Acellular Tissue-Engineered Vascular Grafts from Polymers

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1. Introduction

Vascular malfunctions contribute to various maladies and have emerged as a leading cause of global mortality. Specifically, as much as 32% of deaths worldwide were attributed to cardiovascular diseases (CVD) in 2019 alone ^[1]. At the same time, CVD and associated risk factors are causing substantial morbidity in patients worldwide ^[2]. Current treatments for CVD include medications, surgery, medical implants, mechanical devices, and rehabilitation ^{[3][4][5]}. Moreover, conventional surgical procedures such as grafting bypass, improve vessel patency, and vascular repair treats more severe conditions of CVDs, e.g., stroke and heart attack ^[6]. Specifically, innovative approaches like vascular tissue engineering (VTE) have been intensively explored to address the pathophysiology underlying CVD progression and improve the overall life quality of CVD patients through the direct replacement of damaged vessels ^{[7][8]}.

Currently, VTE lies at the intersection of several emerging disciplines including material science, polymers, stem cell biology, and fabrication technologies to support the development of micro/macroscopic artificial and bioartificial vessels ^{[9][10][11][12]}. Different classes of vascular tissue equivalents have been successfully developed as potential replacements for damaged or malfunctioning blood vessels through advancements in human cell biology and cardiovascular physiology ^[13]. To engineer such artificial blood vessels for in vivo transplantation in patients or in vitro models of vascular pathophysiology, appropriate polymeric materials, cell culture technology, controlled microenvironment, and additive manufacturing are required to develop vascular scaffolds with varied complexity ^[11] ^{[13][14][15][16][17]}.

Several natural and synthetic polymers have been applied to fabricate biodegradable and biocompatible vascular scaffolds through combinations of chemical processing and manufacturing technologies such as hydrogelation ^[18], 3D bio-printing, electrospinning, casting/molding, laser degradation, phase inversion, sheet-based fabrication, medical textile (braiding/weaving/knitting), and gas foaming ^{[19][20][21][22][23][24]}. For instance, vascular scaffolds composed of poly(ε-caprolactone) (PCL) and collagen fabricated by electrospinning has shown higher durability

than sole-PCL/poly (lactide-*co*-glycolide (PLGA) scaffolds ^[25]. The PCL/collagen composite scaffolds could bear long-term high pressure caused by loaded-volume blood flow and provide a favorable environment for vascular cell growth ^[26].

Scientists have also used poly-L-lactic acid (PLLA)/PCL added with heparin to create small-scale vessel substitutes through electrospinning and extrusion ^{[27][28]}. Apart from these examples, other polymers such as PU ^[29], gelatin ^[30], chitosan ^[31], PVA ^[32], PEG ^{[33][34]}, PLCL ^{[35][36][37]}, PGA ^[38], and PET ^[39] have been utilized to create multi-scale blood vessel replacements. The general strategy combines two or more of those polymers and fabricates them via the techniques above ^[14]. Moreover, these polymer-based tissue-engineered vascular grafts (TEVG) have enhanced biomechanical properties, which can better withstand in vivo blood pressures and establish sustainable cellular environments over long periods. Nevertheless, TEVGs must mechanically match the region of interest for transplantation purposes to provide the necessary degree of structural integrity, biocompatibility, biodegradability, and physiology functions ^[21]. Based on the numerous conditions that must be satisfied, research is still required to optimize TEVG technologies.

2. Characterization of Synthetic TEVGs in Clinical Use

Polyethylene terephthalate (PET, Dacron), expanded polytetrafluoroethylene (ePTFE), and polyurethane (PU) are the three major TVEGs that are invested in clinical use [40][41][42][43]. Clinically available Dacron grafts are fabricated via either weaving or knitting in an over-and-under pattern, leading to minimal porosity and creep [44]. Dacron is stable and can persist for more than 10 years after implantation without significant deterioration when applied as macro-scale vascular replacements. They have poor clinical performance and cause thrombus, inflammation, and compliance mismatches when used as small-diameter vascular grafts [45][46]. The compliance of current commercial Dacron TEVGs is $2.0 \times 10^{-2}\%$ mmHg⁻¹ with 42% of two-year patency [47].

