Review Article

Vascular Tissue Engineering: Recent Advances in Small Diameter Blood Vessel Regeneration

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Cardiovascular diseases are the leading cause of mortality around the globe. The development of a functional and appropriate substitute for small diameter blood vessel replacement is still a challenge to overcome the main drawbacks of autografts and the inadequate performances of synthetic prostheses made of polyethylene terephthalate (PET, Dacron) and expanded polytetrafluoroethylene (ePTFE, Goretex). Therefore, vascular tissue engineering has become a promising approach for small diameter blood vessel regeneration as demonstrated by the increasing interest dedicated to this field. This review is focused on the most relevant and recent studies concerning vascular tissue engineering for small diameter blood vessel applications. Specifically, the present work reviews research on the development of tissue-engineered vascular grafts made of decellularized matrices and natural and/or biodegradable synthetic polymers and their realization without scaffold.

1. Introduction

Cardiovascular diseases are the leading cause of death around the world. In 2008, 17.3 million people died from cardiovascular related reasons; specifically 7.3 million were due to coronary heart disease [1]. Currently, in the United States 17% of overall national health expenditures is linked with cardiovascular diseases [2].

Bypass surgeries are commonly performed to allow the peripheral or coronary revascularization. To date, autografts remain the standard clinical approach for the replacement of small diameter blood vessels (inner diameter (ID) < 6 mm). Nevertheless, autografts (such as saphenous vein, arm vein, mammalian artery, or radial artery [3, 4]) show some drawbacks: considerable morbidity associated with autologous harvest and scarce availability due to diseases or previous organ harvesting [4, 5]. Arterial autografts are more indicated for coronary by-pass surgeries due to their higher mechanical properties [6]; internal mammary artery

showed a higher patency rate than saphenous vein (85% versus 61%, after 10 years) [7]. Synthetic materials, for example, polyethylene terephthalate (PET) and expanded polytetrafluoroethylene (ePTFE), are successfully used for the replacement of medium-large diameter blood vessels (ID > 6 mm), when high blood flow and low resistance conditions prevail [3, 4, 8]. However, synthetic grafts used for below-the-knee vascular by-pass and coronary by-pass (ID < 6 mm) fail for unacceptable patency rates in the long term. Patency of ePTFE prostheses is 40–50% when used to bypass the proximal popliteal artery at 5 years and 20% when used for infrapopliteal bypass at 3 years [3]. The use of PET or ePTFE for small diameter blood vessels leads to several complications like aneurysm, intimal hyperplasia, calcification, thrombosis, infection, and lack of growth potential for pediatric applications [3, 4, 9]. These drawbacks are mainly correlated to the regeneration of a nonfunctional endothelium and a mismatch between the mechanical properties of grafts and native blood vessels [3, 5, 6]. A comparison

between the compliance and the elastic modulus of synthetic prostheses and those of native vessels is reported in Table 1.

Causes of graft failure may be classified into early, midterm, and late [10, 11]. Early failures (within 30 days after the implantation) are related to technical complications, flow disturbances, or acute thrombosis [10, 11]. Midterm failures (3 months to 2 years after the implantation) consist of lumen occlusion due to intimal hyperplasia, while late failures (>2 years) are related to atherosclerotic disease [10, 11].

With the aim to improve the scarce patency of synthetic grafts, Deutsch et al. [12] developed a procedure for the autologous *in vitro* endothelialization of ePTFE prostheses (ID = 6–7 mm). Specifically, the inner side of ePTFE prostheses was coated with fibrin to allow for seeding of autologous ECs in *in vitro* rotating conditions; after about 9 days of *in vitro* culture, 341 grafts were implanted as infrainguinal bypass in 310 patients [12]. During 15 years of clinical use, endothelialized ePTFE prostheses showed the presence of endothelium 2–4 years after the surgery and a patency rate similar to vein grafts [12]. The patency rate of 7 mm prostheses was significantly higher than that of 6 mm grafts (78% versus 62% at 5 years, 71% versus 55% at 10 years) [12]. Nevertheless, vascular tissue engineering has become a promising approach to overcome the limits of autografts (e.g., morbidity and scarce availability) and the inappropriate properties of synthetic grafts.

This review summarizes the most relevant and recent studies on vascular tissue engineering for small diameter blood vessel regeneration, focusing on the development of scaffolds made of decellularized matrices and natural and/or biodegradable synthetic polymers and on the realization of tissue-engineered vascular grafts (TEVGs) without scaffold (Figure 1).

2. Vascular Tissue Engineering

As reported by Couet et al. [6], vascular tissue engineering “aims to apply the principles of engineering and life sciences towards the development of a vascular construction that demonstrates biological and mechanical properties as close as possible to those of a native vessel”.

The requirements of an ideal tissue-engineered vascular graft (TEVG), with both large or small diameter, are summarized in Table 2 [3, 5, 13, 14]. Among all requirements for an ideal TEVG (Table 2), the strictest requisites are correlated to the regeneration of a functional endothelium and the similarity between the mechanical proprieties of TEVG and natural blood vessels. These two requirements are related to the failure of PET and ePTFE prostheses for small caliber vessels.

The basic strategy for vascular tissue engineering consists of the design and the production of appropriate scaffolds for vascular cell adhesion, proliferation, and differentiation and the choice of cell type. For human applications, the ideal cells should be nonimmunogenic, functional, and easy to isolate and expand [10]. Two different approaches are mainly developed; the first method consists of the bioreactor uses to generate physiological-like stimuli onto cell seeded scaffolds for *in vitro* TEVG maturation, before the *in vivo* implantation. The latter approach regards the direct implantation of cell

seeded scaffolds in the body that acts as a bioreactor for TEVG maturation. Recently, some studies are focused on the need for off-of-the-shelf grafts for the regeneration of small diameter blood vessels, analyzing the possibility to directly implant acellular scaffolds in the body. The aim of this approach is to develop readily available grafts for urgent vascular surgery.

In the last years increasing interest has been paid to develop an appropriate substitute for small diameter blood vessel replacement. The next paragraphs are focused on the most relevant and recent approaches for vascular tissue regeneration.

2.1. Scaffolds from Decellularized Matrices. Decellularization process aims to remove all cellular and nuclear matter minimizing any adverse effects on the composition, biological activity, and mechanical integrity of the remaining extracellular matrix (ECM) for the development of a new tissue [15–17]. The process usually consists of mechanical shaking, chemical surfactant treatment, and enzymatic digestion [4]. As potential sources of ECM, many organs and tissues (such as skin, ureter, and liver) from humans and animals (such as bovine, sheep, monkeys, pigs, and rabbits) have been decellularized for different applications, such as skin, bone, and valvular heart regeneration [15, 16]. Decellularized matrix advantages are correlated to its natural three-dimensional ultrastructure and its structural and functional proteins, essential for cell adhesion, migration, proliferation, and differentiation [10, 17, 18]. However, the specific composition of the ECM is related to the tissue source. ECM matrices demonstrated to be mainly affected by the age and health status of the animal at harvest and by the manufacturing process, influencing their quality, mechanical and biochemical properties, biocompatibility, and clinical performance [15]. The presence of potential antigenic compound traces (e.g., lipids, DNA, and glycosylation products) may cause an inflammatory response [15, 17]. Furthermore, decellularization procedures may remove desirable ECM components, such as collagen, thus decreasing mechanical properties [15–17]. Decellularized matrices can be stored in hydrated state or in dehydrated lyophilized form. Hydrated ECM matrices demonstrate excellent biomechanical characteristics and improved cellular ingrowth rates; in contrast lyophilized ECM matrices show long shelf life and easy transportability [15, 17].

From a historical context, decellularized matrices derived from many animal organs and tissues (such as porcine small intestinal submucosa, porcine aortas) were widely investigated. In 1999, Sullivan's group [19] developed TEVGs made of porcine small intestinal submucosa and type I bovine collagen. Specifically, porcine small intestinal submucosa was chemically decellularized, wrapped around a 4 mm mandrel, and impregnated with bovine collagen in the TEVG lumen [19]. Then, the collagen layer was crosslinked and coated with heparin-benzalkonium chloride complex [19]. TEVGs were interpositionally implanted in the common carotid artery of rabbits for 90 days [19]. These TEVGs demonstrated excellent patency without hyperplasia and aneurysm formations and allowed the SMC cellularization and the endothelialization, showing physiological vasoreactivity to agonists [19]. In 2000,

TABLE 1: Compliance of natural vessels and synthetic prostheses.

Graft type	Compliance	Elastic modulus	Reference
Artery	$5.9 \pm 0.5\%/100 \text{ mmHg}$	—	[41]
	677%/MPa	0.455 MPa	[42]
Saphenous vein	$4.4 \pm 0.8\%/100 \text{ mmHg}$	—	[41]
PET	$1.9 \pm 0.3\%/100 \text{ mmHg}$	—	[41]
	145%/MPa	1.9 MPa	[42]
ePTFE	$1.6 \pm 0.2\%/100 \text{ mmHg}$	—	[41]
	124%/MPa	2.2 MPa	[42]

FIGURE 1: Sketch of the review content.

TABLE 2: Requirements of an ideal TEVG, in particular small diameter vessels.

