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Biomaterials to Prevascularize Engineered Tissues

Lei Tian · Steven C. George

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Abstract Tissue engineering promises to restore tissue and organ function following injury or failure by creating functional and transplantable artificial tissues. The development of artificial tissues with dimensions that exceed the diffusion limit (1–2 mm) will require nutrients and oxygen to be delivered via perfusion (or convection) rather than diffusion alone. One strategy of perfusion is to prevascularize tissues; that is, a network of blood vessels is created within the tissue construct prior to implantation, which has the potential to significantly shorten the time of functional vascular perfusion from the host. The prevascularized network of vessels requires an extracellular matrix or scaffold for 3D support, which can be either natural or synthetic. This review surveys the commonly used biomaterials for prevascularizing 3D tissue engineering constructs.

Keywords Prevascularization · Tissue engineering · Natural · Synthetic · Scaffold

Introduction

Tissue damage and organ failure create a significant burden, both financially and in lost lives, each year in the world [1]. While tissue transplantation can partially solve the problem [2], there remains a significant discrepancy between the demand and supply of transplantable tissues [3]. Over the past two decades, this discrepancy has fueled the field of tissue engineering to create functional and transplantable tissues for organ replacement.

Currently, the dimension of most functional engineered tissues is limited to a few hundred microns [4, 5] due to the diffusion limit of oxygen in cell-dense (e.g., muscle) tissues [6]. Implanted tissues usually take days or weeks to develop new blood vessels in vivo; during this process, an insufficient supply of nutrients and oxygen predisposes the tissue to ischemia [7] and nutrient depletion, which can compromise cell viability and function [8, 9]. Hence, only thin tissues or those with a small metabolic demand such as skin [10], cartilage [11], and cornea [12] have been successfully engineered. To design larger tissues, nutrients and oxygen need to be delivered via perfusion (or convection) rather than diffusion alone. One strategy is to prevascularize tissues; that is, create a network of blood vessels within the tissue construct prior to implantation.

Prevascularization generally refers to the formation of a well-connected capillary or microvessel network within an implantable tissue prior to implantation. The microvessels can be created by either angiogenesis or vasculogenesis. Angiogenesis is the growth of new blood vessels from pre-existing vessels [13]. Vasculogenesis is the spontaneous de novo formation of undifferentiated endothelial cells to blood vessels [14]. Following implantation, a small number of anastomoses with the host circulation can then rapidly deliver nutrients and oxygen and remove waste products throughout the tissue construct [5, 15]. Prevascularization shortens the time of functional vascular perfusion significantly [16] compared to alternative methods such as delivering exogenous growth factors (e.g., vascular endothelial growth factor, VEGF), within an acellular scaffold [17]. For the latter, host blood vessels need to invade into the center of engineered tissue, a process that can take days to weeks [18, 19].

L. Tian · S. C. George
The Edwards Lifesciences Center for Advanced Cardiovascular Technology, University of California,
Irvine, CA, USA

L. Tian · S. C. George
Department of Biomedical Engineering, University of California,
Irvine, CA, USA

S. C. George (✉)
Department of Chemical Engineering and Material Science, University of California,
Irvine, CA, USA
e-mail: scgeorge@uci.edu

To prevascularize a tissue (Fig. 1.), the first step is to encapsulate endothelial cells together with stromal cells, such as fibroblasts or smooth muscle cells, within a scaffold. The scaffold can initially be in liquid form; for example, in collagen and fibrin scaffolds cells are encapsulated in the liquid matrix prior to polymerization or gelation. The scaffold can also be a pre-formed porous structure, such as polylactic–glycolic acid (PLGA), where cells are seeded onto the scaffold after polymerization. In either case, the cellularized scaffold is then cultured in growth factor supplemented media to encourage the development and maturation of an in vitro vessel network. After a well-connected capillary network is formed, the engineered construct is implanted into the host (e.g., mouse) to allow rapid anastomosis with the host circulation system.

Several endpoints have been used generally to quantify the vessel network for both in vitro and in vivo studies. Examples of endpoints include the time to blood perfusion of the implant with host circulation after implantation, changes in endothelial-cell-specific marker expression, total vessel length per unit area (e.g., centimeters per square centimeter) based on bright field microscopy or endothelial cell surface antibody staining (e.g., anti-human CD31), vessel density per unit cross-section area (e.g., vessel per square centimeter) after histology staining, and even evidence of new collagen synthesis in the implant [20–22].

A wide range of biomaterials have been used to prevascularize an implantable tissue (Fig. 2). An ideal biomaterial should mimic structural and functional properties of the natural extracellular matrix (ECM). This includes providing appropriate (1) binding sites for cell–material interactions [23], (2) mechanical properties to maintain cell phenotype and function prior to host remodeling, and (3) biodegradation in terms of rate and biocompatible breakdown products. This review surveys the most commonly used biomaterials used for prevascularizing 3D tissue engineering constructs, with an emphasis on cellular scaffold applications.

