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A review on fibrous scaffolds in cardiovascular tissue engineering.

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Abstract

It has been long and widely known that cardiovascular diseases (CVD) are among the leading causes of the death worldwide. The current treatments have come a long way to reduce mortality and improve life quality of those affected, but they still present drawbacks and limitations, and that is why cardiovascular tissue engineering is thought to be the great promise for the future. Fibrous scaffolds have been a trendy topic in tissue engineering (TE) for years now, and several techniques have been developed and optimized to produce fibers of various biomaterials – a microstructure that is particularly important for valvular and vascular tissues due to their unique composition and organization. This review aims to explain the rationale behind the use of fibrous scaffolds in cardiovascular TE, the main biomaterials and techniques that have been employed to produce such scaffolds and how close they are to clinical applications.

Keywords: *Cardiovascular Tissue Engineering; Fibrous scaffolds; Heart valve regeneration; Blood vessels regeneration*

Introduction

Cardiovascular diseases (CVDs) are among the main causes of mortality in modern society, coming to reach more than 30% of registered deaths worldwide in 2015, what encourages a great effort of researchers towards changing such reality [1, 2].

Among these CVDs, ischemic heart diseases alone are responsible for 15,5% of these deaths (being the single largest cause of death globally), and ischemic strokes account for 5,2% [1]. One thing these cardiovascular events share is that both can be a complication of atherosclerosis, a chronic disease of the arterial wall: when an atherosclerotic plaque ruptures, is triggers the formation of a thrombus that can obstruct the vessel lumen and then impede blood flow, leading to tissue ischemia. Although myocardial infarction (MI) and stroke are the main complications involved, thrombi can eventually reach other vessels [3], 4]. Restoration of blood flow may involve procedures such as angioplasty, stent insertion or atherectomy, but vascular grafts may be used to replace or bypass the affected vessel too.

In terms of numbers, conditions which affect heart valves (such as congenital heart diseases and rheumatic heart disease) may seem less alarming, but they are just as important. For these cases, the replacement of damaged valves by using mechanical or biological prostheses is currently the most common treatment [5-8].

Despite the advances in treatment of all the problems mentioned above, they all present several drawbacks and limitations. In this context, tissue engineering (TE) represents a promising alternative for restoration or regeneration of damaged cardiovascular tissue, offering the possibility of developing non immunogenic and fully functional bioartificial substitutes for injured tissues [9-13]. Essentially, these substitutes are created using three-dimensional biomaterial scaffolds in association with biomolecules and/or other type of stimuli that establish a microenvironment similar to natural tissue [14-20].

With the advancement of biomaterial technology, as well as the expansion of the knowledge of cellular biology and mechanisms that govern the natural process of tissue repair and regeneration, TE has experienced significant expansion and progress over the past decades. Nonetheless, matching applications, materials, and fabrication processes to best suit the needs of the tissue to be regenerated is not an easy job and there are still many challenges that need to be faced [18].

Throughout these years, intensive interdisciplinary, translational research into cardiovascular regenerative implants has been undertaken to improve surgical outcome and provide better quality of life for patients with cardiovascular defects [9, 11, 13, 21–23]. How-ever, while several replacement products and related therapies for skin, bone and cartilage, are in clinical trials or already approved for clinical use, the cardiovascular area accounted for less than 5% of total sales in the TE industry in 2011. This is a result of both biological and financial factors, such as tissue complexity, capacity of regeneration, scale-up, clinical performance, marketing and cost-effectiveness [24, 25].

This work aims to explain the main problems in the current treatment of CVDs, describe how TE could contribute to overcome them, the obstacles to clinical translation, and, most importantly, present a state-of-the-art review on the use of fibrous polymeric scaffolds for regeneration of heart valves and blood vessels.

Cardiovascular Tissue Engineering

Cells, signals and scaffolds are often referred to as the TE triad. The scaffolds act as an artificial extracellular matrix (ECM), providing structural support for cell attachment and tissue formation, and and they are often combined with biomolecules or biophysical stimuli

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(using bioreactors) to facilitate cell differentiation. The general requirements for a scaffold usually include citocompatibility, biodegradability, adequate mechanical properties and porosity, but the tissue-specific considerations are highly important and must always be taken into account [26, 27].

Biological grafts and bioresorbable biomaterials, both naturally occurring and synthetic, have been widely studied for applications in TE and this knowledge has been applied to cardiovascular TE as well [14, 23, 26, 28-30]. The pros and cons of each of them, consi-dering the context of cardiovascular TE, are shown in Table 1.

Several reviews on cardiovascular TE, focusing on different aspects, have been published thus far and are recommended for a more comp-rehensive understanding of the subject. Recent advances in biomater-ials and cell sources have been a frequent topic. In 2011, Bouten and coworkers presented the state-of-the-art in the area of cardiovascular TE at that time, emphasizing key aspects in the development and use of scaffolds for the regeneration of blood vessels, heart valves and other tissues [11]. Capulli et al (2016) provided a similar review, but focusing specifically on fibrous scaffolds [32]. Generali, Dijkman and Hoerstrup (2014) reviewed and discussed the diversity of natural and synthetic bioresorbable materials investigated

Table 1. Summary of the main advantages and disadvantages of biological grafts, synthetic and natural polymers as scaffold materials for cardiovascular TE.