Biocompatibility	Nontoxicity
	Nonimmunogenicity
	Nonthrombogenicity
	Nonsusceptibility to infection
	Ability to grow for pediatric patients
Mechanical properties	Maintenance of a functional endothelium
	Compliance similar to native vessel
	Burst pressure similar to native vessel
	Kink and compression resistance
Processability	Good suture retention
	Low manufacturing costs
	Readily available with a large variety of lengths and diameters
	Sterilizable
	Easy storage

Haverich's group [20] developed a trypsin-based decellularization procedure to remove cells from porcine aortas and, then, a recellularization method using a bioreactor, human peripheral venous ECs, and myofibroblasts. To study the *in vivo* immune response, decellularized aortas were subcutaneously implanted in the rat model showing a reduced presence of t-lymphocytes and leukocytes in comparison with the control (not decellularized porcine aortas) [20]. They reported for the first time the complete *in vitro*

endothelialization and the *in vitro* intramural myofibroblast repopulation into decellularized matrices using a bioreactor and human cells [20]. In 2001, Mayer's group [21] chemically decellularized porcine iliac vessels and noninvasively isolated endothelial progenitor cells (EPCs) from peripheral blood of sheep. EPCs were *in vitro* cultured and rotationally seeded into the decellularized matrices. After 4 days in a laminar flow bioreactor, decellularized vessels were *in vivo* implanted into the common carotid arteries of sheep by an end-to-end anastomosis [21]. Decellularized vessels remained patent for 130 days due to the presence of EPCs; in fact not seeded decellularized vessels occluded within 15 days [21]. Furthermore, EPCs-decellularized matrices demonstrated contractile activity and nitric oxide mediated vascular relaxation that was similar to native carotid arteries [21]. In 2003, for the first time, Niklason's group [22] developed a decellularization strategy for TEVGs obtained by culturing bovine aortic or porcine carotid SMCs onto polyglycolic acid (PGA) meshes. This approach avoided the use of allogeneic or xenogeneic tissue, eliminating the risk of viral disease transmission due to the possible utilization of highly screened cells [22]. The group improved this approach in the next years as reported in the following (Table 3).

Table 3 reports the most relevant and recent (2008–2013) studies on decellularized matrices for vascular tissue engineering, focusing on the material source, decellularization method, and mechanical and biological performances.

Among all the approaches reported in Table 3, particular attention is paid to an interesting strategy developed by Niklason's group [23] (Figure 2). This approach consisted of a

TABLE 3: Studies of the scientific literature (2008–2013) on decellularized matrices for vascular tissue engineering.

Organ/material	Decellularization method	Decellularization results	ID	Mechanical results	Biological <i>in vitro</i> results	Biological <i>in vivo</i> results	Reference
Bovine jugular vein	Multistep detergent enzymatic process + photooxidatively crosslinking	Integrity of collagen fibrils and elastic fibers	Patch	UTS~6* MPa significant higher than native bovine veins (~5* MPa)	Coating with fibronectin, gelatin, and collagen IV; SEM images and histological analysis (7 days): confluent layer of HUVECs	Model: rat (12 weeks); implanted subcutaneously; graft stability; chronic inflammatory response	[59]
Rat aortic conduit graft	Detergent-based protocol	—	—	—	—	Model: rat (8 weeks); fibronectin-coated grafts (on both graft surfaces); fibronectin surface coating persistent for at least 8 weeks; luminal endothelialization accelerated by fibronectin; local myofibroblast hyperplasia increased by fibronectin; cell invasion from the adventitial layer into the media increased by fibronectin; enhanced matrix metalloproteinase activity by fibronectin; no inflammatory cell markers; no signs of thrombosis	[60]
Urinary bladder	Soaking in saline + mechanical delamination + soaking in phosphate-buffered saline [61]	Intact basement membrane; bimodal surface	Patch	1 layer: UTS = 1.9–2.3 MPa, $\epsilon_b = 38\text{--}40\%$; 4 layers: UTS = 21–30 MPa, $\epsilon_b = 38\text{--}40\%$	SEM and confocal microscope images (5 days): excellent adhesion, spread, and proliferation with phenotype preservation of HAoECs and HAoSMCs	—	[62]
Porcine abdominal aorta and carotid	Detergent-enzymatic process	Preserved ECM structure with no residual cells; removal of the majority of DNA content	2–11 mm	Circumferential tensile tests $E = 0.22^a$ MPa, UTS = 2.01^a MPa, $\epsilon_b = 1.35^a$, $C = 2.27 \times 10^{-3^a}$ l/mmHg, BP = 2560^a mmHg, SRS = 732^a g.	—	—	[18]

TABLE 3: Continued.

Organ/material	Decellularization method	Decellularization results	ID	Mechanical results	Biological <i>in vitro</i> results	Biological <i>in vivo</i> results	Reference
				<i>stress-relaxation tests</i> $\sigma_{\text{residual}} = 0.78\%$; similar to native porcine arteries (except ϵ_b , σ_{residual}): $E = 0.19^a$ MPa, UTS = 1.55^a MPa, $\epsilon_b = 1.83^a$, $C = 2.61 \times 10^{-3}^a$ l/mmHg, BP = 2331^a mmHg, SRS = 882^a kPa, $\sigma_{\text{residual}} = 0.58\%$			
Porcine abdominal aorta	Nonionic detergent process + lyophilization	Intact collagen and elastin	7-8 mm	—	Smooth muscle-like and endothelial-like cells differentiated <i>in vitro</i> from autologous BMMNCs (3 weeks)	Model: pig (18 weeks); before implant: seeding of SMCs and ECs on grafts (1 week of culture); no sign of thrombus formation, dilatation, or stenosis; regeneration of endothelium, tunica media, and adventitia; presence of collagen and elastin; growth potential	[63]
				BP = 840.37 ± 114.67 mmHg, UTS = 1618.21 ± 691.26 kPa, $E = 7.41 \pm 3.85$ MPa, $C = 4.26 \pm 2.96\%$ /100 mmHg; similar to <i>native human umbilical arteries</i> : BP = 969.66 ± 154.42 mmHg, UTS = 1372.23 ± 809.30 kPa, $E = 13.33 \pm 6.85$ MPa, $C = 5.84 \pm 3.10\%$ /100 mmHg		Model: rat (8 weeks); acellular grafts implanted; 5 rats died within few hours after implantation due to thrombosis; other 6 TEVGs remained patent; thrombosis at the proximal anastomosis; no rupture or aneurysm formation	[64]
Human umbilical artery	Detergent process	Integrity of extracellular collagenous matrix; maybe partial reduction of elastin	1.5 mm		—		

TABLE 3: Continued.

Organ/material	Decellularization method	Decellularization results	ID	Mechanical results	Biological <i>in vitro</i> results	Biological <i>in vivo</i> results	Reference
PGA tubular scaffold cultured with human allogeneic or canine SMCs using a bioreactor (7–10 weeks)	Detergent process	—	3.4 mm canine TEVG 6 mm human TEVG	<i>Canine TEVGs</i> BP = 1618 ± 67^b mmHg; <i>human TEVGs</i> SRS = 178 ± 11^b g; BP = 3337 ± 343^b mmHg; C = $3.3 \pm 0.8^b/100$ mmHg; similar to native human blood vessels	<i>Canine TEVGs</i> before implantation: culture with autologous ECs; ~21 days for EC expansion and 2 days for seeding and preconditioning in a bioreactor	<i>Canine TEVGs for coronary artery bypass</i> model: dog (12 months); excellent long-term patency, and no stenosis or dilatation, and no intimal hyperplasia; 1 animal died with a patent graft; 1 TEVG occluded at 1 week; <i>canine TEVGs for peripheral artery bypass</i> model: dog (1 month); only 1 animal died with a patent TEVG (1 day); <i>human TEVGs for arteriovenous access for hemodialysis</i> ; model: baboon (6 months); patency: 88% (7 of 8); 1 TEVG with thrombosis at 3 months; no aneurysmal dilatation, and no calcification, and no substantial intimal hyperplasia	[23]

UTS: ultimate tensile strength; ϵ_f : strain at break; E: elastic modulus; BP: burst pressure; C: compliance; SRS: suture retention strength; * values were graphically read; ^a median; ^b mean $\pm$ standard error of the mean; σ : stress.
HUVES: human umbilical vein endothelial cells; HAoSMCs: human aortic smooth muscle cells; HAoECs: human aortic endothelial cells; BMVNCs: bone marrow mononuclear cells.

first step for growing allogeneic SMCs onto a PGA tubular scaffold in a bioreactor (Figure 2(a)). The PGA scaffold rapidly degrades, while cells secrete ECM proteins for tissue regeneration (Figure 2(b)). After the maturation period (7–10 weeks), the construct was decellularized and stored in a buffer solution at 4°C until the *in vivo* implantation (Figure 2(c)). TEVGs with ID ≥ 6 mm were readily available for the implant (Figure 2(d)); in contrast TEVGs with ID = 3–4 mm were seeded with autologous ECs isolated by a biopsy (Figure 2(e)), before implantation, to provide an antithrombotic luminal surface, minimizing the risk of graft occlusion [23]. Niklason's group used PGA scaffolds only as a support for the ECM deposition; therefore, the final decellularized constructs did not include the synthetic polymer. This approach may allow the production of many TEVGs using only one human donor [23]. In addition, TEVGs may be produced with the appropriate diameter and ready available due to use of allogeneic cells and decellularization [23].

2.2. Scaffolds from Natural Polymers. Natural polymers generally show excellent biological performances; specifically, they do not activate chronic inflammation or toxicity [24].

Among the natural polymers studied for application in vascular tissue engineering, this paragraph is focused on fibrin, elastin, hyaluronan, silk fibroin, and collagen that show interesting properties for vascular tissue engineering applications and are now the main studied natural polymers.

Fibrin is an insoluble body protein entailed in wound healing and tissue repair [6, 25]. Fibrin clot, obtained by fibrinogen polymerization due to thrombin, is a fibrillar network gel that provides a structural support for adhesion, proliferation, and migration of cells involved in the healing [6, 25]. Finally, fibrin clot is resorbed through the fibrinolysis, a fibrinolytic process that breaks down fibrin fibrils [6, 25]. Fibrinogen may be purified from autologous blood and used for scaffold fabrication avoiding immunological problems [6, 25].