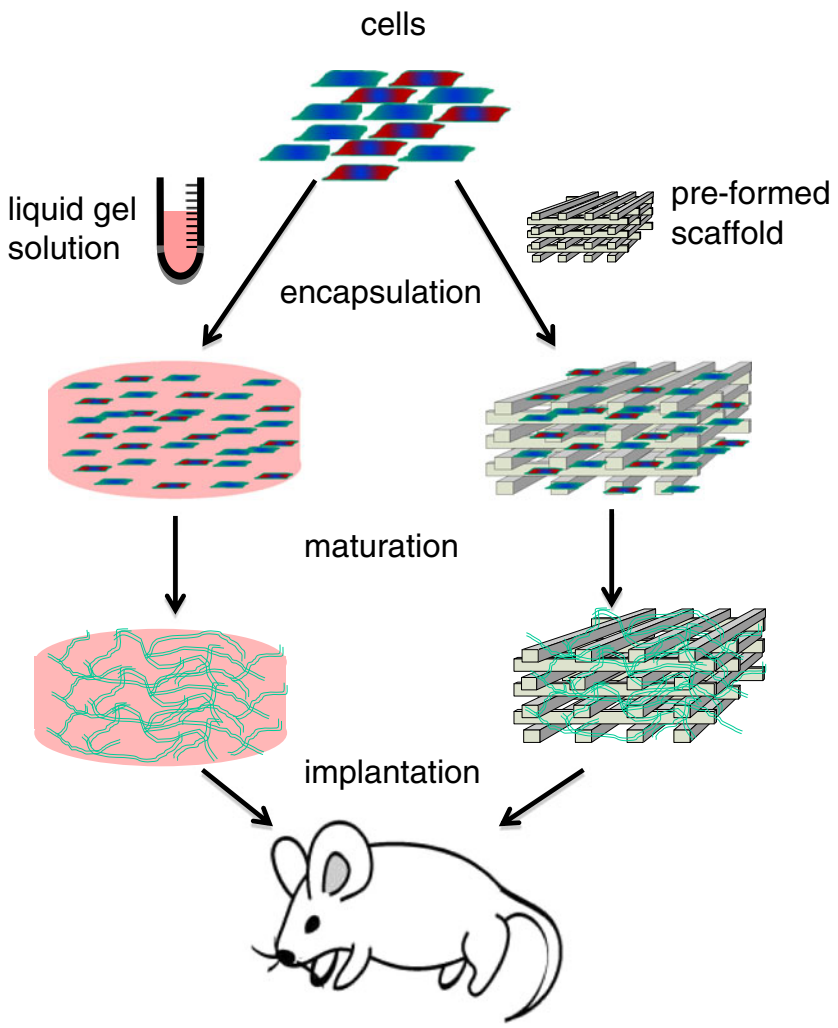

Fig. 1 Schematic diagram of strategies to prevascularize an implantable tissue. The first step is to encapsulate cells (e.g., endothelial cells) within a scaffold and allow a network of vessels to develop in vitro. The scaffold can be either a liquid solution which is gelled or polymerized following mixing with cells, or a pre-formed porous solid scaffold that promotes cells attachment. Next, a continuous vessel network develops and matures in vitro. Following in vitro development, the tissue construct is implanted into an appropriate host (e.g., mouse)