Material	Advantages	Disadvantages
Biological grafts [27], [33]	• Excellent immune compatibility when allogenic/autologous • Presence of structural and functional biomolecules • Analogous tissue structure	• Limited donor availability • Incomplete decellularization may cause immune reactions • Variable properties – source and decellularization protocols
Synthetic polymers [26], [28], [29]	• Smaller risk of thrombogenesis or immunogesis • Easily obtained in large scale • Can be manipulated by various fabrication techniques • More controllable properties	• Do not provide biological cues, so cell attachment and growth can be more difficult • Degradation products can cause undesired reactions, such as inflammation
Natural polymers [26], [28], [29]	• Excellent immune compatibility when allogenic/autologous • Presence of structural and functional biomolecules	• Poor mechanical properties • Variable properties – source and obtaining method • When of xenogeneic origin can cause immunologic responses • Potential for calcification

For cardiovascular TE, physiological conditioning is also particularly relevant because the tissue organization and the mechanical behavior of the heart valves and the blood vessels all result from the mechanical and biological cues they are submitted to. Although this is also true for other types of tissue, in the case of cardiovascular TE these studies tend to be more complex and the effects of the simulated stimuli on the cells are not yet completely elucidated [28, 31, 32].

That is why the in vitro approach, considered the original TE paradigm, is the most commonly used: roughly speaking, it consists of harvesting the patient's cells, seeding them onto a scaffold and growing the tissue in vitro (where conditions can be closely monitored and controlled), and then implanting the construct back into the patient. The in situ strategy bypasses the in vitro phase and uses the patient's own body as a bioreactor for tissue growth, which is considered more clinically viable due to off-the-shelf availability and more accurate in terms of stimuli, but also more difficult, because controlling cell capture in the body is not trivial [31].

for use as scaffold in cardiovascular TE [23]. Truskey (2016) described how new technologies regarding cell differentiation and tissue decellularization have contributed to the advances seen in cardiovascular TE over the past few years. [13].

Regarding research funding and clinical translation of TE technologies, Lundberg (2013) reviewed the TE programs fostered by the National Heart, Lung, and Blood Institute (NHLBI), which support the translation of novel approaches for the treatment of CVDs. The author described NHLBI-supported cardiovascular TE research programs, their recent advances and some of the major questions they have encountered but have not been able to completely elucidate thus far [22]. Lee and colleagues (2014) also listed the various preclinical studies and clinical trials that already employ TE, showing that tissue-engineered materials are a viable option for surgical repair, but still require refinement to reach their clinical potential [9]. Best et al presented a review of the status of the in vivo tissue-engineered blood vessels, heart valves, and myocardium, discussing the criteria and

and limitations for their clinical use [21]. Seifu et al argue that the lack of standardization and various factors relating to processing, sterilization, and packaging currently hinder the advancement of cardiovascular TE although the authors focus on vascular grafts, their comments can be extended to cardiovascular tissue engineered constructs in general. The long period required to synthesize tissue-engineered constructs and high costs associated with both technical development and health care are barriers to their clinical use [34].

This review intends contribute to the area by showing why fibrous scaffolds have been particularly interesting for cardiovascular TE, and the next sections focus on what biomaterials and fabrication technologies are being employed, what the current stage of research is and what is necessary to move forward.

Fibrous Scaffolds for TE

Extensive studies have been performed to allow the understanding of the interactions of cells with its surrounding microenvironment. The extracellular matrix (ECM) is the network where most of the cells are anchored, and it is composed of two main classes of macromolecules: fibrous proteins and proteoglycans [27, 35]. But these components are not simply a passive support for cells, they are responsible for providing key mechanical properties for the tissues, biological cues which are important for cell adhesion, proliferation and differentiation, and more importantly, they are very dynamic and adapt to the cells' needs [27, 36]. Having said that, it is not difficult to understand why a TE scaffold should mimic these characteristics to achieve satisfactory results [19, 37].

Although each tissue presents a specific ECM composition, its major constituents are the proteins, that form a fibrillar network whose fiber diameter varies between 50–500 nm that is, 1-2 orders of magnitude smaller than the cell itself [36, 38-40]. Yannas and coworkers were the first to demonstrate that the presence this network and its orientation are important features for TE scaffolds [41, 42] and because of this, several techniques for obtaining three-dimensional fibrous structures have been developed, using both natural and synthetic polymers [43-45]. Further, studies have shown that fiber diameter and orientation also have considerable influence over the cell's phenotypic expression [46], what have caused manufacturing techniques to turn to nanotechnology, aiming to produce aligned nanofibrous scaffolds [12, 20, 32, 40, 47, 48]. For cardiovascular TE, the importance of such microstructure has also been emphasized in several papers [12, 32, 49, 50].

