular tissue engineering [53].

3.4. ES increases the synergistic promotion of co-cultured vasculogenesis/angiogenesis-related cells

Most current tissue-engineered blood vessels are kind of pure composed by ECs in the tubes, which are more like the original fistula. These tubular structures are extremely unstable due to the absence of basement membrane, pericyte cells and other structures. Using a group of cells with different functions can simultaneously improve the efficiency of blood vessel formation and stabilization. In particular, those stem cells have the potential to differentiate into ECs, pericytes, smooth muscle cells and other vascular cells. For example, MSCs can promote angiogenesis in many ways, including differentiation into ECs and pericytes, secretion of bFGF, VEGF etc. and vessel stabilization (Table 2).

MSCs are multipotent stem cells and have been isolated from different tissues like bone marrow (bone marrow-derived mesenchymal stem cells, BMSCs), dental pulp (dental pulp stem cells, DPSCs), adipose tissue (adipose-derived mesenchymal stem cells, ADSCs), amniotic membrane (amniotic mesenchymal stem cells), etc. [64]. Co-culture of MSCs and ECs or HUVECs on 3D scaffolds improved MSC survival and vasculogenesis, suggesting that these different cell populations have a synergistic promoting effect [65,66]. After co-culture of HUVECs with human periodontal ligament stem cells, the expression of angiogenic genes was 6~9 times of the monoculture group, and capillary-like structures were formed in 14~21 days [67]. When DPSCs and HUVECs were encapsulated in GelMA hydrogel, highly cellular and vascularized dental pulp tissue were formed *in vivo*, which promoted cell attachment to the dentin surface and cell extension to dentin tubules to form a restorative dentin matrix [68].

Interestingly, ES provides superimposed promoting effects on the vasculogenesis function of these cells. When fibroblasts and HUVECs were exposed to EFs, ES promoted HUVECs migration and vasculogenesis by inducing fibroblasts to secrete FGF2 and VEGF [44]. Beugels et al. [48] conducted a 72-hr ES on ADSCs, and the results showed that ES could further promote the expression of VEGF and MCP-1 in ADSCs, inhibited the expression of anti-angiogenic factors Serpin E1/PAI-1, and eventually increased the vascular density, total vascular network length, and the number of branching points.

The role of ES in regulating group of cells to promote vasculogenesis was further demonstrated *in vivo*. Jeong GJ et al. [69] applied ES that produced by a wearable solar cell to induce

Table 1
ES-regulated vascularization and involved mechanisms.

ES type	Parameters	Results	Mechanisms	Refs
Sinusoidal EF	30 kV/m, 50 Hz, 24 h	Prolong Ca^{2+} transients of HUVECs	Involving activation of P2Y	[43]
DC EF	200 mV/mm, 4 h	Facilitates the angiogenesis of HUVECs by stimulating FGF2 secretion.	MAPK/ERK signaling pathway	[44]
DC EF	50~250 mV/mm, 10~24 h	1. Induce HUVECs directional migration to the cathode (voltage dependent). 2. Stimulate ET-1 and NO release.	None	[45]
DC EF	150~400 mV/mm, 24 h	Induce directed migration, reorientation, and elongation of all the vascular cells.	None	[46]
DC EF	70 mV/mm, 6 h	Regulate endothelial permeability.	Akt signalling pathway.	[47]
DC EF	150 mV/mm, 6 h	Promote blood vessel formation by activating migration and proliferation of ECs.	VEGF/VEGFR2 pathway	[39]
Biphasic electrical current	4 V/cm, 2 Hz, 6 ms pulses	Increase the vascular density, total vascular network length and number of branching points.	Promote angiogenic factors, Inhibit anti-angiogenic factors	[48]
DC EF	150 mV/mm, 1.5 mA, 12 h	Result in elongation of ECs.	None	[49]
DC EF	75~100 mV/mm, 4, 12, 24 h	Direct the reorientation, elongation and migration of ECs.	VEGF/VEGFR, PI3K-Akt, Rho-ROCK pathways	[50]
DC EF	200 μA, 4 h	Increase calcium deposition and CD31 expression.	BMP-2 and VEGF pathways	[51]
DC EF	50~300 mV/mm, 3 h	Direct migration of HUVECs and HMECs, increase proliferation and upregulate chemokines CXCR4 and CXCR2.	VEGF/VEGFR, PI3K-Akt, Rho-ROCK pathways	[12]

HMECs: Human microvascular endothelial cells.

Table 2
Potential ability of MSCs in vascularization.