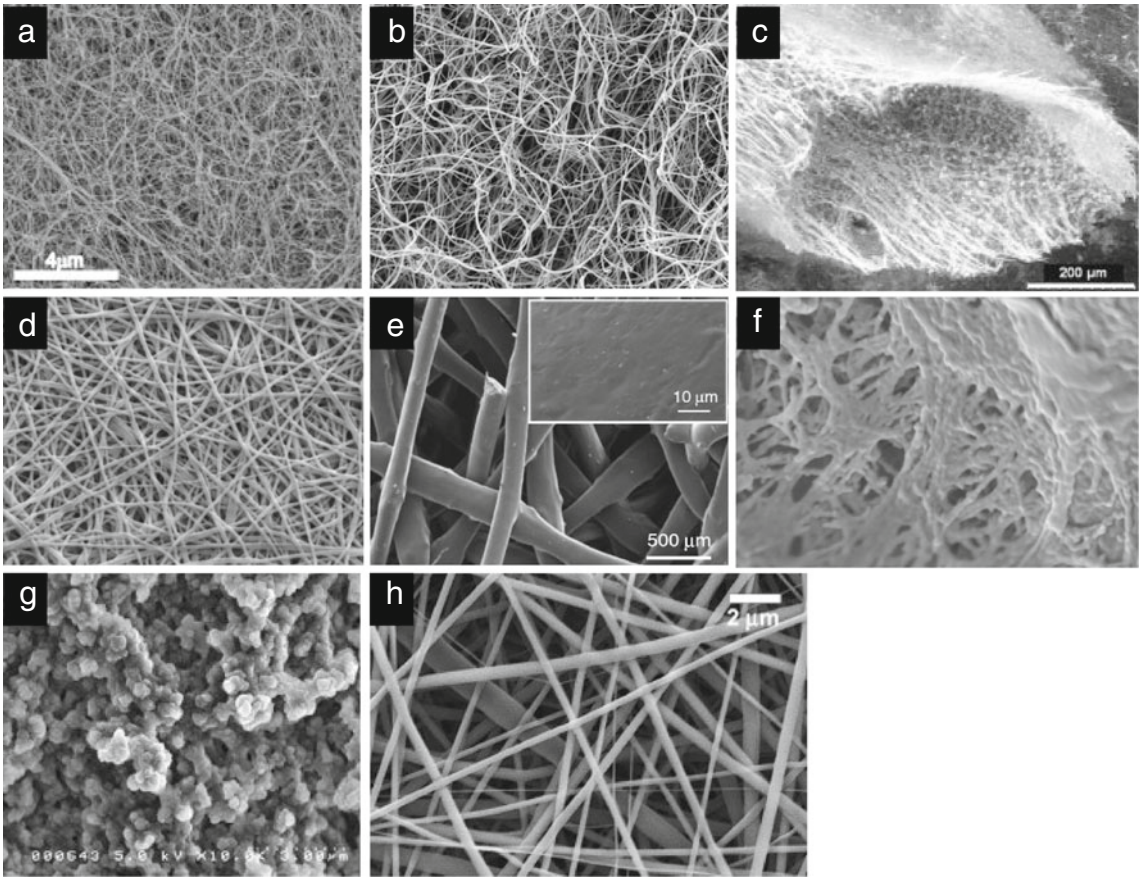

Fig. 2 Scanning electron microscope figures of various biomaterials that have been successfully utilized to prevascularize engineered tissues. The images highlight similarities and differences in the microstructure of the materials. **a** Collagen [163], **b** fibrin [164], **c** decellularized matrix [105], **d** silk fibrion [165], **e** SPCL [83], **f** Matrigel [166], **g** PEG [167], and **h** PLGA [168]

Biomaterial Properties

Biomaterials used to prevascularize engineered tissues can be divided into two types: natural and synthetic. Natural materials include any material that appears in the natural world, whereas synthetic is limited to materials created by chemical synthesis of smaller precursor compounds in a laboratory setting. Whether natural or synthetic, there are several key properties to consider when selecting materials for tissue engineering, especially for prevascularizing engineered tissues:

1. Biocompatibility

   Cells must proliferate, migrate, and function normally upon attachment to the material. Additionally, there should be no adverse foreign body reaction to the material upon implantation. If synthetic materials are used, the degraded by-products should be non-toxic to the cells and limit the inflammatory response [24].
2. Controlled biodegradation

   The host will generally remodel an implanted tissue construct in process that degrades the implanted material. The degradation rate of the biomaterial should match the production rate of new extracellular matrix protein by the host. If the degradation rate is too slow, then nascent tissue formation could be impeded; in contrast, if the degradation rate is too fast, then the mechanical stability, and thus function, of the engineered tissue could be compromised [25]. One way to control the degradation rate is to crosslink enzyme-sensitive peptides to the scaffold, such as tagging matrix metalloproteinase (MMP) to a polyethylene glycol (PEG) hydrogel [26, 27]. Other ways include adjusting the ratio of two composites during polymerization, such as varying polyglycolic acid (PGA) and polylactic acid (PLA) in poly(lactic-co-glycolic acid) formation [28].
3. Surface property

   A biomaterial will interact with host cells and impact their activities [29]. The biomaterial should contain ligands (binding sites and signaling peptides) that facilitate these interactions such as cell surface adhesion [30]. Natural biomaterials possess these ligands, whereas synthetic materials may require modifications such as linking with gelatin [31],

fibronectin-mimetic protein fragments [32, 33], or Arg-Gly-Asp (RGD)-like cell attachment sites [34].

4. Mechanical property

   The mechanical property of the biomaterial should mimic the mechanical environment of the target anatomical site [35]. The mechanical property can many times be characterized by Young's modulus, *E*. *E* relates the stress (force per unit area) within a material to the strain (displacement). A "stiffer" tissue will have a smaller displacement for a given stress, and thus a larger *E*. Material stiffness can be easily modulated by varying the concentration of polymer (e.g., fibrinogen concentration in fibrin gel) [36], hybridization with other materials (e.g., to natural polymer [37]), or cross-linking following polymerization [38].