Elastin is one of the major ECM proteins in the arterial wall [6, 25–27] that confers elastic recoil, resilience, and durability [26–28]. It is an important autocrine regulator to SMC and EC activity, inhibiting migration and proliferation of SMCs and enhancing attachment and proliferation of ECs [26, 28]. Elastin, as a coating of vascular devices (made of ePTFE [29], PET, a copolymer of ePTFE and polyethylene, and a polycarbonate polyurethane [30]), demonstrated low thrombogenicity with reduced platelet adhesion and activation [26, 27].

Hyaluronan is an anionic nonsulfated glycosaminoglycan (GAG) that consists of glucuronic acid and *N*-acetyl glucosamine [6]. It can be produced in large amount by microbial fermentation [25]. Furthermore, hyaluronic acid is hydrophilic, nonadhesive, biocompatible, and biodegradable [6, 25].

Silk fibroin is a protein produced by silkworms and spiders [31]. The amino acid structure of silk fibroin from *Bombyx mori* is composed mainly of glycine (43%), alanine (30%), and serine (12%) [31]. It shows excellent mechanical properties and biocompatibility [31]. Silk degrades slowly and

FIGURE 2: Sketch of the innovative strategy developed by Niklason's group, from [23] (reprinted with permission from AAAS).

proteolytically *in vivo*, maintaining more than 50% of its mechanical properties after 2 months [32].

Collagen is the major ECM protein in the body that supplies mechanical support to many tissues [6]. Collagen demonstrates low antigenicity, low inflammatory response, biocompatibility, biodegradability, and excellent biological properties [6, 25, 33]. Collagen type I is one of the main components of the vascular wall, whereas it is widely used as scaffold for vascular tissue engineering applications [6].

Seminal studies on the use of natural polymers for TEVG are concerned with the development of scaffolds made of collagen or fibrin. In 1986, Weinberg and Bell reported the design and the fabrication of the first TEVG made of collagen gel and cells [34]. Specifically, they developed a tubular graft by casting and gelling bovine aortic SMCs and collagen in an annular mold at 37°C, to mimic the tunica media [34]. Then, a mixture of collagen and bovine aortic adventitial fibroblasts was cast around the first tube to recreate the tunica adventitia and, finally, bovine aortic ECs were seeded into the lumen [34]. Unfortunately, the burst pressure of the obtained TEVG was very low and less than 10 mmHg [34]. Therefore, a PET mesh was added allowing for an increase of the burst pressure (40–70 mmHg) [34]. Furthermore, a TEVG composed of three layers of collagen gel and two PET meshes reached a burst strength of 120–180 mmHg [34]. In 2000, Nerem's group [35] designed a dynamic mechanical conditioning method to improve the mechanical properties of adult rat aortic SMCs entrapped in a collagen-gel scaffold. The TEVG was cultured onto an inflatable silicone tube allowing for the transmission of the mechanical stimuli (i.e.,

cyclic strain) [35]. Due to the mechanical conditioning, SMCs increased their circumferential orientation, leading to an improvement of mechanical properties (higher yield stress, ultimate stress, and elastic modulus) [35]. In 2002, Tranquillo's group [36] demonstrated that neonatal aortic rat SMCs embedded in fibrin gel were stimulated to increase the collagen production in comparison with SMCs entrapped in collagen gel. Furthermore, they inhibited the rapid fibrin degradation by SMCs, due to the addition of ϵ -aminocaproic acid to the culture medium that avoids the binding of plasmin or plasminogen to fibrin [36]. After 6 weeks of *in vitro* incubation in TGF- β and insulin, tubular fibrin gel with entrapped SMCs exhibited ultimate tensile strength and elastic modulus similar to those of rat abdominal aorta [37]. In 2005, Andreadis's group [38] developed scaffolds based on ovine SMCs embedded in fibrin gels, using aprotinin as fibrinolysis inhibitor. After 2 weeks in culture, ovine ECs were seeded on the outer surface of the TEVG and cultured for 3 or 10 days [38]. After the reversion of TEVGs (i.e., ECs in the TEVG lumen), TEVGs were interpositionally *in vivo* implanted in jugular veins of 12-week-old lambs for 15 weeks [38]. TEVGs remained patent and showed blood flow rates similar to those of the native jugular vein [38]. Furthermore, TEVGs allowed the production of elastin and collagen fibers, the SMC circumferential alignment, and the presence of a uniform endothelium [38].

In the last years (2008–2013, Table 4), many research groups studied the possible use of natural polymers for TEVGs, following the first results previously obtained [34–38].

Among the number of recent studies developed to fabricate TEVGs using natural polymers, particular attention is paid to structural proteins, such as fibrin [39, 40], elastin [27], and collagen [33], and to the methods to improve their mechanical properties [39, 40] (Table 4). Simultaneously, silk fibroin is widely investigated for vascular application due to its higher mechanical properties in comparison to other natural polymers, such as fibrin [39] (Table 4). However, it is difficult to compare mechanical properties among different studies because of the different conditions used for mechanical characterization, such as crosshead speed, sample shape (rectangular, tubular), and applied load direction.

2.3. Scaffolds from Biodegradable Synthetic Polymers. Biodegradable synthetic polymers generally demonstrate tailorable mechanical properties and high reproducibility and, compared to natural polymers, can be produced in large amounts [27, 43].

Among the biodegradable synthetic polymers under study for application in vascular tissue engineering, this paragraph is focused on polyglycolic acid (PGA), polylactic acid (PLA), poly- ϵ -caprolactone (PCL), and polyglycerol-sebacate (PGS).

PGA is a semicrystalline, thermoplastic aliphatic polyester synthesized by the ring-opening polymerization of glycolide [6, 25]. It degrades rapidly *in vivo* by hydrolysis to glycolic acid, metabolized and eliminated as carbon dioxide and water, and completely degrades *in vivo* within 6 months [6]. PGA is a Food and Drug Administration (FDA) approved polymer [10] for human clinical use.

PLA is a thermoplastic aliphatic polyester synthesized by ring-opening polymerization of lactic acid [6, 25]. It demonstrates good biocompatibility and mechanical properties and the ability to be dissolved in common solvents for processing [44]. PLA is more hydrophobic than PGA, leading to a slower degradation rate [6, 25]. It is a FDA approved polymer [10] for human clinical use. PLA is a chiral molecule: poly-D-lactide (PDLA) and poly-L-lactide (PLLA) are the enantiomeric semicrystalline forms; in contrast the racemate, poly-D,L-lactide (PLA), presents an amorphous structure [6, 44]. PLLA takes months or even years to lose its mechanical integrity [25].

PCL is a semicrystalline, aliphatic polyester synthesized by the ring-opening polymerization of ϵ -caprolactone [6, 25, 45]. It shows good mechanical properties, specifically high elongation and strength, and good biocompatibility [45, 46]. Furthermore, PCL degrades very slowly *in vivo* by enzymatic action and by hydrolysis to caproic acid and its oligomers [6, 45]. It takes more than 1 year to completely degrade *in vivo* [45, 46]. PCL is a FDA approved polymer [6].

PGS is an elastomer synthesized by polycondensation of glycerol and sebacic acid [47]. It demonstrates good biocompatibility and good mechanical properties, specifically high elongation and low modulus, indicating an elastomeric and tough behavior [47]. PGS degrades *in vivo* by hydrolysis in 2 months [47]. The FDA approved glycerol and polymers containing sebacic acid for medical applications [47].

Seminal studies are mainly concerned with the development of scaffolds made of PGA. In 1997, Langer's group [49] designed PGA mesh tubular scaffolds coated with PLLA or 50/50 PLGA copolymer to stabilize PGA meshes. TEVGs were implanted into the omentum of rats demonstrating the *in vivo* maintenance of their structure. Furthermore, TEVGs were *in vitro* seeded and cultured with bovine aortic SMCs and ECs, demonstrating good adhesion and cell proliferation up to 14 days. In 1999, they developed PGA scaffolds chemically modified with sodium hydroxide to increase hydrophilicity [50]. Bovine aortic SMCs were *in vitro* seeded and cultured onto PGA scaffolds under pulsatile radial stress using a bioreactor [50]. After 8 weeks, SMCs migrated in the TEVG wall thickness and bovine aortic ECs were seeded and cultured into the TEVG lumen under continuous perfusion [50]. The TEVG demonstrated good mechanical strength [50]. TEVG was interpositionally implanted into the right saphenous artery of miniature swine and remained patent for 4 weeks, even though there was a decrease of the blood flow [50]. Furthermore, histological analysis showed highly organized structure with minimal inflammation [50]. In 1999, Mayer's group [51] designed scaffolds made of a copolymer of PGA and polyhydroxyalkanoate (PHA). Specifically, ovine carotid arteries were harvested and cultured *in vitro* [51]. After 6–8 weeks, autologous mixed cell population of ECs, SMCs, and fibroblasts was seeded and cultured onto TEVGs [51]. Then, TEVGs were implanted in the abdominal aortas of lambs for 5 months [51]. All TEVGs remained patent without aneurysm formations and histological analysis showed the presence of elastic fibers in the tunica media and ECs in the tunica intima [51]. During the implantation period, the TEVG mechanical properties changed and became similar

TABLE 4: Studies of the scientific literature (2008–2013) on TEVGs fabricated with natural polymers.

