Review Article

Advantages of selected natural and synthetic materials as scaffolds in vascular tissue engineering

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ABSTRACT

The development of tissue engineering provides various opportunities to vascular tissue engineering. Scaffold plays an essential role in vascular tissue engineering. The selection of biomaterials used as scaffolds will determine the success of vascular tissue engineering. The structure of vascular system, which consists of three layers, is embedded in extracellular matrices that provide the mechanical properties of the system. Therefore, tissue engineering of a vascular structure needs various suitable biomaterials as scaffold that can support vascular system mechanical properties and function. Various materials were used for 3D printing and electro-spinning with good results, including collagen, gelatin, and alginate. Varying sizes of blood vessels require scaffolds with biomaterials that could adapt to their shape, size and approximate the mechanical properties of the blood vessels.

***Keywords*:** Biomaterial, angiogenesis, collagen, alginate

INTRODUCTION

Vascular system is a critical issue in tissue regeneration/repair and, lack of vascular system is a challenge in the survival of tissue engineering product translation *in vivo* [1]. The vasculature plays a fundamental role in supporting survival of

transplanted tissue engineering product and tissue repair by providing blood supply, which contains various beneficial elements, i.e. nutrients and oxygen to the cells in the tissue and organ [2]. In the body, most cells are located around 100–200 μm from the closest blood supply to confirm optimal delivery of oxygen and nutrients by diffusion to support cellular metabolism [2]. Therefore, angiogenesis is a critical issue that is needed for the success of engineered tissues. To aid angiogenesis, a functional tissue engineering scaffold is needed to provide a suitable cellular microenvironment that can be loaded with therapeutic molecules that are necessary for the stimulation of angiogenesis [3].

The term tissue engineering was first introduced in 1987, which was defined as the use of a combination of multidisciplinary approaches to improve or replace damaged tissues/organs [4]. In recent years, rapid development in tissue engineering technology leads to vascular tissue engineering that aims to repair blood vessel damage, where scaffolds play a crucial role. The purpose of scaffold use is to mimic the structure and function of the natural Extracellular Matrix (ECM), which can provide a three-Dimensional (3D) environment and physical properties to promote the adhesion, proliferation, and differentiation of cells to form a desired tissue for tissue repair.

As scaffold plays an essential role in the engineering of vascular tissue, choosing a scaffold material is very important. Material choices of scaffolds are synthetic or natural material, including biomaterials. Most of the synthetic materials are easy to manipulate in term of their macroscopic and microscopic structure, but they are biologically inert, have less interaction with surrounding tissues and might even be toxic to the human body. Therefore, combination with biomaterials is used to improve the overall scaffold property. To date, various materials have been used in vascular tissue engineering. Therefore the aim of this review was to give a brief image of the vascular system, and recent advances in vascular tissue engineering, which was the novelty of this review. For that purpose, we discussed the structure, mechanical properties of vascular system, vascular tissue engineering, and various materials that can be used as vascular tissue engineering scaffolds.

Structure of vascular system

The whole vascular (circulatory) system inner side is lined by a monolayer lining, which is an epithelium that is called

endothelium. The endothelial surface consists of 1 to 6 x 10^{13} cells and weighs about 1 kg [5,6]. The entire circulatory system has a basic structure that consists of three layers: the tunica intima that contains the endothelium, which is supported by a basement membrane and collagen fibers, a muscular intermediate layer, the tunica media, and an outer layer of supporting tissue that is called tunica adventitia [6]. The cells of the vascular system are of mesodermal origin, including Endothelial Cells (ECs), Smooth Muscle Cells (SMCs), pericytes, fibroblasts, nerve endings, and various blood cells that occupied the lumen [7]. Vascular system consists of macrovascular and microvascular vessels. The macrovascular vessels are large arteries like aorta and carotid artery, which have an internal diameter larger than 100 μm, medium and small arteries [7]. Large arteries are elastic reservoirs that store blood during systole and release blood during diastole to guarantee a continuous and steady blood flow that involves large volume changes but experiences little variation in pressure. The structure of the large arteries is dominated by elastic fibers to ensure mechanical strength. The outer most layer of the vessel wall, the tunica adventitia, which is composed of collagen rich Extracellular Matrix (ECM) that is build up by a heterogeneous population of myofibroblasts, helps to avoid rupture within areas of high pressure. The tunica media of medium artery in vertebrates is organized mainly by vascular smooth muscle cells [8]. A variety of large human arteries exhibit a microvasculature in their adventitial layers termed vasa vasorum, which is mainly responsible for nutrient transport to the vessel wall. In contrast to macrovessels, the endothelial cells of microvessels are surrounded by solitary vascular smooth muscle cell-like cells called pericytes that share the basement membrane with the endothelium [9]. Pericytes are related functionally and suspected to belong to the same cell lineage as vascular smooth muscle cells, but differ in their distance to the endothelium, in their morphology, and to some extent in their expression of specific markers. Small arteries or arterioles have a tunica media that consists of vascular smooth muscle cells and therefore primarily control the blood volume for blood-tissue metabolite exchange that is going to metarteriole and capillaries to enter post-capillary venules. Post-capillary venules are thin and mainly composed of ECs lacking pericytes, which give them the ideal properties for regulating the metabolite exchange process [7].