5. Pore density and structure

   Biomaterials need to have interconnected pores to facilitate vessel growth as well as nutrient and waste product transport [39]. Porosity is generally characterized by the volume fraction of the materials that is pores and interconnectivity of the pore architecture. The porosity requirement of biomaterials is highly tissue specific, since different cell types prefer different pore sizes [24, 40]. Pore size also can be easily modulated in synthetic materials. For example, the centrifugation method can alter the pore size of polycaprolactone (PCL) scaffolds, and freezing time can alter the pore size of the collagen-glycosaminoglycan scaffold [41, 42]. Uniform pore size and a highly interconnective pore structure are desirable for uniform cell seeding [43, 44].

Natural materials

Natural materials have several distinct advantages. Their intrinsic structure provides both chemical and physical cues to promote cell adhesion and cell growth [45]. They are biocompatible, biologically active, and porous. They are also easily degraded by the host, thus facilitating tissue remodeling. Examples of natural materials that have been used extensively in the vascularization of tissue engineering constructs include collagen, fibrin, starch, matrigel, and more recently, decellularized extracellular matrix, and silk fibrion [46].

Collagen

As the most abundant protein in mammalian extracellular matrix, collagen is ideal for tissue engineering. It has high mechanical strength (compared to fibrin) and low antigenicity [47]. The latter is important for xenograft applications (e.g., rat tail collagen can support the growth and differentiation of human cells). Collagen degrades faster than most synthetic materials, and its degradation can be controlled by mixing with other polymers, such as chitosan and PLGA [20, 48]. In addition, the pore size and mechanical properties of collagen can be manipulated by varying pH or temperature during polymerization [36, 49], or by mixing other biomaterials with collagen [42]. Collagen has been widely used to prevascularize tissues. For example, a mixture of collagen and human endothelial progenitor cells (EPCs) injected subcutaneously into the rat or mouse demonstrates anastomosis of human vessels with the host circulation by 7 days with 30–60 vessels/mm^2 [50, 51].

Collagen can synergize with other materials to promote angiogenesis. For example, a hydrogel comprised of cross-linked collagen–chitosan seeded with human umbilical vein endothelial cells (HUVECs) will develop a rudimentary capillary-like network in vitro in 2 days, compared to 7 days in collagen alone [20]. In addition, collagen with hyaluronic acid can stimulate revascularization faster than collagen alone, demonstrating a sixfold higher vessel density at the same culture time [52].

One drawback of collagen, similar to other natural materials, is the requirement of additional chemical or polymer crosslinking to confer mechanical strength. Collagen conjugated with glycosaminoglycan demonstrates a coarser and stiffer architecture, which is ideal for bone tissue engineering. When this scaffold is seeded with MSCs, vasculogenesis is initiated in vitro after 1 or 2 weeks [53].

Fibrin

Fibrin is a natural protein that plays critical roles in blood clotting and wound healing [54]. It can be easily harvested from a patients' own peripheral blood, therefore eliminating the possibility of a foreign body reaction or disease transmission [55]. Fibrin has good surface properties, offering various binding sites that facilitate cell adhesion [56]. Fibrin is biodegradable. Its degradation can be modulated by crosslinking with other polymers, like polyethylene glycol or adding protease inhibitors, such as aprotinin [57, 58]. The major weakness of a fibrin-based construct is low mechanical stiffness, which can be improved by mixing with collagen, or crosslinking with factor XIIIa [59, 60].

Fibrin's porosity can be modulated. A fibrin gel is formed by mixing fibrinogen and thrombin. Increasing the fibrinogen concentration reduces the porosity, while increasing the density and stiffness of the fibrin matrix. Although increasing the matrix density increases the number of cell-matrix binding sites, the decrease of

porosity and enhanced stiffness are dominant leading to reduced angiogenesis [61].

Fibrin has been widely used to create vascularized tissue constructs for skin [62, 63], adipose tissue [63–66], bone [67–69], cartilage [70], skeletal muscle [71], retina [72], liver [73], and cardiovascular [74, 75] applications. The wide use of fibrin in vascularized tissues is due to its excellent pro-angiogenic properties. For example, HUVECs or hMSCs cultured in fibrin gel demonstrate initial capillary growth after 2 days of in vitro culture [76].