Material	Fabrication method	ID	Mechanical properties	Biological <i>in vitro</i> results	Biological <i>in vivo</i> results	Reference
Fibrin	Two-layer grafts: SMCs embedded in fibrin hydrogel + fibrin layer composed of high concentration fibrinogen	4 mm	<i>After 2 weeks</i> two layers UTS ~ 90* kPa, BP = 177 ± 5.3 mmHg; single layer UTS ~ 65* kPa, BP = 18 ± 7.7 mmHg; UTS calculated from circumferential tensile tests	SMCs isolated from 3-day-old lambs; <i>histological analysis</i> after 2 weeks of culture: SMCs distributed uniformly in the inner layer, SMCs did not migrate into the outer fibrin layer, and SMCs maintained their contractile properties inside gels	—	[39]
			<i>After 7–9 weeks</i> <i>circumferential tensile tests</i> <i>E</i> ~ 2500* kPa, UTS ~ 1800* kPa; <i>axial tensile tests</i> <i>E</i> ~ 900* kPa, UTS ~ 1200* kPa; <i>ID</i> = 2 mm, BP = 1366 ± 177 mmHg, SRS = 0.19 ± 0.05 N, <i>C</i> ~ 4.5*/100 mmHg; <i>ID</i> = 4 mm BP = 1542 ± 188 mmHg, SRS = 1.32 ± 0.58 N (with entrapped PLA cuffs), <i>C</i> ~ 2.5*/100 mmHg; <i>ovine femoral artery</i> BP = 2297 ± 207 mmHg, <i>C</i> ~ 3.25*/100 mmHg BP = 575.67 ± 17.47 mmHg, <i>C</i> = 3.51 ± 0.42%/100 mmHg; <i>circumferential tensile tests</i> DR = 5 mm/min: UTS ~ 2.4* N, ϵ_b ~ 57*%; DR = 25 mm/min: UTS ~ 1.8* N, ϵ_b ~ 37*%; DR = 50 mm/min: UTS ~ 1.2* N, ϵ_b ~ 34*%	<i>Histological analysis</i> after 7–9 weeks of static + dynamic culture: circumferential collagen fiber alignment, minor residual fibrin in 2 mm grafts, none evident in 4 mm grafts, and nhDFs evenly distributed across the thickness	—	[40]
Silk fibroin	Electrospinning using a rotating translating mandrel	6 mm		<i>MTT assay</i> (7 days): linear increase of NIH/3T3 viability over time; <i>SEM images</i> (7 days): NIH/3T3 attached, spread, and grew; cell motility through matrix	—	[65]

TABLE 4: Continued.

Material	Fabrication method	ID	Mechanical properties	Biological <i>in vitro</i> results	Biological <i>in vivo</i> results	Reference
Silk fibroin	Gel spinning	1, 1.5 mm	<i>Silk</i> $E = 2.20 \pm 0.90$ MPa, $UTS = 0.273 \pm 0.11$ MPa; <i>native rat aorta</i> $E = 2.44 \pm 0.76$ MPa, $UTS = 0.519 \pm 0.11$ MPa; <i>PTFE</i> $E = 918 \pm 52.9$ MPa, $UTS = 43.4 \pm 4.6$ MPa	<i>Protein adsorption:</i> lower on silk than PTFE; <i>platelet adhesion and spreading:</i> silk and PTFE relatively nonthrombogenic; <i>confocal images</i> (overnight incubation): higher HCASMC and HUVEC attachment on silk than PTFE; <i>Pico Green DNA assay</i> (for 14 days): higher rate of HCASMC and HUVEC proliferation on silk than PTFE	Model: rat (4 weeks); no occlusion, clotting, or ischemia; vascular cell remodeling: confluent endothelium, SMCs migration and proliferation	[66]
			BP = 894.00 ± 24.91 mmHg, $C = 3.24 \pm 0.58\%/100$ mmHg; <i>circumferential tensile tests</i> DR = 5 mm/min $UTS = 3.17 \pm 0.32$ N, $\epsilon_b = 58.96 \pm 4.07\%$; DR = 25 mm/min $UTS = 2.76 \pm 0.39$ N, $\epsilon_b = 35.6 \pm 2.13\%$; DR = 50 mm/min $UTS = 2.5 \pm 0.12$ N, $\epsilon_b = 39.04 \pm 5.31\%$	<i>MTT assay</i> (7 days): linear increase of NIH/3T3 viability over time, collagen increase metabolic activity at the early time points; <i>SEM images</i> (7 days): NIH/3T3 attached, spread, and grew	—	[33]
	Electrospinning using a rotational mandrel + dynamic dipping	6 mm				
Hyaff-11 (hyaluronan- based material)	Coating + coagulation bath (ethanol)	4 mm	—	—	Model: porcine (5 months); fibrin glue: sealed anastomotic sites; animal survival: 100%, no signs of vascular insufficiency; 2 grafts completely occluded after 2 and 3 months, 1 graft partially occluded after 4 months, and 7 grafts showed no occlusion or intimal hyperplasia; grafts almost completely degraded; formation of neotissue (SMCs, collagen, elastin fibers organized in layers, complete endothelium)	[28]

TABLE 4: Continued.

Material	Fabrication method	ID	Mechanical properties	Biological <i>in vitro</i> results	Biological <i>in vivo</i> results	Reference
Recombinant human tropoelastin [#]	Electrospinning using a rotating translating mandrel	4 mm 1 cm	$C = 20.2 \pm 2.6\%/100 \text{ mmHg}$, BP = $485 \pm 25 \text{ mmHg}$; <i>circumferential tensile tests</i> UTS = $0.34 \pm 0.14 \text{ MPa}$, $\epsilon_b = 79 \pm 6\%$, $E = 0.15 \pm 0.05 \text{ MPa}$; <i>axial tensile tests</i> UTS = $0.38 \pm 0.05 \text{ MPa}$, $\epsilon_b = 75 \pm 5\%$, $E = 0.15 \pm 0.03 \text{ MPa}$;	<i>Confocal images</i> after 48 h of culture: BMEOCs attached, spread, and grew; cells formed a confluent monolayer	—	[27]
			native porcine carotid arteries			
			$C = 3.4 \pm 0.5\%/100 \text{ mmHg}$; <i>circumferential tensile tests</i> UTS = $2.59 \pm 0.31 \text{ MPa}$, $\epsilon_b = 125 \pm 15\%$, $E = 0.41 \pm 0.09 \text{ MPa}$;			
			<i>axial tensile tests</i> UTS = $0.95 \pm 0.13 \text{ MPa}$, $\epsilon_b = 105 \pm 11\%$, $E = 0.20 \pm 0.06 \text{ MPa}$			

[#] monomer unit of elastin, cross-linked tropoelastin mimic native elastin fibers.

UTS: ultimate tensile strength; ϵ_b : strain at break; E : elastic modulus; C: compliance; BP: burst pressure; SRS: suture retention strength; DR: deformation rate; * values were graphically read.
nDFs: neonatal human dermal fibroblasts; BMEOCs: porcine bone marrow-derived endothelial outgrowth cells; NIH/3T3: murine fibroblast cell line; HUVECs: human umbilical vein endothelial cells; HCASMCs: human coronary artery smooth muscle cells.

to those of native vessels [51]. In 2001, Shin'oka's group [52] performed the first transplantation of a TEVG in four-year-old girl. The TEVG consisted of PGA mesh coated with a 50 : 50 copolymer of L-lactide and ϵ -caprolactone, previously *in vitro* seeded with autologous vascular cells isolated from peripheral vein biopsies [52]. After 7 months, there was no evidence of aneurysm or stenosis formations [52]. The clinical trial performed by Shin'oka's group is widely described in Section 4.

Among the several recent studies (2008–2013, Table 5) developed to fabricate TEVGs using biodegradable synthetic polymers, particular attention is paid to PCL due to its deformability and mechanical strength (Table 5). PCL was studied alone [46, 53, 54], as multilayers (PGA-PCL-PGA [55]), electrospun coating on PGS [56], or copolymer with L-lactide [57, 58] (Table 5). For example, the use of PCL as PGS tube coating increased the mechanical properties of the substrate, specifically suture retention strength, elastic modulus, and ultimate strength stress [56].

2.4. Synthetic versus Natural Scaffolds. TEVGs should have appropriate biological behavior and mechanical properties to reach the clinical use. TEVG biological performance should support the complete integration of the grafts in the human body, avoiding the induction of a chronic inflammatory response during the material degradation process. Furthermore, TEVG mechanical properties should match those of the native blood vessels, specifically in terms of deformability, compliance, and strength.

One of the essential principles of tissue engineering is the choice of a scaffold with appropriate degradation rate during the *in vivo* remodeling process. The speed of the scaffold degradation has to match the tissue-dependent regeneration rate to develop a functional substitute. For vascular tissue engineering, the key point is the maintenance of the structural and mechanical integrity of the neovascular tissue over all the regeneration process.

Generally, natural polymers lack mechanical performance, but they exhibit excellent biological behavior. As reported in Table 4, tropoelastin electrospun scaffolds showed low mechanical strength compared to native blood vessels, specifically, in terms of ultimate tensile strength (0.34 ± 0.14 MPa) and burst pressure (485 ± 25 mmHg) [27]. However, *in vitro* cell culture demonstrated a good interaction with porcine bone marrow-derived endothelial outgrowth cells, forming a confluent monolayer after 2 days [27].

Normally, natural scaffolds are seeded and cultured *in vitro* with cells prior to *in vivo* implantation to obtain a partial remodeling of the structure due to ECM deposition. This *in vitro* remodeling usually leads to an increase of the graft mechanical properties. As reported in Table 4, fibrin gels with entrapped SMCs were dynamically cultured in a bioreactor for several weeks to achieve a circumferential alignment similar to native arteries [40]. This alignment improved the mechanical properties of the grafts, reaching values similar to native blood vessels [40]. To date, studies related to natural materials for vascular applications are focused on the improvement of graft mechanical properties. To reach this

goal, some approaches consist of tissue *in vitro* maturation [40]. Other methods combine the use of natural materials with synthetic polymers (such as tropoelastin with PCL [26]), reaching sometimes promising results in terms of mechanical properties and cell/material interaction.