Polytetrafluoroethylene (PTFE) was patented first in 1937 as Teflon. Expanded ePTFE (Gore-Tex) is the material employed on vascular grafts and manufactured using heating, stretching, and extruding processes, creating a microporous scaffold for firm cell adhesion ^{[41][48]}. An ePTEE vascular graft is non-woven, with a node–fibril structure, and performs well as aortic replacements having a 5-year primary patency rate of 91% to 95% but a lower patency rate for being analogs of substitutes with small ID ^{[49][50]}. The compliance of ePTEE is 1.5×10^{-2} % mmHg⁻¹ with 42% of two-year patency ^[51]. Specifically, both Dacron and ePTEE can be bonded to heparin ^[52]. Heparin-bonded ePTFE aortic grafts presented decreased thrombogenicity and enhanced patency rates at 8 weeks ^[53]. Heparin-bonded Dacron grafts are commercially available in Europe ^[44]. Significantly, the heparin-bonded Dacron showed promising wide application of SDVGs such as femoropopliteal bypass grafting, with eye-catching patency rates at 1, 2, and 3 years of 70%, 63%, and 55%, respectively ^[54].

Researchers prefer using PU for microcapillary scaffolds due to their microstructure ^[55]. Polyurethanes can be divided into fibrillar or foamy structures, and both tend to lack communicating spaces for potential capillary ingrowth ^{[56][57]}. In microporous foamy PU with a 15 μ m pore size, relatively little capillary ingrowth can be achieved. Whereas once the pore size increased up to 157 μ m, capillary sprouting occurred ^{[58][59]}. Although PU grafts

possess many exciting features, such as EC growth under inferior hemodynamic conditions, excellent healing, subtle surgical handling, and low suture bleeding, sufficient evidence of the spread use of PU vascular grafts as human peripheral bypasses remains in scarcity because of lacking investigations ^[60].

3. Key Challenges Limiting the Translation of Polymer-Based TEVGs

Ideally, bioartificial blood vessels should possess the structural and functional capacities of native structures ^[61]. Therefore, identifying the conditions that may lead to deviation from these ideal characteristics is vital for reducing the potential of device failure. It is also essential that these structures be rendered with bio-inertness for supporting somatic growth post-transplantation ^[62]. To this end, pinpointing the key challenges the current polymeric TEVGs face in clinical translation is extremely necessary.

As we all know, the endothelium is essential in restricting the movement of water, cells, and protein between intravascular and interstitial compartments ^{[63][64]}. Based on the characterization demonstrated in below table, TEVGs solely composed of natural polymers have better performance regarding biological aspects ^[61]. These microscale vascular conduits are free of considerations regarding biocompatibility, degradability, and cytotoxicity. They are highly supportive of cell repopulation and nutrition exchange. Besides, different natural polymers will create vascular substitutes with specific physical performance. For instance, collagen type I exhibited a vital barrier function after cell seeding.

Moreover, the endothelium has to align on the basement membrane, where collagen type I is the essential component and regulator ^{[18][65]}. This characteristic explains why vascular replacements consisting of collagen type I have a vital barrier function and indicates the potential for endothelium regeneration ^[66]. However, the mechanical properties of these natural polymer scaffolds require significant improvement. Going back to collagen type I, the stiffness of collagen type I is 0.1–18 kPa when the concentration is 3–20 mg/mL ^[67]. Based on the fact that compliance is the inverse of stiffness ^[68], the compliance of collagen type I is around 10^{-2} cm/s. The compliance of vascular conduits made by collagen type I conducted with microfluid/hydrogelation is close to 10^{-6} cm/s. The compliance of native micro-vessels with the same dimension is unknown, but the compliance of this polymer has been highly reduced when formed into microvascular constructs.