Mechanical properties of vascular system

The mechanical properties of a vascular system depend on its composition, structure, and ultrastructure. The properties of an artery depend not only on how much collagen it has but also on how the collagen fibers are arranged in the tissue [10]. Due to their strategic location, vascular ECs are able to sense hemodynamic changes and blood-borne signals and to respond by releasing vasoactive substances. Under physiological conditions, endothelium-derived relaxing and contracting factors are balanced, so that vascular homeostasis is maintained marginally in favor of vasodilation. The endothelium is exposed to different mechanical and hemodynamic forces i.e. : radial forces, which are caused by intravascular pressure, tangential forces in the vessel wall, which are caused by the balance between cell-cell contacts and vaso-motion of the vessel, and axial shear forces that are caused by the friction of the flowing blood against the vessel wall [11]. The characteristics of various components of a vascular wall show unique mechanical features in response to the physiological forces such as non-linearity, anisotropy, visco-elasticity and compliance. The non-linearity occurs in the pressure-diameter curve of an artery, which is a result of the wavy and disorganized configuration of elastic and collagen fibers when not under pressure. As the pressure increases, the fibers start to gradually straighten. At a low physiological pressure (80 mmHg), elastic fibers become nearly straight. An increase in pressure results in the stretching of elastic fibers. Continuous straightening of collagen fibers occur up to the upper limit of physiological pressure (120 mmHg). Beyond the upper limit, collagen and elastic fibers are fully stretched. Therefore, at lower pressures the mechanical behavior is dominated by elastic fibers, which are less stiff and more elastic. In the physiological range of pressure, the load transits between the elastic and collagen fibers. At high pressures, the mechanical behavior is dominated by the rigid collagen fibers, where a greater amount of load is necessary for a change in the diameter. The stiffer collagen fibers prevent the damage and/or rupture of blood vessels when the pressure is increased [12].

Material components of vascular system extracellular matrix

Material components of the vascular system extracellular matrix play a crucial role in the selection of scaffold materials for vascular tissue engineering [4]. The tunica intima that consists of a single layer of ECs sits on the internal elastic lamina

that is aligned parallel to the blood flow. The tunica media consists of concentric layers of smooth muscle cells that are arranged between sheets of elastic fiber sheets (elastic lamina), and collagen fibers. The collagen and elastic fibers are arranged to form a 'two-phase' system, in which circumferentially aligned collagen fibers of high tensile strength and elastic modulus bear most of the stressing force at and above physiological blood pressure. Elastic fibers, which are distensible and have a low tensile strength, function as elastic reservoir and distribute stress evenly throughout the wall and onto collagen fibers [13]. The main components of vascular system are elastic and collagen fibers, as well as smooth muscles. Elastin is a biological material of elastic fibers with an almost linier stress-strain relationship. Collagen is the basic structural protein that gives strength and stability and is present in almost all parts of the body. A collagen molecule consists of three helically wound chains of amino-acids. These helices are collected together in micro fibrils, which in their turn form sub-fibrils and fibrils. The fibrils have a diameter of 20-40 nm, depending on species and tissue. Smooth muscle cells appear in the inner part of the tunica media and are oriented longitudinally, circumferentially or helically [10,14]. The tunica adventitia is made up of myofibroblasts that are embedded mainly in collagen fibers [13].