Ability	Species	Models	Events and Results	Refs
Differentiation into ECs	Human	In vitro	11 genes were found that may be involved in the differentiation of MSCs into ECs.	[54]
	Human	In vivo	DPSC-derived cells were found tightly associated with the endothelial layers of brain vasculature, forming full blood vessels.	[55]
	Human	In vitro	BMSCs and DPSCs had positive paracrine effects on endothelial cell migration and *in vivo* blood vessel formation.	[56]
Differentiation into pericytes	Human	In vitro	BMSCs appear more suitable for engineering of mature vascularized networks than DPSCs.	[57]
Secretion bFGF	Human	In vivo	DPSC expressed bFGF, matrix metalloproteinases, and IGF-binding protein-3, which caused a significant increase in blood vessel count.	[58]
Secretion VEGF	Human	In vivo	DPSCs increased early vascular network formation by increasing VEGF expression.	[59]
	Human	In vivo	Secreted VEGF ligands and associated with vessels resembling pericyte-like cells.	[60]
	Human	In vitro	VEGF expressed by DPSCs can facilitate chemotaxis, cell growth, and cell differentiation in an autocrine fashion	[61]
	Human	In vitro	Secreted more vascular endothelial growth factor compared to ADSCs.	[62]
Vessel stabilization	Human	In vitro	Represented an effective source of pericytes and a promising ability to stabilize vessels and promote vascular maturation.	[63]

HMEC Human microvascular endothelial cell.
HUVEC Human umbilical vein endothelial cell.
BMSCs Bone marrow-derived mesenchymal stem cells.
DPSC Dental pulp stem cell.
FGF Fibroblast Growth Factor.
VEGF Vascular endothelial growth factor.
IGF insulin like growth factor.

angiogenesis in ischemic tissue. Solar cell-generated ES promoted the migration of MSCs toward the ischemic site, upregulated the expression of angiogenic paracrine factors, promoted the formation of capillaries and arterioles at the ischemic region, attenuated muscle necrosis and fibrosis, and eventually prevented loss of the ischemic limb.

Taken together, ECs and MSCs can secrete a series of pro-angiogenic factors to promote the formation of blood vessels. When the two are co-cultured, on the one hand, the extracellular matrix environment provided by ECs can promote the proliferation and differentiation of MSCs. Moreover, as pericytoid cells, MSCs can provide a local microenvironment for endothelial vascularization and enhance the stability of neovascularization. What's more, the addition of ES further promotes vasculogenesis of both types of cells.

3.5. Potential regulated mechanism of ES-promoted vasculogenesis/angiogenesis

It was well documented that ES activated downstream signaling pathways by activating ion channels or ES-sensitive genes, thereby affecting cell behavior. The VEGF signal is undoubtedly the primary mechanism of vasculogenesis/angiogenesis induced by ES. ES up regulated the expression of CXCR4 and CXCR2 chemokine receptors and promoted their phosphorylation, thereby promoting the release of VEGF and the generation of blood vessels during the process of wound healing [12]. Additionally, ES could induce the secretion of FGF-2 through the NOS pathway and activate the MAPK/ERK signaling pathway, subsequently promoting the expression of VEGF and influencing the migration, invasion and angiogenesis of HUVECs [44].

Furthermore, endogenous EFs could promote vascular formation of ECs by activating the VEGF receptor (VEGFR) signaling pathway directly [39]. EF treatment resulted in activation of VEGFR2, Akt, extracellular signal-regulated kinase 1,2 (Erk1/2), and the c-Jun NH2-terminal kinases (JNKs). This was further confirmed by the results in which a self-powered piezoelectric composite hydrogel induced activation of the AKT and ERK1/2 signaling pathways [70].

A recent study has shown that the directed migration of ECs in angiogenesis was associated with autophagy, which could be enhanced by EF. EFs induced the migration of HUVECs to the cathode by activating of ROS/SIRT1/FOXO1 pathway and enhanced autophagy. Genetic ablation of autophagy by silencing autophagy-related gene 5 (Atg5) eliminated EFs-directed HUVEC migration, suggesting that autophagy was required for EFs-guided cell migration [41].

Altogether, ES provides synergistic and superimposed promoting effects on the vasculogenesis function. It can not only induce the migration, proliferation and invasion of ECs by regulating different growth factors and modifying their downstream signaling pathways, but also can promote MSCs to differentiate toward ECs and pericytes, to further promote and stabilize vasculogenesis/angiogenesis.

ES has the ability to speed up vascularization in the following ways: (1) ES increases the proliferation of ECs; (2) ES promotes the migration of ECs; (3) ES promotes elongation and connection of ECs; (4) EFs promotes vascular formation of ECs by activating the VEGFR signaling pathway; (5) ES significantly increases the number of junctions and total branching length of the endothelial network; (6) ES also increases the expression and secretion of vasculogenesis/angiogenesis related factors, which including VEGF, bFGF; (7) ES promotes the differentiation of MSCs toward ECs and pericytes (Fig. 2).

Therefore, we believe that ES can shorten the time of blood vessel formation, although the specific time scale has not been proved experimentally, some existing studies have pointed out that ES significantly promoted the migration speed to 100 μm/h and increased the number of junctions and total branching length of the endothelial network by about 4 times [46].