Angiogenesis in fibrin gels can be further promoted by embedding growth factors or co-culturing with other cells. Adding a low concentration of growth factor such as VEGF or fibroblast growth factor-1 leads to a persistent and normal vascular response [77–79]. Co-culturing endothelial cells with mural cells (pericytes, smooth muscle cells) significantly accelerates the prevascularization process in fibrin gels (16 vessels per unit area for co-culture compared to nine to 12 vessels for individual culture), and it can also stabilize the capillary once formed [76, 80]. Moreover, an in vitro prevascularized fibrin gel using EPC–ECs and fibroblasts implanted subcutaneously into an immune-compromised mouse will anastomose with the host within 27 h after implantation with an average of 150 vessels/mm^2 [16].

Starch

Starch is one of the most abundant polysaccharides found in nature. It is made of α-amylose and amylopectin, hence, it is biodegradable and easy to manipulate [81]. This review will focus on SPCL (starch and polycaprolactone).

SPCL is a blend of starch with polycaprolactone (30/70 wt.%) obtained by a fiber bonding process [82]. It is biocompatible in vitro, and is a highly interconnected porous scaffold with good mechanical properties [83, 84]. HUVECs can be cultured on a SPCL scaffold resulting in capillary-like structures and expression of endothelial specific markers [85, 86]. However, SPCL induces a moderate inflammatory reaction following subcutaneous and intramuscular implantation [87]. SPCL is susceptible to enzyme degradation by α-amylase and lipase which can facilitate the enzymatic hydrolysis of SPCL both in vitro and in vivo [88]. Degradation of SPCL has an advantage in that it increases porosity, providing more space for cell migration and ingrowth [89], although mechanical strength may be compromised. Cell adhesion to SPCL can be altered by coating SPCL with other materials such as fibronectin for vascularization applications [85].

Recently, SPCL has shown great potential in prevascularizing tissue constructs. For example, human dermal microcapillary endothelial cells (HDMECs) and primary human osteoblast (hOBs) seeded on SPCL scaffolds form microcapillary-like structures with lumens after in vitro culture for 21 days [90]. Human outgrowth endothelial cells (hOECs) and hOBs, co-cultured in a prevascularized construct embedded in Matrigel prior to implantation, anastomoses with the host circulation after 14 days. The hOBs in this model also demonstrated pericyte-like behavior, providing additional structural support for the vessels [91]. By improving OEC-induced vessel formation, anastomosis can be achieved within 48 h after implantation, and demonstrated 25 vessels/mm^2 with 14 days [92].

Matrigel

Matrigel is extracted from Engelbreth-Holm-Swarm mouse sarcoma cells [93]. It is one of the most abundantly used natural materials in tissue engineering. It is pro-angiogenic, and widely used in angiogenesis assays. For example, EPCs and MSCs can be suspended in Matrigel and implanted subcutaneously into a host. After 7 days of in vivo culture, vessels in the Matrigel implant anastomose with the host and demonstrate an extensive blood vessel network [94]. However, a major disadvantage of Matrigel is batch to batch variability and other ill-defined properties, such as possible tumor formation after implantation, making it unfavorable for translational applications of implantable vascularized tissues [40, 95].

Decellularized matrix

Decellularized ECM is attractive because it removes most, if not all, antigens that invoke a host-immune response following whole-organ transplantation. The process also retains the biocompatible and biodegradable features of collagen and fibrin, but also maintains 3D features of the ECM such as intact vascular spaces to facilitate endothelial cell attachment and angiogenesis. Early attempts at decellularizing tissues were limited to thin tissues such as skin [96, 97], blood vessels [98, 99], heart valve [100], and urinary bladder [101]. In these examples, delivery of oxygen and nutrients by diffusion is adequate during the recellularization process [1]. More recently, thick cellular scaffolds have been created through whole-organ decellularization of heart [102], liver [103–106], and lung [107–109]. To adequately recellularize these scaffolds, oxygen and nutrient delivery will require convection through functional blood vessels.

There are many methods to decellularize tissues. For example, chemical reagents such as alkalines, acids, detergents, and other solvents are used to solubilize cytoplasmic components and disrupt nucleic acids and

lipids. Biological reagents, such as enzymes (e.g., trypsin), cleave specific peptide bonds and remove residual cells. Physical processes such as freeze–thaw and external forces can also be used to efficiently lyse cells [110, 111].

The surface and mechanical properties of the decellularized matrix are influenced by the decellularization processes. For example, detergents can disrupt native tissue structure, damage collagen, and remove GAGs [112, 113]. However, if treated carefully, a decellularized matrix can preserve its 3D architecture (ultrastructural and macroscopic), native matrix compositions, porosity, as well as biological signaling cues that facilitate in vitro vascularization and in vivo implantation [1].