Currently, there are several methods to produce fibrous scaffolds, for example self-assembly, phase separation, and freeze drying and electrospinning. However, some of them cannot be scaled up, present difficult processability and/or are not able to produce nanometric fibers. Electrospinning is commonly used because it meets all these demands [46], but recently other techniques such as jet spinning, airbrushing and solution blow spinning have been attracting researchers' attention and have already been applied in several biomedical applications – among them, TE [51-56].

Heart Valve Regeneration

Heart valves (HV) are the components that maintain unidirectional blood flow through the heart. The human heart has four valves, that can be divided into atrioventricular or inflow (mitral and tricuspid) and semilunar or outflow (aortic and pulmonary). There are several conditions that can impair their functioning, resulting in either an obstruction to flow (stenosis), or in backflow across the valve (regurgitation) - in either case, the patient will eventually need medical treatment [57-59].

Despite their vital role to the appropriate function of the heart, valvular heart disease (VHD) has been long neglected by society, especially because of its lower prevalence when compared to other cardiovascular problems [60, 61]. But when we consider the need for long- term follow-up, complex decision-making for intervention and its high cost, the impact of HVD on healthcare systems is not proportional to the attention drawn to it [61, 62].

Nonetheless, over the past decades VHD has become a subject of growing attention because of the changes in its epidemiology and patient care. Since 1950s, the incidence of acute rheumatic fever (ARF), one of the main causes of valve malfunction back then, has dramatically declined in industrialized nations. Nowadays, the greatest burden of VHD in these countries is attributable to degenerative diseases, correlated with increasing age – specialists even believe this can beb the 'next cardiac epidemic' [60, 62, 63]. The picture in most low-income countries, however, has not changed. The incidence aof ARF is still considered high, and so is its associated mortality, since most patients cannot afford medical care. This means several children and teenagers aged 5-14 years, the peak age group to develop ARF, are still severely affected and need age-appropriate solutions, different from those utilized for the elderly [58, 62].

Adding to that, there are the patients with congenital heart diseases among these cases, bicuspid aortic valves (BAV) are important due to their frequency and their late complications. Its incidence is underestimated, because it usually takes decades for the patients to be symptomatic, What keeps it from being detected in pediatric studies [64]. Even when the BAV functions adequately, the changes in anatomical configuration ultimately lead to calcification [58].

Although the currently available prosthetic valves and surgical techniques have advanced in many ways, they have not yet given these young patients a suitable solution. Children who need valve replacement must often undergo multiple procedures because their prosthesis cannot keep up with their growth. Even for the adults, there are limitations: for the mechanical valves, there is the need for long-term anticoagulation to avoid thrombogenesis, leading to the consequent risk of hemorrhagic complications, and the biological prosthesis are susceptible to early calcification and degradation, what may lead to its failure and then the need for reoperation [65, 66].

HVs open and close over 30 million times per year and, in the case of the aortic valve, it is submitted to relatively high pressure and severe shear stress – not coincidentally, it is the most frequently diseased alongside the mitral valve. Yet its thin leaflets may function properly for over 70 years - the secret to that, and what all the current valve replacement technologies lack, is that it is a living structure that communicates with the body, adapts and renovates itself [31, 58, 67]. That is why tissue engineering is considered the great promise for this problem: a tissue engineered heart valve (TEHV) would solve the pediatric issues, and be free from the drawbacks mentioned, for it would be capable of growth, remodeling, and would be non-thrombogenic [66, 68].

This section of the review, like the majority of the research on the subject, will be focused on semilunar valves, particularly because of the clinical impact of aortic stenosis that accounts for 45% of VHD-related deaths in the USA [57, 62]. Pulmonary valve TE is

This section of the review, like the majority of the research on the subject, will be focused on semilunar valves, particularly because of the clinical impact of aortic stenosis that accounts for 45% of VHD-related deaths in the USA [57, 62]. Pulmonary valve TE is covered here because it is often considered a first target for heart valve tissue engineering (HVTE). It is less challenging than aortic valve TE due to the lower pressure it is submitted to and its easier surgical access, but it shares several similarities with the aortic valve, being a reasonable starting point [69, 70].

Fibrous scaffolds for HVTE

Contrary to popular belief, HVs are not entirely passive components. Their histological structure is tightly linked to their function and it has evolved to guarantee optimal hemodynamic flow and durability. To successfully create a tissue engineered TEHV, one must first thoroughly understand how all the native components of the valves work together [57, 67, 71].