Synthetic polymers show controllable physical and mechanical properties but lack in biological performances. The products of PGA degradation could lead to a SMC dedifferentiation and to a pH decrease, potentially inducing an undesired inflammatory response [10, 39, 68]. For example, as reported in Table 5, PCL electrospun scaffolds demonstrated high elongation, similar to that of native arteries, and slow degradation, allowing for cell infiltration and tissue regeneration [46, 53, 54]. A long-term *in vivo* study of the PCL electrospun tubes was performed in the rat aorta for 18 months [46]. During the first 6 months, PCL electrospun scaffolds induced a promising tissue regeneration with a rapid colonization and a wide neovascularization [46]. In fact, fibroblasts and macrophages infiltrated and proliferated in the PCL graft mainly from the adventitial side; the fibroblasts become myofibroblasts that deposited a natural ECM in the PCL graft wall and the macrophages secreted angiogenic factors to induce neovascularization [46]. After 6 months, tissue regeneration showed a clear regression with a reduction of capillaries and fibroblasts, an increase of calcification, and a cell regression in intimal hyperplasia [46]. The tissue regression was caused by macrophage disappearance, inducing the neovascularization decrease and the consequent myofibroblast reduction, due to the decrease of oxygen and nutrient presence [46]. Furthermore, calcifications were related to the process of chondroid metaplasia that initially regards the transdifferentiation of smooth muscle cells into chondrocytes in the intimal hyperplasia layers [46]. Therefore, PCL electrospun tubes developed by de Valence et al. [46] demonstrated insufficient regeneration of the blood vessels on the long term (18 months); this result was unexpected because of the promising conclusions obtained in a previous study [79] regarding the implantation of PCL electrospun graft in rat model for 6 months. Specifically, the PCL electrospun grafts demonstrated patency, structural integrity, faster endothelialization, ECM formation, and fiber degradation during 6 months [79].

2.5. Hybrid Scaffolds from Synthetic and Natural Polymers. Recent studies have proposed hybrid scaffolds, made of synthetic and natural polymers, to combine the adequate mechanical properties of synthetic materials with the excellent biological behavior of natural polymers.

Table 6 reports the most relevant and recent (2008–2013) studies on TEVGs fabricated using hybrid scaffolds made of natural and biodegradable synthetic materials, focusing on the fabrication method and mechanical and biological performances.

Among the several recent studies aimed to fabricate TEVGs using hybrid scaffolds, particular attention is paid to the use of PCL with natural polymers such as elastin [26, 68] and collagen [45, 68, 70] (Table 6). As an example, the addition of PCL to tropoelastin electrospun scaffolds improved

TABLE 5: Studies of the scientific literature (2008–2013) on TEVGs fabricated with biodegradable synthetic polymers.

Material	Fabrication method	ID	Mechanical properties	Biological <i>in vitro</i> results	Biological <i>in vivo</i> results	Reference
PCL	Electrospinning of 2 layers with different porosity using a cylindrical rotating translating mandrel; OLLP = inner layer with lower porosity; OLLP = outer layer with lower porosity	2 mm	<i>ILLP</i> UTS = 3.67 ± 0.76 MPa, $\epsilon_b = 900 \pm 229\%$, $E = 8.8 \pm 0.9$ MPa, BP = 2845 ± 241 mmHg, SRS = 556 ± 47 gf, WL = 33.2 ± 4.6 mL cm⁻² min⁻¹, BL = 0.25 ± 0.06 mL cm⁻² min⁻¹; <i>OLLP</i> UTS = 3.66 ± 0.30 MPa, $\epsilon_b = 862 \pm 142\%$, $E = 8.5 \pm 0.8$ MPa, BP = 2688 ± 192 mmHg, SRS = 490 ± 41 gf, WL = 39.3 ± 2.8 mL cm⁻² min⁻¹, BL = 0.89 ± 0.39 mL cm⁻² min⁻¹; UTS, E, and ϵ_b calculated from axial tensile tests	—	Model: rat (12 weeks); endothelialization nearly complete; ILLP: allowed good cell invasion from the adventitia; OLLP: inhibits graft cellularization; perfect patency with no thrombosis	[53]
					Model: rat (18 months); patency = 72.5%; no aneurysm formation; no calcification; scaffolds did not disappear completely; endothelium: complete regeneration; media: partial regeneration	[54]
					Model: rat (18 months); graft <i>in vivo</i> C = $7.8 \pm 0.9\%$/mmHg, proximal native artery <i>in vivo</i> C = $21.0 \pm 2.8\%$/mmHg; excellent structural integrity; no aneurysmal dilation, perfect patency with no thrombosis, and limited intimal hyperplasia (maximum 5% lumen loss); promising tissue regeneration until 6 months, after 6 months a clear regression; calcifications: spread transmurally at 18 months; limited vascularization at 18 months	[46]
PCL	Electrospinning using a cylindrical rotating translating mandrel	2 mm	BP = 3280 ± 280 mmHg, SRS = 936 ± 32 g, WL = 32.1 ± 1.3 mL cm⁻² min⁻¹, BL = 0.87 ± 0.08 mL cm⁻² min⁻¹; <i>axial tensile tests:</i> UTS = 4.1 ± 0.5 MPa, $\epsilon_b = 1092 \pm 28\%$	—		

TABLE 5: Continued.

Material	Fabrication method	ID	Mechanical properties	Biological <i>in vitro</i> results	Biological <i>in vivo</i> results	Reference
PGA or PLLA nonwoven felts + PCL/PLA sealant solution	Dual cylinder chamber molding system	PGA-PCL/PLA = 0.9 mm; PLLA-PCL/PLA = 0.7 mm	PGA-PCL/PLA: BP = 2710 ± 282 mmHg, SRS = 3.13 ± 0.72 N, E = 33.0 ± 7.0 MPa, UTS = 5.3 ± 0.72 MPa; PLLA-PCL/PLA: BP = 2790 ± 180 mmHg, SRS = 4.37 ± 0.67 N, E = 24.0 ± 5.9 MPa, UTS = 3.7 ± 0.53 MPa	MTT after 24 h: no difference in HAoSMC viability between PGA-PCL/PLA, PLLA-PCL/PLA, and TCP; <i>histological analysis</i> after 72 h: HAoSMCs penetrated and adhered to both scaffolds	Model: mice (6 weeks); acellular grafts implanted; foreign body reaction with giant cell formation (at 3 weeks); encapsulation around the external periphery of PLLA-PCL/PLA grafts; no thromboembolic complications or aneurysm formation; organized vascular neotissue (endothelium, media, and collagen)	[57]
PGA nonwoven mesh + PCL porous sheet + PGA nonwoven mesh	PGA and PCL seeded with SMCs + after 4 days SMC-PGA wound around a silicone tube + after 3 days SMC-PCL wound around SMC-PGA + after 3 weeks PGA seeded with fibroblasts + after 2 days fibroblast-PGA wound around SMC-PCL (2 days) + silicone tube removed + seeding of ECs in the lumen + static culture (2 days) + pulsatile bioreactor (14 days)	6 mm	Circumferential tensile tests after 14 days of culture: UTS = 827 ± 155 kPa, E of collagen region = 3.75 ± 0.78 MPa, E of elastin region $\sim 0.1^*$ MPa, $\epsilon_b \sim 0.42^*$; only polymeric scaffold: UTS = 91 ± 21 kPa, $\epsilon_b \sim 0.6^*$; bovine native arteries: UTS = 882 ± 133 kPa, E in collagen region = 3.31 ± 0.56 MPa, E in elastin region $\sim 0.075^*$ MPa, $\epsilon_b \sim 0.6^*$	SMCs, fibroblasts, and ECs isolated from calf; <i>histological analysis</i> (after 14 days in the bioreactor): formation of 3 layers, luminal area: presence of middle layer: presence of abundant elastin, well-organized dense collagen ECM, SMCs, and PCL, outer region: presence of loose collagen ECM	—	[55]
Heparin-coated PGS porous tube wrapped with a PCL thin electrospun layer	Salt fusion method + electrospinning	720 μ m	PGS + PCL SRS = 0.45 ± 0.031 N, E = 536 ± 119 kPa, UTS = 3790 ± 1450 kPa; PGS SRS = 0.11 ± 0.0087 N, E = 243 ± 71.8 kPa, UTS = 76.6 ± 15.7 kPa; E, UTS calculated from circumferential tensile tests; <i>neoartery</i> BP = 2360 ± 673^a mmHg, C = $11 \pm 2.2^a\%$/100 mmHg; <i>native aorta</i>	—	Model: rat (3 months); no aneurysm and stenosis; regular, strong, and synchronous pulsation of grafts; confluent endothelium, contractile smooth muscle layers, and coexpression of elastin, collagen, and GAG;	[56]

TABLE 5: Continued.