Several milestone studies have been published recently describing the decellularized matrix of whole organs such as heart, liver, and lung. Ott et al. [102] decellularized an intact rat heart by coronary perfusion, and subsequently re-endothelialized the organ; endothelial cells formed a single layer in coronary vessels after 7 days. The decellularized matrix of a liver was seeded with hepatocytes or human hepatic stem cells by different perfusion strategies [103–106]. Upon transplantation, the recellularized liver tissue was perfused with the host circulation within 5 min and remained metabolically active, indicating the ECM remained intact through the decellularization procedure [103]. A decellularized lung matrix was recently repopulated in vitro with epithelial and endothelial cells. After 5 days, cells were observed in large and small conducting airways, alveoli, and vasculature. Upon transplantation, functional gas exchange was also observed [107, 108].

Despite recent advancements in decellularizing tissue to create a biomaterial scaffold for tissue engineering, many technical difficulties remain. Very little information is available on how to maintain the ultrastructural features of the natural ECM, which could be critical to maintaining a normal cellular phenotype. To restore tissue functionality, the recellularization process will need to include both nonparenchymal cells and parenchymal cells (such as hepatic cells for liver) [1]. In addition, access to enormous numbers of cells of various types will be needed in a relatively short period of time to recellularize an entire human organ for clinical purposes. In the end, the choice of cell source (autologous or allogeneic cells), cell type (stem or progenitor cells), and culture system (bioreactor or perfusate) will need to be considered in a case-specific manner.

Silk Fibrion

Silk fibrion is a fibrous protein produced by arthropods such as silkworms (*Bombyx mori*) and spiders (*Nephila clavipes*) [114]. It is useful as scaffolding material for tissue engineering applications due to its low toxicity, anti-inflammatory properties, low degradation rate, and excellent elastic properties [115]. Silk fibrion is a tunable material. Plasma treatment, genetic engineering, or cross-linking with a cell-binding domain such as RGD, can improve cell attachment to the silk surface [116–118]. The pore size of a silk fibrion gel can be modulated by varying the fibrion concentration, hybridization with collagen, or by altering the polymerization process [119, 120].

However, several challenges need to be addressed. When cultured with $HepG_2$ and HeLa cells, silk fibrion shows relatively weak cell infiltration and vascularization compared to other biomaterials [121]. Due to its good elasticity and slow degradation rate, silk fibrion is still considered to be more appropriate for tissues with low oxygen and nutrient requirements (like bone, tendon, and ligament), rather than highly vascularized systems (e.g., liver) [122, 123].

Recently, the vascularization of silk fibrion-based constructs was improved both in vitro and in vivo by co-culture of endothelial cells with other cell types, the latter of which can provide essentials pro-angiogenic growth factors. Human aortic endothelial cells and human coronary artery smooth muscle cells seeded in a silk fibrion scaffold develop an interconnected vessel network after 7 days of in vitro culture [124]. In addition, a prevascularized co-culture tissue of HDMECs and hOBs will demonstrate microcapillary formation by 7 days following subcutaneous implantation. After another 14 days, the tissue construct demonstrates anastomosis with the host with 108 vessels/mm^2 [125, 126]. A similar in vivo result can be obtained using a co-culture of hOECs and primary human osteoblasts in silk fibrion. This model characterized the percentage of cells that stain positive for von Willebrand Factor (vWF) as the primary endpoint [127, 128].

Synthetic Materials

Synthetic materials are chemical compounds synthesized from one or more precursors, and have several distinct advantages in the field of tissue engineering and vascularization. They can be reproduced consistently by large-scale production. They generally have tunable mechanical properties, degradation rates, and a porous microstructure [129]. In addition, they do not introduce any potential risk of disease transmission.

Synthetic materials possess several drawbacks, thus limiting the in vivo potential. They do not possess intrinsic surface ligands (biological recognition sites) for cell attachment [130]. Therefore, they must be chemically modified such that they contain protein sequences such as RGD (Arg-Gly-Asp) or coated with serum proteins to obtain desirable properties that promote cell–scaffold interactions [131]. An additional concern is the fate of the

degradation products, which have the potential to impact normal cell function; however, most commonly used synthetic materials, have been designed with naturally occurring degradation products such as lactic acid [132].

Finally, although the porous microstructure can be controlled, creating well-defined, interconnected pore architectures and complex geometries has proven technically challenging [133]. Nonetheless, in the past decade significant progress has been made as 3D porous synthetic scaffolds have been fabricated with a variety of techniques such as solvent casting and particulate leaching, electrospinning, and gas foaming [43]. All of these techniques can manipulate porosity, pore size, and interpore connectivity of the scaffold.

Many synthetic materials have been used to prevascularize tissue engineering constructs, such as polyethylene glycol, polylactic acid, polyglycolic acid, polylactic–glycolic acid, polycaprolactone, polyurethane, and polyhydroxyalkanoate. This review will focus on the two most commonly used in vascularization applications: PEG and PLGA.