Semilunar valves consist of three semilunar cusps (or leaflets) attached to the inner wall of the vessel. Microscopically, the cusps are composed of three different layers: fibrosa, spongiosa and ventricularis, each one with a different ECM profile that serves a specific purpose. The fibrosa is the one closest to the outflow surface, and is composed of densely packed collagen fibers aligned parallelly to the free edge of the cusp. It provides the strength and stiffness that maintain the coaptation during the diastole and transfers some load from the leaflet to the wall of the aortic root. The spongiosa is the middle layer, and it is mostly composed of proteoglycans and glycosaminoglycans (GAGs), generating an amorphous substance that act as a cushion. The ventricularis, located closest to the left ventricle, is rich in elastin fibers that are perpendicular to the collagen fiber in the fibrosa. The elastin extends and contracts during the cardiac cycle, preventing permanent deformation of the leaflets [66, 68, 71, 72].

It is thanks to the mechanical properties, the unique fiber alignment and the organization of these ECM layers, that work in conjunction one another, that the valves are able to guarantee optimal hemodynamic function and also withstand the harsh conditions they are subjected to [66, 71] and that is why fibrous scaffolds are so interesting for this application.

From this perspective, a decellularized native valve would be the best scaffold for HVTE, for it would possess all its structural features [58, 72]. In fact, the first tissue engineered valve to reach clinical trials was a decellularized porcine valve, Synergraft™ (Cryolife Inc., USA). Although the results in adults have been encouraging, in children they have been catastrophic [58, 73]. Although much progress has been made ever since, there are still drawbacks and uncertainties, such as the risk of zoonosis for the xenografts and the limited donor availability for allografts [71, 72]. This will not be further explored in this article, but such concerns fuel the search for other alternatives, like the ones shown in Table 2.

The projects mentioned in Table 3 were considered relevant because of the material and/or the technique employed, due to promising results and/or advanced research stage. They will now be further detailed in the next sections.

Synthetic polymer scaffolds

Shinoka and colleagues were the first to present feasibility studies that proved that bioresorbable polymers could be used as scaffold materials for HVTE. In their first study, a polyglactin woven mesh surrounded by PGA nonwoven meshes were used as a scaffold and it was seeded with autologous cells. The constructs were used to replace one resected pulmonary leaflet in lambs. After 21 days of implantation, the tissue engineered leaflets still functioned appropriately and maintained their shape and size [113]. Even though this was a pilot study, it is still considered a milestone for HVTE. In another study by the same group, they used a scaffold made of nonwoven PGA and a PGLA woven mesh. In that work, they tested seeded and unseeded (blank) scaffolds.

The results showed that the pre-seeded leaflet was functional, but did not move as freely as the native ones. The blank ones, however, became smaller over time and eventually became dysfunctional [114]. Stiffness, thickness and nonpliability were considered the major problems with this model, leading to the choice of other polymers [115].

Afterwards, the most prominent results came in 2000 with the work of Hoerstrup et al. who proposed a new scaffold based on a nonwoven PGA mesh dip coated with P4HB [103]. At the time, this was considered the most promising material available and it was extensively assessed, being probably the most thorough work in the field. Different cell sources, bioreactor conditioning and implantation methods were tested for both pulmonary and aortic position [104-110, 116, 117]. In the first moment, this scaffold was employed using the conventional in vitro TE approach [103, 104]. Years later, to reduce the observed cell-mediated retraction of the leaflets, researchers decellularized the construct before implantation [33]. This method will be further discussed in the "Hybrid Approaches" section. Another alternative to reduce leaflet retraction was the choice of another polymer: the group acknowledged that PGA-P4HB may not be completely suitable for a classical approach in HVTE, particularly because of its uncontrollable degradation, and have been studying the use of slow-degrading polymers, such as PCL [74, 75, 116].

In parallel, with the advancements in biomaterials for TE, different groups began exploring other polymers as potential substrates for HVTE and novel fabrication techniques as well. Elastomers, thermoplastics and hydrogels have been of interest, as sole materials or combined.

Del Gaudio et al. have also been investigating the use of PCL for HVTE. They fabricated a scaffold by using a custom-made aluminum target in the shape of a trileaflet HV to collect electro spun PCL fibers. Preliminary hydrodynamic assessment showed a proper functioning of the device, with satisfactory and synchronous opening and closing. More recently, a particle image velocimetry (PIV) study provided more details of its mechanical performance [76, 77]. Also, Sohier et al have used the technique of jet-spraying to produce fibrous PCL mats with anisotropic properties that influenced cell differentiation, and its satisfactory porosity and pore sizes also allowed good proliferation of stromal cells and ECM production [78].

Thermoplastics of the lactide family have also been explored. Schmidt et al shaped PLDLLA multifilament fibers into a trileaflet scaffold and coated it with electro spun fibers of the same material to mimic native valve architecture. In vivo studies performed for up to 4 weeks in sheep were uneventful - however, further studies using this construct were not found [79]. Gottlieb et al fabricated PGA/PLLA nonwoven scaffolds, by cutting the meshed into leaflet shape and suturing them. The cell-seeded constructs were implanted in sheep, but after 6 weeks, significant regurgitation was observed [80]. The limitation mentioned by the group is that PGA/PLLA do not permit large deformations and are not able to duplicate valve motion [118].

Table 2: Summary of main publications regarding fibrous scaffolds for HVTE.