However, the mechanical properties of polymers are flexible and changeable by distinct ways of fabrication, physical/chemical reactions, and incorporation with other materials ^[69]. The HA vascular micro-tubes in Table have a stiffness from 19 to 32 kPa, while, when it combines with PVA as a composite hydrogel, the stiffness can be extended to 200 kPa ^[70]. Other similar examples provide future research directions on amplifying the mechanical properties of natural polymer-based vascular homologs but also bring new challenges of choosing to fabricate techniques, a combination of polymers, and methods of modifications ^[69]. These problems and confusion can only be solved with arduous academic work. Besides that, the mechanical properties of these natural polymeric vascular substitutes still need to be discovered, which implies a shortage of small- and macro-scale vessel analogs generated by natural polymers.

For vascular scaffolds created by synthetic polymers, their dimensions become multiple at the micro-scale level, and the small ID vascular structures have been formed through the braiding of PET/PLGA and the casting/electrospinning of PLGA/ P(CL/LA). Except for this, the morphology of blood vessel conduits is not limited only by straight but also by branched tubes ^[69]. Most scaffolds' mechanical features are available and are highly hopeful of reaching that of native blood vessels, as listed in **Table 2**. For example, in **Table 2**, the saphenous vein's longitudinal elastic modulus (stiffness) can be 130 or 23.7 MPa. The mean diameter of the usual great saphenous vein (GSV) is 5.0 ± 2.4 mm. The mean diameter of a typical small saphenous vein (SSV) is 3.1 ± 1.3 mm ^[71].

Regarding the dimensions, various polymers and corresponding fabrication skills presented in **Table 1** can meet the requirement, such as silicone, PU, heparin-releasing PLLA/PCL, and PEG/collagen/PU. For the stiffness, 10% (*w*/*v*) P(CL/LA)/PGLA (sealed) and 15% (*w*/*v*) P(CL/LA)/PGLA (sealed) are capable of matching the stiffness of native small saphenous with 23.7 MPa ^[69]. However, most synthetic polymers' stiffness lies in the range of kPa. Apart from that, polymers' suture retention strength and burst pressure are still predominantly lower than native vessels. Compared to single synthetic-polymer-made scaffolds, a mixture of polymers with or without biological molecules/natural polymers demonstrated potential neovascularization ability ^[72]. Therefore, new challenges arise in this field, and these issues are becoming more specific and detailed. How do we control the components' percentage of composite polymer families? The methods and choices are increasing, but at the same time, the complexity of studies and characterization of those synthetic polymer-based scaffolds are also being augmented. Similar to vascular replacements created by natural polymers, more exploration and studies should be conducted and established to develop acellular vessel prostheses with small- and macro-dimensions.

	Polymers	Applied Technology	Characterization References
Natural Polymers Collagen type I		Hydrogelation/microfluid;	Strong barrier function after being seeded with human vascular cells; compliance coefficient of [18][63] BSA: $5.5 \times 10^{-6} \pm$ 3.5×10^{-6} cm/s (n = 3) at days 3– 4 and 7.9 × 10 ⁻⁶ \pm 3.5 × 10 ⁻⁶ cm/s at days 6–7; ID = 116 µm
Gelatin	Hydrogelation/laser degradation	D = 50 μm ^{[73][74]}	
	Gelatin	Hydrolyzation/microfluid	Good fluidic ^[30] access and

Table 1. Polymer-Based TEVGs and Characterizations.

	Polymers	Applied Technology	Characterization R	eferences
			cytocompatibility to murine mammary epithelial cells; microscale	
	Silk	Braiding	Implanted as a rodent abdominal aorta with ECs/SMCs migration and alignment observed; ID = 1.5 mm	[<u>75]</u>
	Polysaccharides: HA	Molding/microfluid/hydrogelation	Efficient delivery of nutrients Stiffness: 19–32 kPa; microscale	[<u>76</u>]
	Polysaccharides: alginate/Cacl2 (addition)	Extrusion/injection 3D printing	Stiffness < 500 kPa; short maturation of SMCs; D = 1–3 mm BT; D = 2 µm ST L = 2 µm T = 2 µm	[77]
	Fibrin	3D-quasi microfluid	Strong ADSCs attachment, regrowth, and differentiation; microscale	[<u>78]</u>
Synthetic Polymers	PCL/PVA	Extrusion3D printing	Porosity: 61% with strand space 0.7 mm; 74% with strand space 1 mm; D = 2–4 mm BT	[<u>32]</u>
	PCL/chitosan	Electrospinning/extrusion 3D printing	ST	<u>[31]</u>
	PCL/GeIMA-gellan/alginate	Extrusion 3D printing	D = 4 mm ST	[<u>32</u>]
	PDMS/fibrin		A tissue ring of SMCs after being seeded with	[<u>79]</u>