Polyethylene Glycol

PEG is an inert hydrophilic polyether. The chemical structure is shown in Fig. 3a. Its hydroxyl end can be functionalized through conjugation with other polymers [134]. PEG is an uncharged hydrophilic molecule, and thus attracts water molecules. This feature can be exploited to create a hydrogel that is resistant to protein absorption and cell adhesion [135, 136]. As a result, PEG has been commonly used as an immunoprotective barrier between encapsulated cells and host immune cells in vivo [137]. However, in order to be used for vascularization applications, PEG must be modified [138]. Various molecules have been coupled to PEG to enhance cell attachment. For example, human foreskin fibroblast spreading increased significantly in a PEG gel with high RGD concentration [139]. In another example, gelatin macromere coupled with PEG will promote valvular interstitial cell function [140].

PEG is not naturally degradable, but its degradation can be improved by incorporating with other materials. Collagen- and fibrin-derived short peptides can introduce MMP-sensitive and plasmin-sensitive regions in PEG, respectively [134, 141]. Addition of PLA or poly(vinyl alcohol) into the PEG scaffold can also tailor the degradation rate [142, 143]. PEG's mechanical property can be modulated during polymerization. PEG polymerization can be photoinitiated by two approaches, chain growth and step growth [144]. Chain growth can form gels under physiological and biocompatible conditions [145]; step growth can offer more homogeneous network structures and thus, better mechanic properties [146]. PEG's mechanical property can also be altered by crosslinking with other polymer such as poly(vinyl sulfone) [147].

PEG is widely used to prevascularize tissues [148]. For example, a porous PEG hydrogel supports extensive vessel formation when co-culturing HUVECs with human umbilical artery smooth muscle cells. Interestingly, PEG pore sizes seems to have a positive effect on the collagen content; PEG with larger pores (100–150 μm) demonstrated a 23% collagen content 3 weeks post-implantation, compared to pores 25–50 μm in size, which had only ~12% collagen [149]. PEG has also been hybridized with other materials to induce angiogenesis. HUVEC (in fibrin) and fibroblast cells (in PEG) co-cultured with PEG/fibrin ribbon hydrogel will form a capillary network after 7 days in vitro [138].

PEG has also been shown to impact stem cell differentiation, which has significant potential in the vascularization of engineered tissues. For example, adipose-derived mesenchymal stem cells differentiate into endothelial cells in PEGylated fibrin gels (covalent coupling of PEG with fibrinogen) and will form tube-like structures resembling crude capillaries after 11 days of in vitro culture. The primary endpoint in this study was CD31 and vWF staining, which increased 25- and 45-fold, respectively [150].

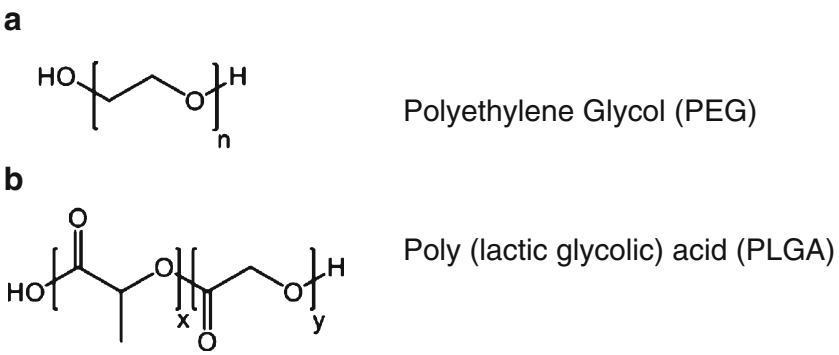

Fig. 3 Chemical structure of PEG and PLGA. **a** PEG, *n* represents the number of oxyethylene groups. **b** PLGA, *X* represents the number of units of PLA, *Y* represents the number of units of PGA

Poly (Lactic Glycolic) Acid

PLGA is the copolymer of PLA and PGA, and one of the most popular synthetic materials used in tissue engineering [151]. The mechanical strength, porous structure, and porosity, and degradation rate are all tunable. PLGA degrades to non-toxic naturally occurring compounds (lactic acid and glycolic acid), and is thus biocompatible. Furthermore, PLGA can absorb proteins (e.g., fibronectin [152]) or be coupled with RGD-like short synthetic peptides to provide cell attachment sites [153].

PLGA's degradation rate and mechanical strength can be manipulated by the ratio of PLA/PGA. The chemical structure is shown in Fig. 3b. PGA is a simple linear aliphatic polyester with high mechanical strength and fast degradation rate (<20 days [154]). In contrast, PLA has an extra methyl group in the repeating unit, and is thus more hydrophobic and degrades slower. Mixing PGA with PLA reduces the crystallinity of the materials, thus increasing the degradation rate due to autocatalytic hydrolysis. For example, PLGA (50:50 PLA/PGA) usually takes 1 or 2 weeks to degrade, while PLGA (85:15) takes more than 5 weeks [155].