Biomaterial/Shape/Fabrication technique	Latest stage*	Assessed position	Ref.
Synthetic polymer scaffolds			
Electrospun PCL mats – shaped and sutured as a valve	Material characterization and *in vitro* biological evaluation	-	Brugmans MM et al. [74, 75]
PCL electrospun in trileaflet shape	Material characterization and *in vitro* functionality test	Pulmonary and aortic	Del Gaudio C et al. [76, 77]
PCL jet-sprayed mats	Material characterization and i*n vitro* biological evaluation	-	Sohier J et al. [78]
Poly(L-lactide-co-D,L-lactide) (PLDLLA) meshes shaped as a valve and coated with electrospun PLDLLA fibers	*In vivo* studies in sheep – 4 weeks follow-up	Pulmonary	D. Schmidt et al. [79]
PGA/PLLA nonwoven mats – shaped and sutured as a valve	*In vivo* studies in sheep – 20 weeks follow-up	Pulmonary	D. Gottlieb et al. [80]
Trilayered structure with electrospun PGS:PCL and microfabricated PGS layers	*In* vitro biological evaluation and ex *vivo* functionality test as a single leaflet	Pulmonary	N. Masoumi et al. [81, 82]
Electrospun poly(ester urethane)urea (PEUU) mats	Material characterization	Pulmonary	John E.MayerJr., C. M. Hobson Amoroso NJ et al. [83–85]
PU sprayed in trileaflet shape	*In vitro* biological evaluation	Aortic	G. Aleksieva, N. Thierfelder [86, 87]
PCL fibers embedded in Poly(ethylene glycol) (PEG) hydrogel	Material characterization and *in vitro* biological evaluation	Aortic	H. Tseng et al. [88]
Poly(ethyleneglycol) (PEG) dimethacrylate/PLLA electropun mats	Material characterization and *in vitro* functionality test as leaflets	-	S. Hinderer et al. [89]
Electrospun PC-BU mats – shaped and sutured as a valve	*In vivo* studies in sheep, 12 months follow-up	Pulmonary	J. Kluin et al. [90]
Airbrushed PCL trileaflet shape	Material characterization	-	M. M. O. Simbara et al. [91]
Natural polymer scaffolds			
Trilayered structure composed by collagen fiber bundles, GAGs and elastin sheets	Material characterization and *in vitro* biological evaluation	-	Y. Shi, A. Ramamurthi et al. [92, 93]
Trileaflet fibrin hydrogel	*In vivo* studies in sheep – 3 months follow-up	Pulmonary	T. C. Flanagan et al. [94, 95]
Lyophilized chitosan scaffold shaped as a valve and reinforced with extruded chitosan fibers	Material characterization	-	Albanna MZ et al. [96]
Hybrid scaffolds			
Collagen-silk fibroin-PGS electrospun mats	Material characterization, *in vitro* biological evaluation	-	Wang R et al. [97]
Jet spun poly(4-hydroxybutyrate) (P4HB)/gelatin, trileaflet shape	*In vivo* studies in one sheep – 15 hours (proof-of-concept)	Pulmonary	Kevin Kit Parker et al. [51]
Methacrylated hyaluronic acid (HAMA)/methacrylated gelatin (GelMa) hydrogel reinforced with PGS-PCL electrospun fibers	Material characterization, *in vitro* biological evaluation	-	M. Eslami et al. [98, 99]
Decellularized porcine valve coated with electrospun P4HB/chitosan fibers	Material characterization and *in vitro* biological evaluation	-	[Hong H et al. 100, 101]
Decellularized bovine pericardium with electrospun PCL/chitosan fibers	Material characterization and *in vitro* biological evaluation	-	] Jahnavi S et al. [102
Nonwoven PGA mesh dip-coated with P4HB – shaped as a trileaflet valve and stented	*In vivo* studies in sheep, 24 weeks follow-up in pulmonary position/2 weeks follow-up in aortic position	Pulmonary and aortic	Dijkman PE, Schmidt D, S. P. Hoerstrup ,E. Rabkin, A. Mol, M. a J. Cox,M. Y. Emmert , B. Weber et, A. Driessen-Mol et al. [33, 79] [103–112]

Sant et al. have explored the use of PGS in HVTE, combining PGS with PCL to facilitate electro spinning. Results showed PCL indeed enabled fiber formation and that the mechanical properties of the scaffold were in the range of those of native human aortic valves. Also, PGS improved cell attachment and proliferation when compared to pure PCL because it increases hydrophilicity [119]. When seeded with VICs and submitted to degradation, the cells were able to balance the effects of the loss of mechanical properties, reinforcing its pote ntial for HVTE [120].

Masoumi et al have extensively studied PGS as well, using different fabrication techniques. In a work published in 2014, they report the fabrication and characterization of electrospun PGS/PCL scaffolds. Using different polymer ratios and producing both random and aligned fiber mats, they created anisotropic composites with enhanced mechanical properties (comp-ared to PGS alone), but small pore size prevented cell migration and 3D tissue formation [81]. More recently, they combined these microfibers with micro fabricated PGS to build trilayered constructs, aiming to solve this problem and achieve a closer resemblance to the semi lunar valves [82].