Material	Fabrication method	ID	Mechanical properties	Biological <i>in vitro</i> results	Biological <i>in vivo</i> results	Reference
			BP = 3415 ± 529 ^a mmHg, C = 6.7 ± 2.3 ^a %/100 mmHg		graft extensive degradation + ECM synthesis within 14 days; remodeling still active at 3 months	
PCL/PLA	Patient-specific scaffolds: rapid prototyping (using radiographic X-ray slices) + lost wax + dip coating + selective dissolution + salt leaching	>1 mm	E = 0.6 to 5.2 MPa; higher E for lower porosity and higher concentration	PCL/PLA scaffolds coated with collagen; <i>fluorescent images</i> (4 days): HUVEC attachment; material biocompatibility confirmed	—	[58]
PGA nonwoven mesh	Seeding of SMCs + culture for 7 days in static condition + mesh wound around a silicone tube + pulsatile bioreactor (8 weeks)	4 mm	<i>After 8 weeks of culture</i> UTS = 0.62 × 10 ⁶ Pa, E = 10.5 ± 1.25 MPa, SRS = 1.26 ± 0.16 N, BP ~ 1.2* MPa; ~50–60% of those of human saphenous veins: UTS ~ 0.95 × 10 ⁶ Pa, E ~ 14.5* MPa, SRS ~ 2.25* N, BP ~ 1.2* MPa; UTS, E calculated from circumferential tensile tests	SMCs differentiated from hASC (7 days); <i>histological analysis</i> (after 8 weeks in the bioreactor): no remnant of undegraded PGA fibers, dense structure with well-populated SMCs, SMCs orientated in layers, and no elastic fibers	—	[67]

PCL/PLA: 50:50 copolymer of ε-caprolactone and L-lactide.
UTS: ultimate tensile strength; E: elastic modulus; ϵ_p : strain at break; SRS: suture retention strength; BP: burst pressure; C: compliance; WL: water leakage at 120 mmHg; BL: blood leakage at 120 mmHg; * values were graphically read; ^a mean ± standard error of the mean.
hASCs: human adipose-derived stem cells; HAoSMCs: human aortic smooth muscle cells; HUVECs: human umbilical vein endothelial cells; GAG: glycosaminoglycan; TCP: tissue culture plastic.

TABLE 6: Studies of the scientific literature (2008–2013) on TEVGs fabricated with natural and synthetic polymers.

Material	Fabrication method	ID	Mechanical properties	Biological <i>in vitro</i> results	Biological <i>in vivo</i> results	Reference
Blend of PCL + elastin + collagen type I	Electrospinning of 3 layers at different polymer ratios using a cylindrical rotating translating mandrel	2 mm	SRS = 91–189° gf, BP = 2387–>3000° mmHg; C = 2.83–0.77°%/100 mmHg, increase of PCL + decrease of elastin: increase of SRS and BP, decrease of C	—	—	[68]
Recombinant human tropoelastin (inner layer) + PCL (outer layer)	Electrospinning using a rotating mandrel; cross-linking: glutaraldehyde vapors	2.8 mm	$E \sim 300^* \text{ kPa}$, BP $\sim 1900^* \text{ mmHg}$, $C \sim 0.065^* \text{ kPa}^{-1}$, HP = minimal; <i>similar to internal mammary artery</i> : $E \sim 267 \pm 46 \text{ kPa}$, BP $\sim 2267 \pm 215 \text{ mmHg}$, $C \sim 0.09^* \text{ kPa}^{-1}$	SEM and fluorescence microscope (3 days): HUVECs attached and proliferated; <i>platelet attachment</i> : reduced, compared to ePTE and PCL	Model: rabbit (1 month); maintenance of physical integrity; no evidence of dilatation, or anastomotic dehiscence, or seroma; mechanical properties of implanted grafts remained similar to controls	[26]
PGA + PLLA + collagen type I	Collagen microsponge + woven tube made of core-sheath yarns (PLLA and PGA, resp.) fabricated by air-jet spinning	4 mm	<i>Axial tensile tests</i> before implantation : UTS $\sim 30^* \text{ MPa}$ versus, $E \sim 210^* \text{ MPa}$; after implantation : UTS $\sim 5\text{--}9^* \text{ MPa}$, $E \sim 70\text{--}100^* \text{ MPa}$; native carotid artery : UTS $\sim 7.5^* \text{ MPa}$, $E \sim 60^* \text{ MPa}$	Cell culture with NIH/3T3 or HUVEC (3 days); SEM analysis: higher cell number and better adhesion for grafts with collagen	Model: dog (12 months); acellular grafts; 100% patency, no thrombosis or aneurysm; excellent tissue regeneration: complete endothelialization, SMCs, elastin, and collagen fibers; PGA fibers completely absorbed at 2 months, PLLA fibers unabsorbed at 12 months	[69]
Blend of PCL + collagen type I	Electrospinning using a rotating mandrel	4.75 mm	SRS = $3.0 \pm 1.1 \text{ N}$, BP = $4915 \pm 155 \text{ mmHg}$, $C = 5.6 \pm 1.6\%/100 \text{ mmHg}$; <i>axial tensile tests</i> : UTS = $4.0 \pm 0.4 \text{ MPa}$, $E = 2.7 \pm 1.2 \text{ MPa}$, $\epsilon_b = 140 \pm 13\%$; maintenance of tensile properties in a perfusion bioreactor system under overphysiological conditions for 4 weeks; native porcine coronary artery : UTS $\sim 2.5^* \text{ MPa}$, $E \sim 1^* \text{ MPa}$, $\epsilon_b \sim 100^* \%$	ECs and SMCs from bovine carotid artery; <i>cytotoxicity via direct contact</i> (7 days—MTS assay): no differences in cell viability compared to TCPs; <i>cytocompatibility</i> (7 days—MTS assay): EC and SMC adhesion and proliferation; <i>cell culture on tubular grafts</i> (48 h—SEM and histological analysis): SMCs formed multiple cell layers on the outer region, ECs formed a monolayer on the inner surface	—	[45]

TABLE 6: Continued.

Material	Fabrication method	ID	Mechanical properties	Biological <i>in vitro</i> results	Biological <i>in vivo</i> results	Reference
Blend of PCL + collagen type I	Electrospinning using a rotating mandrel	4.75 mm	<i>Circumferential tensile tests</i> before implantation: UTS ~ 1.8* MPa; after implantation: UTS ~ 0.8* MPa; comparable to native aorta: UTS ~ 1.2* MPa	ECs derived from endothelial progenitor cells isolated from live sheep; SMCs isolated from sheep artery explants; dynamic culture using a pulsatile bioreactor under physiologic conditions (9 days); SEM + <i>histological analysis</i> : ECs formed a confluent layer on the inner lumen, SMCs assumed a multilayered configuration on the exterior	<i>Platelet adhesion</i> in a live sheep model for 15 min: no adhesion for seeded grafts, abundant adhesion for unseeded grafts; <i>model: rabbit</i> (1 month); acellular grafts; no aneurysmal degeneration; majority of the grafts remained patent at 1 month; absence of inflammatory infiltrate	[70]
PCL/PLA + collagen type I	PCL/PLA electrospinning using a rotating mandrel + collagen coating after air plasma treatment	1, 3 mm	<i>Axial tensile tests:</i> UTS = 7.0 ± 0.4 MPa, $E = 16.0 \pm 7.1$ MPa, $\epsilon_b = 289 \pm 55\%$; <i>circumferential tensile tests:</i> UTS = 3.9 ± 0.3 MPa, $E = 16.6 \pm 4.4$ MPa, $\epsilon_b = 292 \pm 87\%$	HCAECs rotationally seeded in the bioreactor for 4 h + <i>static culture</i> (10 days—SEM and histological analysis); HCAECs evenly distributed and well spread on the lumen	Model: rabbit (7 weeks); acellular grafts without collagen coating; no blood leaking; graft structure integrity and patency; foreign body response: no cell infiltration	[71]
PLDLA + fibrin	PLDLA tubular warp knit structure + coating of fibrin gel with SMCs and fibroblasts	~5 mm	—	ECs, SMCs, and fibroblasts isolated from ovine carotid arteries; ECs seeding in the inner layer using a rotating chamber; dynamic culture in a bioreactor under physiological pressure conditions for 21–28 days; <i>histological analysis:</i> a dense, homogenous distribution of cells throughout the scaffold thickness, extensive collagen	Model: sheep (6 months); ID ~ 6 mm; cellular grafts (dynamic culture for 21–28 days); at 3 months 1 graft with a significant stenosis; other grafts: completely patent, no thrombus formation and aneurysm, infection, or calcification; remodeling: confluent endothelium, collagen and other ECM components, and elastin (not assembled in cohesive sheets);	[43]

TABLE 6: Continued.

Material	Fabrication method	ID	Mechanical properties	Biological <i>in vitro</i> results	Biological <i>in vivo</i> results	Reference
PCL/PLA: 70 : 30 copolymer of ϵ -caprolactone and L-lactide; PLDLA: 96 : 4 copolymer of L-lactide and D-lactide. SRS: suture retention strength; BP: burst pressure; C: compliance; ° range of average values of grafts with 3 different media compositions; HP: hydraulic permeability; UTS: ultimate tensile strength; E: elastic modulus; ϵ_b : strain at break; * values were graphically read. HUVECs: human umbilical vein endothelial cells; HCAECs: human coronary artery endothelial cells; NIH/3T3: murine fibroblast cell line; TCPs: tissue culture plates.						
				formation, and no evidence of calcification	fibrin completely degraded after 1 month, sparse PLDLA material remained at 6 months	

FIGURE 3: Sketch of the *in vivo* bioreactor approach; (a) fibrous capsule formed around a Silastic tubing in the peritoneal cavity; (b) after removal of Silastic tubing, reversion of fibrous capsule, the mesothelium was the inner layer.

