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Vascularization strategies of engineered tissues and their application in cardiac regeneration☆

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ABSTRACT

The primary function of vascular networks is to transport blood and deliver oxygen and nutrients to tissues, which occurs at the interface of the microvasculature. Therefore, the formation of the vessels at the microcirculatory level, or angiogenesis, is critical for tissue regeneration and repair. Current strategies for vascularization of engineered tissues have incorporated multi-disciplinary approaches including engineered biomaterials, cells and angiogenic factors. Pre-vascularization of scaffolds composed of native matrix, synthetic polymers, or other biological materials can be achieved through the use of single cells in mono or co-culture, in combination or not with angiogenic factors or by the use of isolated vessels. The advance of these methods, together with a growing understanding of the biology behind vascularization, has facilitated the development of vascularization strategies for engineered tissues with therapeutic potential for tissue regeneration and repair. Here, we review the different cell-based strategies utilized to pre-vascularize engineered tissues and in making more complex vascularized cardiac tissues for regenerative medicine applications.

Contents

1. Introduction . . . 184
2. Isolated cell systems . . . 184
   2.1. Endothelial cells and endothelial progenitor cells . . . 184
   2.2. Co-cultures . . . 185
      2.2.1. Endothelial cells and perivascular cells (smooth muscle cells or pericytes) . . . 185
      2.2.2. Endothelial cells and mesenchymal stem cells . . . 186
      2.2.3. Endothelial cells and fibroblasts . . . 187
3. Isolated vessel systems . . . 187
   3.1. Large vessel systems . . . 187
   3.2. Microvessel systems . . . 187
4. Tissue complexity: vascularizing cardiac tissues . . . 189
5. Clinical trials . . . 190
6. Future directions and summary . . . 192
Funding sources . . . 192
References . . . 192

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1. Introduction

A functional vascular network is essential to promote oxygen transfer, to deliver nutrients and to dispose of metabolic waste. It also has a fundamental role in promoting the circulation of immune cells, allowing for fast immune cell trafficking to surrounding tissues when needed. It plays a crucial role in a variety of biological process, including the regulation of metabolism [1], development [2], healing [3], regeneration [4], immune response [5] and the progression of many diseases [5,6]. Such multi-function nature derives from its network structure coupled with the presence of specific cell types that form each blood vessel.

There are different types of blood vessels such as arteries, arterioles, capillaries, venules, and veins. Blood flows away from the heart into arteries that branch into smaller arteries, which will eventually branch into arterioles and capillaries. Capillaries are narrow, thin-walled vessels that connect arterioles and venules and, given their thin walls, allow for the exchange of oxygen, nutrients and metabolic waste with the surrounding tissue. Then, blood flows from the capillaries into very small veins called venules, then through veins back to the heart. Thus, the primary function of supplying nutrient and oxygen to tissue occurs at the interface of the microvasculature, which is the network of small blood vessels that include arterioles, capillaries and venules [7]. Vessels are lined by endothelial cells (ECs) which are surrounded by perivascular cells (PVCs) such as smooth muscle cells (SMCs) or pericytes [8]. Such structure is supported by the extracellular matrix (ECM) [9] and is essential for proper vascular function.

The formation of new blood vessels occurs by the de novo formation of blood vessels, or vasculogenesis; which occurs mostly during embryogenesis but can also take place in adult neovascularization. This process relies on the formation of new blood vessels from single ECs in the developing embryo and endothelial progenitor cells (EPCs) in the adult. The formation of new blood vessels from pre-existing ones, known as angiogenesis happens through vessel sprouting and/or in intussusception (a process where the vascular wall expands and divides an existing vessel in two). Vessel sprouting is a highly regulated, multi-stage process where quiescent ECs respond to angiogenic signals such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), and release metalloproteinases (MMPs) that degrade the ECM. Then, an endothelial tip cell leads the way of the newly growing sprout following gradients of growth factor. This migration is mediated by signaling through EC surface proteins including Notch and VEGF receptors [10]. The ECs situated behind the tip cells, referred to as trunk cells, start dividing to increase the new vessel length and to form a lumen. Once the new, immature vessel is formed, ECs are stabilized by the recruitment of SMCs and/or pericytes in response to, for example, transforming growth factor-β (TGF-β) [11] and platelet-derived growth factor B (PDGF-B) [12] and a new, mature, blood perfused vessel is formed.