Vascular tissue engineering

Although demand of bioengineered blood vessels continues to rise, current availability of vascular conduits remains limited. A synergistic combination of advances in scaffold fabrication and stem cell production offers new strategies for tissue engineering of autologous blood vessels that recapitulate the mechanical properties of native vessels as well as their biological function [15]. Within the vasculature hierarchy, blood vessels vary in size. Large vessels (arteries and veins) should ensure efficient transport through their lumen to sites far away, while smaller vessels (capillaries) should ensure optimal trans-mural exchange of nutrients, oxygen, and waste [15].

For small size tissues, diffusion might support areas for cell proliferation, differentiation, and growth, but larger tissues need vascular system. Therefore, vascular tissue engineering, which requires scaffolds, cells, and growth factors are necessary. Scaffold biomaterials should be capable of carrying cells and signals to ensure successful vascular tissue engineering. In addition, a perfect scaffold

should be biocompatible, biodegradable, have appropriate mechanical properties, high porosity with an interconnecting pore structure. In vascular tissue engineering, the new vessels establish their own ECM, and bit by bit the previous scaffold degrades till it is fully replaced by the newly formed ECM. Design and fabrication of suitable customized scaffolds may be obtained by computer-aided producing technology [4]. Therefore, scaffold biomaterials should be suitable for printing and processing.

Various biomaterials as vascular tissue engineering scaffold

Biomaterials such as metals, natural or synthetic polymers, ceramics, and their composites have been widely used in biomedical fields for decades [16]. The use of a certain synthetic polymer scaffold i.e. Polydioxanone (PDO), when implanted, may cause inflammation that leads to Foreign Body Reactions (FBR), fibrosis, and implant failure. A study showed that changing electro-spun PDO scaffold architecture might modulate mast cell responses and promoted regenerative interactions between cells and scaffold. The PDO were solutions of 60 mg/ml or 140 mg/ml, and electro-spinning produced structures with different fiber diameters and pores. Pores that were > 4-6 μm led to less secretion of IL-6 and TNF [17].

Mineralized collagen scaffolds are degradable biomaterials, whose biophysical and integrative parameters can be adjusted to facilitate cell invasion and tissue remodeling [18]. A study reported the potential of these scaffolds to sequester 60–90 % of protein from solution without additional cross-linking treatments and showed high levels of retention for individual (>94 %) and multiple growth factors (>88 %) that can be layered into the material via sequential sequestration steps [18].

Another study developed compound tube-shaped structure networks to study angiogenesis in a 3D human skin model in vitro. When combined with matrigel, the results showed promising results in more than one aspect of angiogenesis, including endothelial migration and tube formation in the human skin model [19].

Table 1 shows various materials that may be used as scaffold in vascular tissue engineering. Scaffold material choice is very crucial, as each material has its own characteristics, advantages and disadvantages [1,3,16,17,19,31].

Tabel 1. Characteristics of biomaterials that can be used as scaffolds for tissue engineering

Biomaterials	Characteristics	Advantages and reason of biomaterial choice	Reference
TCP, alginate= main 3D printing material, gelatin= hardening agent	3D Printed , suitable pore size, interconnected porosity, and geometry	Suitable for cell adhesion and extracellular matrix formation; TCP-alginate is a thick solution – easy to be bio-printed	[1]
PA= matrix, PCL= electro-spun material	Hybrid nanosack PA gel with suitable porosity and electro-spun PCL nanofiber sheet with porous crater-like structures	Enhanced local angiogenesis -by multi-stage delivery of FGF-2; PCL solution is easy to be electro-spun into nanofiber sheet	[3]
SAP (RADA4- SDKP)= hydrogel scaffold	$(RADA)_4$-SDKP hydrogel	Provide pro-angiogenic, anti-fibrotic, and anti-inflammatory activity to be used as a cardio-protective scaffold; SAP is a hydrogel-ready to use as scaffold	[20]
Alginate= 3D printing biomaterial, ECs, SMCs= cells in alginate hollow tubes	Vesseloid that is composed of ECs - SMCs - matrigel filled alginate hollow tube	Provide a one-step process of mature and functional blood vessel (vesseloid) production using a microfluidic co-extrusion device, which is a suitable model to elucidate vascular development and signaling mechanisms of tissue/cell polarization; alginate= suitable solution to be 3D printed into hollow tubes	[21]
Collagen= commercial 3D scaffold biomaterial	Natural organic material (ECM) with excellent biocompatibility	Biocompatible, provides strong cell adhesion, ideal biomimetic biomaterial for cartilage TE; collagen is a thick solution– easy to be bio-printed	[22]