	Polymers	Applied Technology	Characterization R	eferences
			HASMCs; D = 5 mm ST	
	Silicone		Stiffness: 20– 244.78 kPa; Support culturing of HUVECs, HA- VSMCs, HDF-n; D = 0.5–2 mm ST	[<u>80]</u>
	PU		EM = 1.1 MPa No cytotoxicity at highest concentration 26 mgL^{-1} ; ID = 1.5 mm OD = 4 mm ST	[29]
	PPF	DLP 3D printing	P = 0.35 nm for $ID = 2.5 mm$ support cell culturing of HUVECs, hMSCs, HUSMCs; ID = 2.5 or 1 mm t = 0.25 or 0.15 mm ST	[<u>81]</u> [<u>82</u>]
	PTHD-DA	SLA 3D printing	ID = 18 μm T = 3 μm L = 160 μm BT	[<u>83]</u>
		2PP 3D printing	ID = 2 μm T = 2 mm L = 2 mm ST	[<u>83]</u>
	Heparin-releasing PLLA/PCL	Electrospinning/extrusion 3D printing	D = 5 mm L = 6 cm ST	[<u>27][28]</u>
	PGS/PCL/salt	Casting/molding	SRS = $0.45 \pm$ 0.031 N, EM = 536 ± 119 kPa UTS = $3790 \pm$ 1450 kPa BP = 2360 ± 673 mmHg C = $11\% \pm$ 2.2%, transplanted as rat abdominal aorta with progressive vascular	[84]

Polymers	Applied Technology	Characterization R	eferences	
		remolding in 3 months; ID = 720 μm T = 290 μm		
10% (<i>w/v</i>) P(CL/LA)/PGLA (sealed)	Opation/alestroppinging	SRS = $2.16 \pm$ 0.037 N EM = 17.73 ± 3.09 MPa UTS = $2.93 \pm$ 0.26 MPa BP = 1002.17 ± 181.98 mmHg, support HUVECs' attachment and proliferation; ID = 1.02 ± 0.5 cm T = 0.21 ± 0.02 cm		
15% (<i>w/v</i>) P(CL/LA)/PGLA (sealed)	Casting/electrospinning	SRS = $3.20 \pm$ 0.577 N EM = 26.90 ± 6.66 MPa UTS = $4.75 \pm$ 0.97 MPa BP = 1321.66 ± 214.67 mmHg support HUVECs' attachment and proliferation; ID = 1.01 ± 0.08 cm T = 0.19 ± 0.09 cm		
PLCL (inner layer)/PGA/PLA (outer layer)	Casting/electrospinning	Cell infiltration in scaffold observed, transplanted as infrarenal aortic graft in mice, maintaining 8- month survival; Outer layer ID = 600 µm, inner layer ID = 200 µm T = 3 mm	[<u>38]</u>	
PEGDA	LD	Elongated microchannels and molecule	[<u>73][74]</u>	

	Polymers	Applied Technology	Characterization transportation between unconnected microchannels observed; support HUVECs' seeding; Microcapillary	References
	PU/gelatin	PI	$P = 2 \mu m PE =$ 1.2 ± 0.4 $mLmin^{-1} UTS =$ $2700 \pm 400 kPa$ Support hMSCs' adhesion and growth	[<u>86]</u>
	PLLA/inner MSCs	Sheet-based fabrication	Patency of 100% in 8.6 weeks; vascular remolding observed, SMCs alignment in 60 days; ID = 0.7 mm	[<u>35][36][37]</u>
	PLCL/FB/collagen		4-week transplantation, patency unknown; ID = 4.1 mm	
	PET/PLGA	Braiding	Small ID	<u>[39</u>]
	Polyester/PTT	weaving	EM = 1056 MPa under pressure 200 mmHg	[<u>87]</u>
	Spandex (over 80% PU)/polyester	knitting	Transplanted as dog abdominal aorta; D = 8–10 mm	[<u>88]</u>
	PLA/PCL	CO ₂ gas foaming	Recellularized with HUVECs exhibiting high viability and migration; Small ID	[<u>89]</u>