FIGURE 4: Sketch of different cell sheet-based approaches; (a) tissue-engineered vascular media (TEVM) and tissue-engineered vascular adventitia (TEVA) were assembled by rolling a single cell sheet of SMCs or fibroblasts; (b) the standard approach, to produce a TEVMA, required rolling a fibroblast sheet around a SMC sheet (stdTEVMA); (c) the single-step TEVMA was produced due to the presence of SMCs and fibroblasts in the same sheet (ssTEVMA), from [48]. The publisher for this copyrighted material is Mary Ann Liebert, Inc. publishers.

the mechanical properties, making them more similar to native blood vessels [26, 27] (Tables 4 and 6). Specifically, compliance and burst pressure of PCL/tropoelastin scaffolds decreased and increased, respectively, when compared to scaffolds made of tropoelastin alone [26, 27] (Tables 4 and 6).

2.6. TEVGs without Scaffolds. Some studies were focused on fabrication of completely biological TEVGs without the use of scaffolds. This strategy avoids problems that may be related to synthetic materials, such as inflammation, stenosis, and infection, allowing for the complete graft integration and increase of the patency rate [8, 25]. Three main approaches have been developed: the *in vivo* bioreactor, the cell sheet-based, and the cell ring-based methods.

The *in vivo* bioreactor approach consists in the use of the body as a bioreactor, as a consequence of the foreign body reaction due to the implantation of a “nonself” material [6]. A Silastic (silicone compound) tubing was implanted in the autologous peritoneal cavity inducing a foreign body reaction with the formation of a fibrous capsule around the mandrel [8, 25, 80]. The fibrous capsule was composed of myofibroblasts, collagen matrix, and a single layer of mesothelial cells (Figure 3(a)) [8, 25, 80]. After 2–3 weeks, the explanted tube of tissue was removed from the Silastic mandrel and reversed, so the mesothelium represented the inner layer (Figure 3(b)) [25]. The main limitations of this method are the requirement of a double surgery and the possible TEVG adhesion to the peritoneal wall during maturation [8, 80] and, hence, the damage to the mesothelium (the external layer).

The cell sheet-based approach consists of the *in vitro* growing of cells in the presence of ascorbic acid in the culture medium to generate a large production of ECM [8]. After a maturation period, cell sheets were detached from the culture flasks and rolled onto a mandrel (Figure 4) [8]. TEVGs were cultured for about 8 weeks until the layers merged in a uniform structure (Figure 4) [8]. The major limitation of this approach is the long time required for the *in vitro* TEVG growth prior to implantation (6 to 9 months [81]).

The cell ring-based approach consists of the preparation of TEVGs from aggregated cells and cell-derived ECM, due to the use of annular agarose wells [73]. Immortalized rat aortic smooth muscle cells seeded into annular agarose wells with different ID (2, 4, or 6 mm) aggregated and formed thick tissue rings within 2 weeks of *in vitro* static culture (Figure 5) [73]. Tissue rings could be fused to generate tubular constructs. Specifically, tissue rings were placed onto silicone tube mandrels and cultured in close contact for 7 days to prepare cohesive tubular constructs [73].

FIGURE 5: Sketch of cell ring-based approach; (a) schematic of cell seeding and ring self-assembly process; monodisperse cells (black dots) are pipetted into ring-shaped agarose wells; within 24 hours, cells form ring-shaped aggregates (black circles) contracted around center posts; (b) photograph of 2 mm diameter rat smooth muscle cell rings (arrowhead) in agarose wells; (c) schematic of agarose well fabrication process; silicone elastomer (PDMS) is cured on machined polycarbonate molds to form a template; molten agarose is poured into the PDMS template to form agarose wells; polycarbonate mold dimensions (post diameter, p ; well width, w ; and well height, h) can be modified to make cell rings of different sizes (courtesy of Dr. Marsha W. Rolle, Worcester Polytechnic Institute).

The most relevant and recent (2008–2013) studies on TEVG development without the use of scaffolds are reported in Table 7, focusing on the fabrication method and mechanical and biological performances. Cell sheet-based TEVGs, developed by L'Heureux's group and involved in a clinical trial, are widely described in Section 4.

3. Clinical Trials of Vascular Tissue Engineering

Clinical trials were conducted by Shin'oka's et al. [52] and L'Heureux's [82] groups using two different types of TEVGs. Although Shin'oka's group did not develop TEVGs for small diameter blood vessel regeneration (ID = 12–24 mm), their studies are described in this paragraph because they carried out the first successful clinical trial of TEVGs.

In 2001, Shin'oka et al. [52] performed the first successful clinical trial of a TEVG in humans (25 patients), specifically in the high-flow low-pressure pulmonary venous system of pediatric and young patients. The patients were not treated with long-term antiplatelet and anticoagulation therapy [83]. The investigated TEVGs consisted of a polymeric scaffold made of PGA or PLLA fiber-based meshes coated with a 50:50 copolymer of L-lactide and ϵ -caprolactone (PCLA/PGA or PCLA/PLLA) [83]. Specifically, TEVGs were fabricated by pouring and freeze-drying a PCLA solution onto the PGA or PLLA woven mesh (ID = 12–24 mm) [14]. Shin'oka's group designed these scaffolds to improve the poor compliance match with blood vessels and the poor surgical handling properties of PGA fibers [83]. For the first surgeries, grafts were *in vitro* seeded with autologous vascular cells isolated from peripheral vein biopsies [52, 83, 84]. This procedure was time-consuming, limiting its clinical utility: cell expansion took approximately 60 days and cell cultured onto

scaffolds approximately 10 days, increasing the contamination risks. In addition, this methodology demanded an invasive technique for cell harvesting and showed the difficulty to collect healthy cells from diseased patients [83, 84]. Therefore, another cell type was found to avoid the limitations related to the use of autologous vascular cells. The improved procedure consisted of *in vitro* scaffold seeding with autologous bone marrow-derived mononuclear cells, isolated from the iliac crest, instead of autologous vascular cells [84]. Bone marrow mononuclear cells could be harvested the same day of the vascular surgery and required a short scaffold incubation time before the implant (2 hours) [9, 84]. Approximately 2 years after implantation, TEVGs were functional, without any sign of thrombosis, stenosis, obstruction, aneurysm, or calcification [4, 9]. Approximately 7 years after implantation, the long-term followup showed no mortality related to TEVGs. Nevertheless, 16% of patients showed TEVG stenosis that was successfully treated with angioplasty and stenting [4, 9, 14, 83, 84]. This complication was developed in TEVGs with smaller diameter (ID < 18 mm) [83]. The clinical trial demonstrated the TEVG growth potential and also some TEVG limitations: long-term graft stenosis [4, 9, 14, 83, 84] and lack of mechanical properties necessary for a use as high-pressure arterial replacements [5].

L'Heureux's group [82] developed a cell sheet-based technology to generate completely autologous TEVGs and performed a clinical trial for hemodialysis in 10 patients with end-stage renal disease and previous hemodialysis failure. TEVGs were implanted as arteriovenous shunts in the limb that was contralateral to the existing graft, specifically placed in the upper arm, typically between the humeral artery and axillary vein [81]. To fabricate completely autologous TEVGs, dermal fibroblasts were isolated from a skin biopsy and *in*

TABLE 7: Studies of the scientific literature (2008–2013) on TEVG development without the use of scaffolds.

Fabrication method	Cell type	ID	Mechanical properties	Biological <i>in vivo</i> results	Reference
<i>In vivo bioreactor approach:</i> device implanted in the peritoneal cavity to attract cells around a tubular scaffold; scaffold = stretchable urethane + silicone with a coating of nonadhesive material	Autologous tissue	6 mm outer diameter	<i>Axial tensile tests</i> UTS = 0.2 [0.04, 0.45]^b MPa, $\epsilon_b = 0.75$ [0.37, 0.86]^b; <i>circumferential tensile tests</i> UTS = 0.07 [0.02, 0.72]^b MPa, $\epsilon_b = 0.69$ [0.39, 1.09]^b; similar to native ovine blood vessels: <i>axial tensile tests</i> UTS = 5.7 [3.39, 7.12]^b MPa, $\epsilon_b = 0.9$ [0.57, 1.25]^b; <i>circumferential tensile tests</i> UTS = 4.43 [2.76, 5.54]^b MPa, $\epsilon_b = 0.72$ [0.65, 1.15]^b	Model: sheep; <i>device implantation for 10 days:</i> many porous spaces in neotissue; some contractile protein expression; no production of collagen bundles and elastin; <i>carotid artery interposition:</i> quick rupture; <i>patches in carotid artery (2 weeks):</i> 1 graft failed after 1 week; other graft remained patent; aneurysm formation in both cases	[72]
<i>Cell ring-based approach:</i> seeding of cells into annular agarose wells + cell aggregation + formation of thick tissue rings (static culture); tissue rings could be fused to generate tubular constructs	Rat aortic SMCs	2, 4, and 6 mm	<i>Circumferential tensile tests</i> ID 2 mm <i>day 8:</i> UTS = 169 ± 45 kPa, $E = 0.81 \pm 0.28$ MPa, $\epsilon_b \sim 0.46^*$; <i>day 14:</i> UTS = 97 ± 30 kPa, $E = 0.50 \pm 0.09$ MPa, $\epsilon_b \sim 0.5^*$; ID 4 mm <i>day 8:</i> UTS = 339 ± 131 kPa, $E = 1.21 \pm 0.46$ MPa, $\epsilon_b \sim 0.49^*$; <i>day 14:</i> UTS = 201 ± 63 kPa, $E = 0.71 \pm 0.20$ MPa, $\epsilon_b \sim 0.52^*$; ID 6 mm <i>day 8:</i> UTS = 503 ± 76 kPa, $E = 1.98 \pm 0.4$ MPa, $\epsilon_b \sim 0.46^*$; <i>day 14:</i> UTS = 302 ± 42 kPa, $E = 1.08 \pm 0.14$ MPa, $\epsilon_b \sim 0.47^*$	—	[73]
<i>Cell sheet-based approach:</i> production of a completely autologous TEVG (7 months) + air-dried devitalization + freezing + storing for 5 months at -80°C + seeding with autologous ECs (culture for 4 days)	Autologous fibroblasts and ECs isolated from skin and vein biopsies	4.8 mm	BP = 6407 ± 633 mmHg, SRS = 261 ± 20 gf, C = 3.1 ± 0.7%	Clinical trial (1 patient); reduction of the production time (~2 weeks versus ~6–9 months); implanted without complication; no evidence of leakage at anastomoses; at 8 weeks: no complication; graft patency; stable diameter; no adverse reactions	[74]

TABLE 7: Continued.