PLGA= electro-spun material	Increase in bioavailability, biocompatibility, and biodegradability, and has smaller pore size	Provide better cell-scaffold interaction; PLGA solution is easy to be electro-spun into nanofiber scaffolds	[23-25]
PHBV= electro-spun material	Biocompatible, and biodegradable fabricated SVNs	Suitable to study angiogenesis in a 3D human skin model in vitro; PHBV solution is easy to be electro-spun into SVNs	[19]
Alginate, GelMA, photo-initiator irgacure 2959= 3D printing material	Bio-printed 3D micro-fibrous scaffold, which microfibers form the backbone and can gradually migrate to the periphery	Endothelial cell bio-ink can be directly printed within the scaffolds, where migrating microfibers promote a confluent endothelial layer; Alginate, GelMA, photo-initiator irgacure 2959 mixture has suitable properties to be printed into micro-fibrous scaffold	[26]
PMA, PEA= thermal polymerization material on a 3D dissolvable template, FN, VEGF= coating material	Nano-networks with critical binding domains (FN), and synergistic integrin/VEGF signaling in low concentrations of VEGF	Provide in-vitro higher level of angiogenesis, with newly formed vessels clearly visible; PMA-PEA is suitable for thermal polymerization.	[16]
PCL= 3D printing material	3D printed porous scaffolds, biodegradable	Promote replacement by natural ECM; PCL solution has suitable properties to be printed into porous scaffold	[27]
PCL, camphene ($C_{10}H_{11}$)= 3D printing material	3D printed hierarchically structured micro channeled structures	Provide unique biological reactions, including modulated immune/inflammatory responses, promoted angiogenesis and stimulated stem cell recruitment; PCL-camphene solution has suitable properties to be printed into micro channeled structures	[28]

nCS, alginate= disk material, fibrin hydrogel= encapsulating material	Hybrid biomaterial promoting delivery of MSCs, angiogenic factors, VEGF, and FGF9	Promote neovascularisation and bone formation; nCS, alginate mixture has suitable properties to be printed into disks	[29]
Alginate hydrogel= 3D printing material	3D scaffold, biocompatible and nontoxic	Cell bio-ink can be printed into the alginate 3D scaffold (disk); alginate hydrogel has suitable properties to be printed into disks	[30]
ECM powder= gelation material	Scaffold that provide micro-architecture of a native adventitia	Increase proliferation of human adventitia-derived endothelial cells; ECM powder has suitable properties to be used in gelation process into hydrogel scaffold	[31]
PDO= electro-spun material	Scaffold with divergent fibers and suitable pore diameters	Improve wound healing and reduce implant rejection; PDO solution is easy to be electro-spun into fibrous scaffolds	[17]

TCP= tri-calcium phosphate, PA= peptide amphiphile, PCL= poly (ε-caprolactone), SAP= Self-assembling peptide, RADA-SKDP= (N-terminus →C-terminus: Ac-RADARADARADARADAGGSDKP-NH2), ECs= endothelial cells, SMCs= smooth muscle cells, ECM= extracellular matrix, TE= tissue engineering, PLGA= Polylactic-co-glycolic acid, PHBV= poly-3-hydroxybutyrate-co-3- hydroxy valerate, SVNs= synthetic vascular networks, GelMA= gelatine methacryloyl, PMA= Poly(methyl acrylate), PEA= poly(ethyl acrylate), FN= fibronectin, nCS= nano calcium sulfate, MSCs= mesenchymal stem cells, VEGF= vascular endothelial growth factor, FGF9= fibroblast growth factor 9, PDO= Polydioxanone

CONCLUSION

The varying sizes of blood vessels require scaffolds with biomaterials that can adapt to their shape, size and approximate the mechanical properties of the blood vessels. Various biomaterials are suitable to produce scaffolds for vascular engineering.

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