Poly(ester urethane) (PU)-based polymers have been used for HVTE also in light of its elastomeric properties [121]. Courtney et al studied the fabrication of PEUU fibrous scaffolds, and later Amoroso et al have tried to mimic the mechanical properties of a native pulmonary valve by testing PEUU fibers alone, a PEUU: PCL composite and PEUU with poly(ethylene)oxide (PEO) secondary fibers to serve as sacrificial material. They have shown how it is possible to alter some properties - like bending modulus - to achieved desired values, and that pure PEUU mats seemed to be more suitable for HVTE [83, 85]. In 2015, in a continuation of the work, PEUU fibers were aligned in a curvilinear manner that resulted in more uniform strain distribution in response to loading [84]. Akra's group has also evaluated a PU scaffold, produced with a patented spraying technique (further details on material and fabrication are not available). The construct showed biocompatibility and culture in bioreactor improved cell adaptation to shear stress [86, 87].

PEG-based hydro gels are already used in biomedical applications, including TE [122]. PEG presents low strength and isotropic behavior, what hinders its use as the sole scaffold material for HVTE. However, an interesting property is that it allows coupling with strong interfaces, which means it can be used to build layers like the ones of native semi lunar valves. Tseng et al have modified PCL to reduce its hydrophobicity, so that it could be embedded within PEG hydro gels. The electro spun PCL fibers provided an anisotropic characteristic comparable to the aortic valve leaflet and that influenced cell behavior [88]. A PEG dimethacrylate (PEGdma)/PLLA electro spun scaffold has also been reported by Hinderer et al. In this study, they also biochemically modified the scaffolds with ECM proteins, generating a promising material [89].

Kluin et al opted for an in situ approach, where cell-free synthetic fibrous scaffolds were implanted in sheep. This is an interesting strategy, as mentioned earlier, because it is cost-effective and readily available. They employed a novel custom developed bioresorbable elastomer based on bis-urea-modified polycarbonate (PC-BU) to fabricate electro spun mats that were shaped and sutured onto a polyether ether ketone (PEEK) reinforcement ring –they were also fibrin coated before implantation. The 12-month follow-up, probably one of the longest-lasting, showed that the valves maintained functionality throughout the study, neotissue was formed and replaced the polymer (although degradation was not complete), and no calcification was detected [90].

By analyzing these publications, it is undeniable that electro spinning is by far the most utilized method for fabrication of fibrous scaffolds nowadays. However, as mentioned before, other techniques such as jet spinning (and similar ones, such as airbrushing and solution blow spinning) have been calling attention in several fields, including HVTE. Simbara et al have proposed a scaffold prototype for HVTE using the airbrushing technique to fabricate fibrous PCL leaflets, resulting in a very resistant yet plia-ble structure. In silico simulations showed the geometry and the materials chosen allow it to withstand the stresses which they are subjected to [91].

Natural polymer scaffolds

Natural polymers have been widely used in other areas of TE, but their application in HVTE is still limited because they usually present low mechanical properties and therefore are considered inadequate for load-bearing applications [66, 68, 69, 71]. They are most frequently used in the form of hydro gels, foams, sheets, and have also been used as bioinks in bioprinting applications [71, 72, 123, 124].

As collagen represents a large percentage of the composition of the semi lunar leaflets, it is a logical choice for HVTE. Also, it self-assembles at neutral pH, producing fibers that crosslink and ultimately produces a hydro gel in the presence of a water-based solvent, being predominantly employed in that form [125]. Neidert and Tranquillo have fabricated a bileaflet collagen gel valve employing injection molding that was seeded with fibroblasts. Surprisingly, the cells failed to produce other ECM components and its use was dismissed [126]. Vesely et al have employed collagen and other ECM components differently. They have attempted to build a trilayered structure like the one seen in native valves: collagen fibers, GAGs and elastin sheets. Collagen bundles were developed by using the principle of directed collagen shrinkage. Initial findings were published in the early 2000's, and after that the project was said to have entered a slow, refinement phase, but hitherto no other publications were found [92, 93].

As mentioned earlier, fibrin is an appealing material for TE. When it is polymerized in vitro, it produces a stable fibrillar network in the form of a gel– so far, it seems to have been used for HVTE only as a hydrogel. Flanagan et al have fabricated a fibrin-based trileaflet scaffold using an injection molding technique that was cell-seeded and conditioned in a bioreactor to enhance mechanical properties. Constructs were implanted in sheep for up to 3 months, and while ECM deposition was satisfactory, significant shrinkage was observed, an issue that must be solved before taking this research to the next step [94]. It has also been used as a coating for synthetic matrices, to promote a more homogeneous cell seeding [105].