Many diseases result from insufficient blood supply, a condition called ischemia. For example, in cardiovascular diseases such as myocardial infarction (MI), ischemia ensues after blood flow to the heart is stopped due to the occlusion of one or more blood vessels that feed the heart muscle [13]. This can lead to the significant death of the heart contractile cells, called cardiomyocytes, and may result in loss of heart function or heart failure. Heart transplantation is a possible treatment for heart failure. However, given the low availability of donors there is a need for the development of new, alternative treatments. One alternative for tissue regeneration lies in tissue engineering strategies where an organ would be regenerated by generating the desired specific tissue containing all the different cell types organized in a tissue-specific architecture.

One pivotal aspect for the generation of larger scale tissues that can be used for tissue regeneration is the formation of a functional vascular network that will allow the survival of the tissue *in vivo*, by promoting blood perfusion. Thus, different strategies have been pursued to incorporate vascular networks within biodegradable and/or biocompatible biomaterials to generate vascularized engineered tissues and allow for its integration with host tissue after transplantation. Growth factor-based vascularization approaches have been extensively reviewed elsewhere [14–16]. Here, we review cell-based approaches using isolated cell or vessel systems to vascularize engineered tissues, and discuss current methods being used to generate vascularized cardiac tissues for use in tissue regeneration.

2. Isolated cell systems

Multiple cell sources are required for *in vitro* vascular engineering. These include ECs and PVCs. These cells can be obtained from different sources, such as EPCs or embryonic and induced pluripotent stem cells (ESC and iPSC), which can be a source of ECs, SMCs, mesenchymal stem cells (MSCs) or pericytes. Early studies have focused on building a vasculature from isolated cells utilizing only ECs and more recently EPCs (Fig. 1A, B).

2.1. Endothelial cells and endothelial progenitor cells

Primary ECs can be obtained from various tissues, including umbilical cord blood vessels, the dermal vasculature, the lungs and the pulmonary artery. These primary ECs have been widely used in vascular engineering and require little manipulation prior to transplantation. EPCs, such as endothelial colony-forming cells (ECFCs) have also been investigated for vascular tissue engineering. The interactions of ECs with their surrounding ECM are essential to modulate neovascularization *in vitro* and *in vivo* [9]. Thus, the most common *in vitro* angiogenesis models utilize ECs in 3-dimensional (3D) gels made of various ECM components such as fibrin [17], collagen [18–22], fibronectin [23,24] and basement membrane extracts or Matrigel [25].

In an early study, human umbilical vein endothelial cells (HUVECs) were sandwiched between two 3D fibrin gels [17]. When the second fibrin gel was formed overlying the EC monolayer, the cells formed capillary tubes within 5 h in the presence of low serum concentrations [17]. These were fully developed into long and thin tube-like structures by 24 h [17]. Another system for 3D neovessel formation *in vitro* relied on seeding bovine pulmonary artery ECs on gelatine-coated microcarriers, which were subsequently suspended in a fibrin gel [26]. When stimulated with fibronectin, these entrapped ECs assembled to form multicellular capillary-like sprouts by day 6 [26]. Culture with growth factors such as bFGF or VEGF increased capillary formation by day 3 [26]. Such fibrin-based gel systems can also be used to incorporate different ECM components to accelerate or promote angiogenesis *in vitro*. For example, embedding a recombinant peptide of human thrombospondin-1 containing the N-terminal domain of the ECM molecule in fibrin gels accelerated the formation of tube-like structures of HUVECs [27]. This was shown to be dependent of binding to the cell surface receptor syndecan-4 [27]. Yee et al. used a 3D hyaluronic acid (HA) hydrogel scaffolds for formation of capillary-like structures from ECFCs and showed that the process depended on the physical properties of HA hydrogel [28]. Decreased matrix viscoelasticity, i.e., softer matrices, corresponded to a loose ultrastructure and significantly increased ECFC vascular tube length and area [28].

In addition to natural biodegradable materials such as fibrin, synthetic biomaterials can also be used to build scaffolds for EC-based vascularized organoids. Moon et al. developed a synthetic, bioactive poly(ethylene glycol)-diacrylate (PEGDA) hydrogel, which is photopolymerizable, non-cell adhesive and resistant to protein adsorption [29]. Then the surface of PEGDA hydrogels was micropatterned with a cell binding ligand, Arg-Gly-Asp-Ser (RGDS) in various densities and geometries via photolithography [29]. ECs cultured on these RGDS substrates underwent morphogenesis and formed capillary-like structures depending on the geometrical cues (width and density) of the RGDS patterns [29].