	Polymers	Applied Technology	Characterization References
	PEG/collagen/PU	Electrospinning/hydrogelation	Mean pressure = 50 mmHg, peak to through pressure = 20 mmHg, circumferential modulus = 190 kPa, SRS = 406 ± 124 gf, BP = 1440 ± 40 mmHg, C = 5.9 ± 1.4%, support rapid endothelialization; ID = 3.7-4.7 mm
	PLGA/collagen/elastin		Stiffness: 2–137 kPa, 2–901 kPa, support ECs, SMCs growth, dry pore area = 1.92 $\pm 0.23 \ \mu\text{m}^2$ wet pore area = 4.74 $\pm 0.43 \ \mu\text{m}^2$; dry D = 384 \pm 22 nm–1196 \pm 79 nm, wet D = 446 $\pm 69 \ nm-1735 \pm 103$
	PA/PEG	Hydrogelation/molding	P = 35 nm, stiffness:0.1–0.3 kPa, 1–4 kPa, 6– 8 kPa, cell adhesion observed
	PGS	molding	Supported the seeding of [94] hSkMDCs and HUVECs
	PDMS/peptides	microfluid	Enhanced blood biocompatibility and cell adhesion
	PLLA/gelatin	Electrospinning	Supported SMCs [96] and HUVECs alignment and proliferation and improved cell

More importantly, the future perspective for developing synthetic-polymer scaffolds should focus on enhancing biophysical performance, such as neovascularization. Some vascular scaffolds proved insufficient for cell regrowth

	Polymers	Applied Technology	Characterization	References	ed from
[<u>107]</u>			proliferation; ID = 2–6 mm	I	liferation
	PCL/collagen		UTS = 4.0 MPa, EM = 2.5 MPa	[<u>97]</u>	(derived
2. Ricklefs Cardiova Clinical J	[<u>108]</u> PCL/PEO/GCC hydrogel sleeve		C = 4.5%, water permeability = 528 mL/cm ² /min, BP = 695 mmHg, SRS = 2.38 N, supported the seeding and culturing of vascular ECs and SMCs in vitro, quick cell growth, and stable flow perfusion; Small ID	[<u>109</u>] 6 [<u>98</u>]	tegrities, a result, erties of n tissue-
	Elastin/PDO		SRS = 375 gf, C = 3.8%, EM = 9.64 MPa	[<u>99]</u> Ə	ardiol.
	collagen/elastin/PLGA/PLCL		Substantial interactions between SMCs; D = 200–800 nm T = 0.5 mm	[<u>91]</u> (
3. Wilhelm substitut	collagen/elastin/PLLA	rrent strategies. Biomed. Enç	UTS = 0.83 MPa, EM = 2.08 MPa	[<u>72</u>] 3	el 35—

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Vessel Types and Axial Directions	Elastic Modulus ^[100]	Ultimate Tensile Strength ^[100]	Strain at Failure (%)	Burst Pressure (mmHg)	References	eview
Saphenous vein	43/4.2/2.25	3/1.8/4	11/243/180	NA/1680-	[101][102]	e-of-the-

	Vessel Types and Axial Directions	Elastic Modulus ^[100]	Ultimate Tensile Strength ^[100]	Strain at Failure (%)	Burst Pressure (mmHg)	Reference	s 54.
1	circumferential				3900/1250		107-
1	Saphenous vein longitudinal	130/23.7	13/6.3	17/83	NA/NA	[<u>102][103</u>]	nto foi
Т	Left internal mammary artery circumferential	8	4.1	134	2000	[<u>102</u>]	TILS TO
1	Left internal mammary artery longitudinal	16.8	4.3	59	NA	[<u>104</u>]	ring
1	Femoral artery circumferential	9–12	1–2	63–76	NA	[105]	Tissu cesse
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