Although natural scaffolds usually comprise these pure ECM components, other biopolymers such as chitosan are also broadly used for TE. Albanna et al have tried to overcome the low mechanical properties of chitosan by reinforcing a freeze-dried porous scaffold with fibers - both made of chitosan. The fibers were formed by an extrusion/gelation technique and were incorporated in the porous matrix by randomly distributing them in a trileaflet mold before pouring the solution. They demonstrated the ability to control the scaffold's mechanical properties by varying fiber/scaffold mass ratio, fiber length and fiber mechanical properties, all important considerations to the future application of this material [96].

Hybrid Approaches

Natural and synthetic polymers were also combined to modulate various properties and elicit different reactions, usually complementing each other. For example, Wang et al engineered a type I collagen, silk fibroin and PGS electro spun scaffold for potential use in HVTE. The rationale for this choice of polymers was: collagen is a load-bearing component and facilitates endothelial cell adhesion, silk fibroin provides more strengh and a slow degradation rate, and PGS can be a replacement for native elastin [97]. Eslami et al also explored this alternative when proposing another option for the issue regarding Masoumi's PGS-PCL electropun scaffolds, mentioned earlier in the review [82]. They fabricated a HAMA/GelMa hydro gel reinforced with PGS-PCL electro spun fibers, because HA promotes elastin secretion in VICs, GelMA provides a more suitable microenvironment for cells, and fibers provide structural integrity and guidance for cell internal organization. Results showed that cell distribution and ECM secretion were better than those seen in fiber-only and hydrogel-only scaffolds [98, 99].

Capulli et al. have fabricated what they call "JetValves" (made by jet spinning) with P4HB and gelatin - according to the authors, the advantages of their manufacturing process were: rapid production, possibility of shape and size customization and controlled and automated fabrication. The structural, mechanical and biochemical properties were similar to those of the native fibrosa and they seem to be durable and functional according to in vitro and in vivo assessments [51].

Despite the concerns regarding decellularized tissues, they are widely explored both as a sole material and in combination with others. Hong et al combined a decellularized porcine aortic valve with synthetic and biological polymeric fibers. Using the electro spinning technique, they coated the decellularized valves with P4HB and with a P4HB/chitosan blend. Results showed that P4HB enhanced mechanical properties, while maintaining biological characteristics. The incorporation of chitosan also contributed to the mechanical properties but, most importantly, provided an improvement related to hydrophilicity that should be beneficial for cell-scaffold interaction [100, 101]. Following the same strategy, Jahnavi et al have used PCL and chitosan to coat decellularized bovine pericardium [102].

Another interesting approach to highlight in this review is the one followed by a group composed by researchers from Eindhoven and Zürich, including well-known names in the area such as Baaijens, Dijkman, Driessen-Mol, Hoerstrup, Schmidt and Weber. As mentioned in a previous section, they have explored the use of the nonwoven PGA mesh coated with P4HB scaffold, originally seen in the work published by Hoerstrup and colleagues in 2000, but presented a shift in methodology in their latest works [103]. Now, the key feature of that work is that these structures were seeded with cells and were later decellularized: all starts with a synthetic matrix, but in fact a decellularized valve is implanted – what could be interpreted as a hybrid approach [111]. Its great advantage is that it allows the scale-up production of off-the-shelf homologous matrices, what makes this a more feasible alternative in terms of costs and logistics and also eliminates the risk of zoonosis [112]. In vivo studies in sheep were performed and, although the results were very promising, valvular coaptation decreased and lead to moderate regurgitation after 24 weeks. According to the authors, this was most likely due to a design flaw of the prototype that could be solved by incorporating some anatomical characteristics into it [112].

In conclusion, different approaches have been used to create a living functional TEHV, but despite the encouraging results hitherto the challenge seems to be maintaining the characteristics of the tissue over time. Most likely, the key to solving this problem is not concentrated in one sole parameter, but in the combination of an optimal geometry, adequate materials (in terms of mechanical properties, but also chemical and physical such as wettability and degradation rate) and proper pre-conditioning. Hopefully, the advances in molecular biology, biomaterials and in silico simulation will help get a clearer understanding of what is take to create a functional TEHV.

Blood Vessel Regeneration

CVDs such as coronary occlusive disease often require surgical repair using vascular grafts. Although autografts retrieved from mammary arteries and saphenous veins remain the gold standard, their availability can be limited depending on the patient´s age and comorbidities. Due to these inconveniences, synthetic grafts have arisen as a viable alternative - current commercialized prosthetic grafts, mainly those made of expanded polytetrafluoroethylene (ePTFE, Gortex®), polyurethanes and polyethylene terephthalate (Dacron®) have shown encouraging results in the replacement of large blood vessels (>6 mm diameter). However, they have not yet shown clinical effectiveness for small-diameter vessels (<6 mm diameter) because of their poor patency rates, stenosis, intimal hyperplasia, calcification, infection and thromboembolization [127 -130]. To address these challenges, efforts have been direct towards the development of tissue engineered vascular grafts (TEVGs).