Fabrication method	Cell type	ID	Mechanical properties	Biological <i>in vivo</i> results	Reference
<i>Cell sheet-based approach</i> (single step method): tunica media and adventitia produced by rolling a tissue sheet containing both cell types contiguously (SMCs grew beside fibroblasts in the same culture dish)	SMCs isolated from human umbilical cord; TEVMA produced by SMCs and either DFs (TEVMA-DF) or SVFs (TEVMA-SVF)	4.5 mm	TEVMA-DF BP $\sim 1000^*$ mmHg; <i>circumferential tensile tests</i> : UTS $\sim 2.1^*$ MPa, $E \sim 12^*$ MPa, $\epsilon_b \sim 30^*$ %; <i>stress-relaxation tests</i> : $E_i \sim 8^*$ N, $E_e \sim 6^*$ N; TEVMA-SVF BP $\sim 250^*$ mmHg; <i>circumferential tensile tests</i> : UTS $\sim 0.8^*$ MPa, $E \sim 2^*$ MPa, $\epsilon_b \sim 35^*$ %; <i>stress-relaxation tests</i> : $E_i \sim 5^*$ N, $E_e \sim 2^*$ N	—	[48]
			aTEVMA BP $\sim 250^*$ mmHg; <i>circumferential tensile tests</i> : UTS $\sim 3^*$ MPa, $E \sim 2^*$ MPa, $\epsilon_b \sim 30^*$ %; <i>stress-relaxation tests</i> : $E_i \sim 2^*$ N, $E_e \sim 1.5^*$ N; vTEVMA BP $\sim 100^*$ mmHg; <i>circumferential tensile tests</i> : UTS $\sim 1^*$ MPa, $E \sim 0.5^*$ MPa, $\epsilon_b \sim 30^*$ %; <i>stress-relaxation tests</i> : $E_i \sim 1^*$ N, $E_e \sim 0.5^*$ N		
<i>Cell sheet-based approach</i> (single step method): tunica media and adventitia produced by rolling a tissue sheet containing both cell types contiguously (SMCs grew beside fibroblasts in the same culture dish)	SMCs and fibroblasts isolated from 3 distinct human umbilical cords; aTEVMA-arterial TEVMA; vTEVMA-venous TEVMA	4.5 mm	dTEVM-DF UTS $\sim 3.1^*$ MPa, $E \sim 22^*$ MPa, $\epsilon_b \sim 23^*$ %; BP $\sim 2600^*$ mmHg; dTEVM-SVF UTS $\sim 2.2^*$ MPa, $E \sim 20^*$ MPa, $\epsilon_b \sim 20^*$ %; BP $\sim 1800^*$ mmHg; TEVM UTS $\sim 0.8^*$ MPa, $E \sim 5^*$ MPa, $\epsilon_b \sim 3^*$ %; BP $\sim 400^*$ mmHg; UTS, E , and ϵ_b calculated from circumferential tensile tests	—	[76]
			Human arterial SMCs isolated from an umbilical cord; DFs obtained from a reductive breast surgery; <i>dTEVM-DF</i> obtained from DFs; <i>dTEVM-SVF</i> obtained from SVFs; <i>TEVM</i> standard cell-sheet grafts (no decellularization), produced by SMCs		

TABLE 7: Continued.

Fabrication method	Cell type	ID	Mechanical properties	Biological <i>in vivo</i> results	Reference
<i>Microtissue self-assembly approach:</i> microtissues produced higher amounts of ECM + bioreactor to assemble microtissues in a tubular shape + dynamic culture for 14 days (pulsatile flow and circumferential mechanical stimulation)	Microtissues composed of myofibroblasts and ECs; HAFs and HUVECs used for microtissue generation	3 mm	—	—	[77]
<i>Scaffold-free rapid prototyping bioprinting approach:</i> cells aggregated into cylinders + bioprinting of cell cylinders using agarose rods as molding template + 2–4 days in the mold (fusion period) + maturation in a perfusion bioreactor	HUVSMC and HSF aggregated into cylinders	0.9 to 2.5 mm outer diameter	—	—	[78]

TEVMA: tissue-engineered vascular media and adventitia; TEVM: tissue-engineered vascular media.

UTS: ultimate tensile strength; ϵ_f : strain at break; E : elastic modulus; E_i : initial modulus; BP: burst pressure; BPe: estimated burst pressure; * values were graphically read; ^b data expressed as medians (min., max.), SRS: suture retention strength, C: compliance.

SVFs: human saphenous vein fibroblasts; DFs: human dermal fibroblasts; HAFs: primary human artery-derived fibroblasts; HUVECs: human umbilical vein endothelial cells; HUVSMCs: human umbilical vein smooth muscle cells; HSFs: human skin fibroblasts.

vitro cultured with ascorbic acid, facilitating the production of extracellular matrix proteins [5, 81]. Approximately 6 weeks after the seeding, fibroblast sheets were detached from culture flasks, wound around a temporary mandrel (diameter = 4.8 mm), and led to maturation for approximately 10 weeks to generate a homogenous tissue [5, 81]. Then, autologous endothelial cells isolated from peripheral vein or endothelial precursors taken from circulating blood were seeded into the TEVG inner layer devitalized by air-drying, few days before surgery [5]. TEVGs required 6 to 9 months of *in vitro* growth prior to implantation [81]. Primary patency was maintained in 7 of the remaining 9 patients 1 month after implantation and in 5 of the remaining 8 patients 3–6 months after implantation [81]. There were 3 TEVG failures due to thrombosis or aneurysm during the safety phase that was used to evaluate TEVG mechanical stability. In spite of the use of completely autologous grafts, one patient showed a TEVG aneurysm, mediated by an acute immune response that was caused by high levels of immunoglobulin G present in a serum batch during the cell culture [8, 81]. At implantation, TEVGs exhibited a poor compliance ($3.4 \pm 1.6\%/100$ mmHg, experimental evaluation) in comparison with human internal mammary artery; however, 6 months after implantation the TEVG compliance increased and was equal to $8.8 \pm 4.2\%/100$ mmHg (measured by Doppler ultrasound) [85]. Some challenges are related to L'Heureux's clinical trial; participants were end-stage renal disease patients and TEVGs are subject to repeated puncture and great hemodynamic loads typical of the hemodialysis access [5, 8]. To date, L'Heureux's group intends to decrease the cost ($\geq \$15,000$ per graft [23]), the complexity, and the time for the production of their TEVGs [8]. They performed a first human implant of a devitalized frozen graft in a hemodialysis patient: 5 days prior to surgery, the TEVG was rehydrated and its lumen was seeded with autologous endothelial cells [8]. Five months after implantation the TEVG worked with no evidence of failure [8].

4. Conclusions and Future Perspectives

This review is focused on the most relevant and recent advances concerning vascular tissue engineering for small diameter blood vessel applications, specifically the development of TEVGs made of decellularized matrices and natural and/or biodegradable synthetic polymers and their realization without the use of scaffolds. Particular attention is paid to scaffolds made of natural and/or biodegradable synthetic materials, highlighting their advantages and disadvantages and comparing mechanical and biological performances of natural and synthetic materials.

Main failures of ePTFE prosthesis were related to neointimal hyperplasia, remarkable calcification, thrombosis, and foreign body reaction [60]. During clinical trials or *in vivo* animal tests, TEVGs often showed the same failure causes of ePTFE prostheses (Tables 3–7 and Section 4). The challenge of vascular tissue engineering still remains the development of TEVGs that are able to regenerate a functional endothelium and exhibit mechanical properties similar to native blood vessels. Although completely autologous TEVGs have

reached promising results in clinical trials, the development of readily available TEVGs characterized by an economical feasibility still remains a major challenge. In fact, the fabrication of completely biological TEVGs without the use of scaffolds requires long time and high cost of manufacturing (Section 2.6).

Among the different approaches based on the use of scaffolds, the direct implantation of cell-seeded scaffolds without the use of bioreactors simplifies the procedure and reduces the cost related to the use of bioreactors. This method is based on the concept that the cells seeded in scaffold play a key role in the recruitment of the host cells for *in vivo* tissue regeneration, as shown by Shin'oka's group. The implantation of acellular scaffolds for *in situ* tissue regeneration by the host would avoid the limits correlated to the *in vitro* cellularization of scaffolds, specifically the cell choice and expansion, and the use of a bioreactor that is time-consuming and technical complex due to the mandatory application of Good Manufacturing Practice (GMP, EudraLex—Volume 4) for the clinical use. Therefore, both of these approaches lead or would lead to off-of-the-shelf scaffolds for the regeneration of small diameter blood vessels, considerably simplifying the procedure for fabrication of TEVGs and reducing the cost and the time of manufacturing.

Among all the available materials for small diameter blood vessel regeneration, hybrid scaffolds exhibit promising and tailorable results due to the combination of appropriate mechanical properties of synthetic polymers with excellent biological performances of natural polymers (Section 2.5). Furthermore, an important perspective would be the direct introduction of antiinflammatory and anti-proliferative drugs in TEVGs, due to the better knowledge of molecular pathogenesis of artery disease.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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