4. Conclusions and perspectives

Regenerative therapy based on tissue engineering has greatly progressed. However, the engineered tissue constructs that obtained for clinical applications remain limited, in part due to a lack of vascularization. Pre-vascularized engineered tissue may provide better integration and overcome ischemia of defect sites after implantation. Clinically, well-designed pre-vascular structures will also reduce donor site morbidity and shorten operative time. Despite engineered tissue constructed with MSCs and ECs having a certain vascular structure, there is no consensus on the optimal

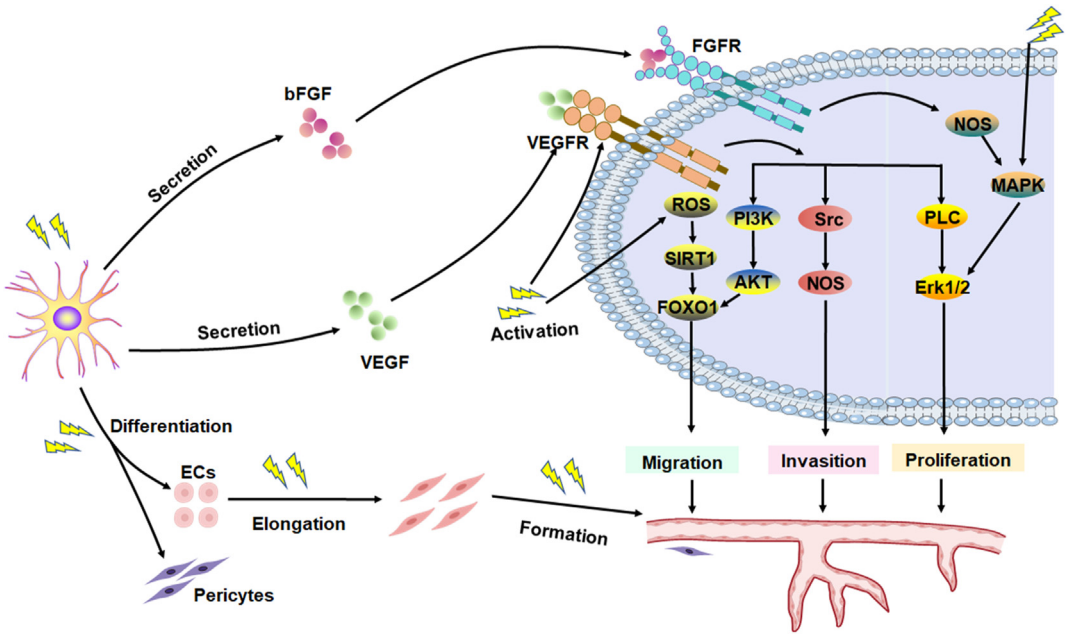

Fig. 2. ES provides synergistic and superimposed promoting effects on the vasculogenesis function. ES can enhance autophagy and promote ECs migration by activating ROS/SIRT1/FOXO1 signal. It can induce the migration/proliferation of ECs by either directly activating VEGFR or by promoting the secretion of VEGF and FGF2 by co-cultured stem cells/fibroblasts. ES can promote elongation and connection of ECs, increase the number of junctions and total branching length of the endothelial network, thus promote vascular formation. ES can promote the differentiation of MSCs toward ECs and pericytes.

procedure for *in vitro* vascularization, especially as the current rate of vascular regeneration of engineered tissue can't meet the nutrient supply of the tissue.

In this review, the authors highlight the potential advantage of a combination of MSCs, ECs and ES to achieve tissue vascularization of engineered tissue. ES can not only affect the directed migration and proliferation of ECs, but also increase the expression of VEGF, thus improving the speed of vasculogenesis/angiogenesis. In addition, ES-promoted differentiation and vasculogenesis/angiogenesis of MSCs may further promote vascularization of engineered tissues. Moreover, the application of ES is also procedurally controlled and systematic, the stimulation parameters can be optimized and adjusted, and it is easy to operate, allows for the systematic regulation of endothelial and stem cell vasculogenesis/angiogenesis and appears to open important avenues that will lead to more predictable and repeatable outcomes. Finally, it safe, cheap and can be applied to either pre-vascularization tissue engineering *in vitro* or directly to stimulate angiogenesis *in vivo*.

The application of ES also has disadvantages. When constructing pre-vascularized tissues, different cell types may require different stimulation parameters and need to be optimized for stimulation procedures, which increases the complexity of electrical stimulation. When ES is administered in the body, invasive stimulation may be required for tissues deep in the body. This may cause some side effects, including itching, tingling, etc. There are also contraindications for the use of ES, for example, patients with a history of seizures. Tissue with complex topography may affect electrical currents and cause unexpected side effects. ES may negatively affect the function of implantable medical devices such as cochlear implants and pacemakers.

Overall, these recent developments in the field of regenerative therapy point to new directions that can accelerate translation into clinical practice and benefit patients. Therefore, this article can certainly help other researchers in the field in the design and execution of their experiments, providing a new idea for the realization of accelerated vascularization of engineered tissue.

Author contributions

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Data availability statement

Not applicable.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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