The PLGA degradation by hydrolysis produces poly (α-hydroxy acids), which reduce the pH, promote further degradation, but can cause local acidosis [132, 156]. As a result, the host inflammatory response can be augmented thus compromising implant integration with the host [157]. Simple pH-compensation fillers can potentially reverse this effect. For example, the careful addition of inorganic compounds, such as sodium bicarbonate [158] and wollastonite [159], have been used to stabilize the pH in a physiological range for several weeks.

For 3D tissue in vitro prevascularization, PLGA has been primarily used as a scaffolding material to provide mechanical strength and 3D structure. PLGA seeded with MSCs and kidney vascular endothelial cells formed a vascularized network upon implantation into a rat thigh. Compared to non-prevascularized PLGA scaffold, there is about a twofold increase in the bone density [160].

PLGA can also be used for in vivo prevascularization. For example, a PLGA scaffold implanted into the flank of donor mice for 20 days develops a pre-formed microvascular network by host cell infiltration. The prevascularized construct can then be excised and transferred to the dorsal skinfold chamber of a recipient host. The implant anastomosis with the host circulation and develops a functional capillary density of 230–310 cm/cm^2 throughout the entire scaffold [161].

PLGA is also used in conjunction with various materials to tailor its properties suitable for prevascularizing engineered tissues. For example, a macroporous PEG/poly-L-lysine hydrobromide hydrogel with PLGA outer scaffold supports the formation of tubular structures in a co-culture of neural progenitor cells and endothelial cells. Upon implantation to a spinal cord lesion, the construct develops stable, functional vascular networks with ~57 vessel/mm^2 within 3 weeks [162].

Future Directions

Current prevascularization methods have successfully demonstrated that co-culturing endothelial cells with stromal cells in 3D biomaterial scaffolds can support vessel formation for both in vitro and in vivo strategies. However, there remain limitations in our understanding of this process that must be overcome before this technology can be clinically relevant. The following represent critical areas of research in the near future.

1. *Mechanical properties.* Currently, engineered tissue is supported structurally by the 3D scaffold, and is generally weaker than load-bearing in vivo tissues. Although the scaffold is necessary for in vitro culture, the scaffold has to be removed eventually. Hence, new strategies must be employed to maintain the mechanical integrity of the tissue without negatively impacting the development of the capillary network. Cell-matrix and cell–cell interaction plays a major role in this remodeling process.
2. *Long-term function of vessels.* Current studies have demonstrated the survival of engineered tissues and the prevascularized vessel network using biomaterial scaffolds for up to several weeks. Long-term studies are necessary to address the ultimate fate of the prevascularized microvessel network (e.g., remodeling and absorption). In addition, current microvessel networks demonstrate evidence of vessels that are more complex than simple capillaries, but a larger (>1 cm) implantable tissue will undoubtedly require more complex vessels such as arterioles and venules.
3. *Incorporate tissue function.* Currently, most prevascularized tissues do not have any specific biological function. Cells with metabolic function (e.g., pancreatic islet) or mechanical function (skeletal muscle) need to be incorporated into future designs. Additional examples of function are contractility of cardiomyocytes, albumin production of hepatocytes, and oxygen exchange of alveolar cells. Appropriate biomaterial selection will likely play an important role in meeting the functional requirements for specific engineered tissue.
4. *True 3D tissues.* Most biological functions (either structural or metabolic) require tissue volumes that exceed the diffusion limit for nutrient and waste transport. One key to thick tissue design is the ability

to deliver nutrients and remove waste products during the in vitro prevascularization process. It is crucial to maintain tissue viability as the interconnected vascular network develops. One approach is to layer multiple tissue constructs; in this case, inter-tissue channels are used to provide nutrient and waste transport during in vitro development. Advances in our ability to create truly thick 3D tissues in vitro will be critical in the design of functional engineered tissue.

Conclusion

A wide range of biomaterials, including natural and synthetic, have been used to prevascularize engineered tissues. An ideal biomaterial should mimic structural and functional properties of the natural ECM. Hence, when selecting materials for specific tissue engineering applications, several key features should be considered such as biocompatibility, controlled biodegradation, surface properties, mechanical properties, and porosity. Generally speaking, natural materials are biocompatible, biologically active, and easily degraded and remodeled by the host. However, because they are derived from biological components, they carry the risk of disease transmission. Compared to natural materials, synthetic materials have tunable mechanical properties, and are easily manipulated by different fabrication techniques. However, they generally have inadequate biological cues to support cell adhesion and growth. Future success in prevascularizing engineered tissues will require the advanced manipulation of both natural and synthetic materials.

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