Different techniques have been explored to produce TEVGs, among which three can be highlighted:

1. Biological TEVGs including cryopreserved allografts and decellularized tissues;
2. Self-assembled cell-sheet-based techniques and 3) bioresorbable synthetic scaffolds [127]. Their advantages and limitations are listed below.
3. Bioresorbable synthetic scaffolds [127]. Their advantages and limitations are listed below.

Cryopreserved allograft veins were developed in the 1980s and have been used in patients where autografts are unavailable, but they were not accepted due to the poor early and late patency rates [131 -134]. As to decellularized vessels, different animal species were implanted with such substitutes, which were patent for several months [135, 136], but several shortcomings have been encountered, including the potential transmission of animal pathogens, ECM with different composition and architecture, structural graft failure and deficient cell migration [137-139]. The cell sheet technique consists in promoting cell self-assembling in autologous cell-derived ECM sheets harvested from in vitro cultures [34, 129, 140, 141]. Peck et al presented a review about their journey from bench-top to bedside in the cell self-assembly approach to develop a TEVG that has shown potential as an arteriovenous shunts for hemodialysis access, showing high patency when tested in a human clinical trial [140]. Although this approach showed promising results in early clinical applications, they failed from both a clinical and financial perspective [142]. Because of the problems associated with these two approaches, the use of synthetic scaffolds has been deeply explored.

Pashneh et al. presented a very complete review about TEVGs, including the history, the different approaches and best results obtai-

obtained by different research groups along the time as well as the challenges and perspectives. According to authors, it is clear that a number of different approaches are being explored to produce a TEVG and, with clinical results being reported for a range of techniques, the best solution is yet to be determined [143].

Fibrous scaffolds for TEVG

The great challenge of TEVG is to produce a graft that meets the biological and mechanical requirements of the application or implantation site. Considering the morphology of natural blood vessels, it is believed that synthetic TEVGs should mimic the fibrous architecture of their ECM, which has led research groups to investigate the performance of fibrous TEVGs.

The blood vessel´s ECM are composed of collagen and elastin where endothelial cells (EC) and smooth muscle cells (SMC) are assembled. The walls blood vessels are arranged into three conce-ntric layers: intima, media and adventitia. The inner layer is the intima, the thinnest layer, composed of a single layer of EC and a small amount of subendothelial connective tissue. The media, the intermediate layer, is the thickest and provides structural support, vaso reactivity and elasticity. It is composed of smooth muscle cells, elastic fibers and connective tissue (mainly by type III collagen, proteoglycans and glycoproteins), which vary in amount depending on the type of vessel. Elastic fibers allow the vessel´s expansion and contraction. And finally, the outer layer is the adventitia, composed of connective tissue (mainly type I collagen) and elastic fibers besi-des nutrient vessels and nerves [144].

Ercolani, Del Gadio and Bianco reported the advancement in vascular tissue engineering, discussing the role of electrospinning as a potential fabrication technique for small-diameter vascular grafts. They focused on the contribution of micro or nanofibers to an effective cell response and on the production of a suitable microenvironment for vessel substitute, showing a large overview of the polymers tested so far, comparing their characteristics in terms of morphology, mechanical properties and cell response. Finally, the authors discuss the potential approaches that can promote the formation of a functional vascular tissue [145].

Considering the purpose of this paper, an overview of the main studies regarding fibrous scaffolds for vascular TE is shown in Table 3 and commented next.

Synthetic polymer scaffolds

A great number of studies has been performed by many researchers around the world aiming the development of TEVGs, including good perspectives in animal models. These studies have shown great variation in relation to the biomaterials employed, scaffold manufacturing methods and cell source [143, 171], but the bioresorbable synthetic polymers are the biomaterials that have been receiving attention in the late years since several already have FDA approval. This review will focus on the most relevant results to the area and on the latest findings; for a complete review on the subject, the review by Pashneh-Tala et al. is suggested [143].

Xu and colleagues produced and tested a nanofibrous scaffold using a [P (LLA-CL)] (75:25) copolymer. The biomaterial was electrospun to result in aligned fibers with average diameter of 500 nm, mimicking the circumferential orientation of cells and fibrils present in the medial layer of a native artery. The scaffold was tested in vitro with SMC cells, which attached and migrated along the axis of the nanofibers and expressed a spindle-like contractile phenotype. The like contractile phenotype. The authors suggests that aligned fibrous scaffolds in nanometer-scale dimension and a architecture replicating the natural vascular structure can perform as an ideal blood vessel scaffold [146].

Wang et al discuss the importance of the porosity in the fibrous scaffolds, since relatively small pores may limit cell infiltration and hinder the regeneration and remodeling of the grafts into neovessels. To solve this problem, the authors fabricated macroporous electrospun PCL scaffolds with thicker fibers (5-6 μm) and larger pores (~30 μm) and performed in vitro cell culture and in vivo tests (replacing rat abdominal aorta). Results indicated that macrophages cultured on thicker- fiber scaffolds presented a tendency for immunomodulatory phenotypic remodeling, while those cultured on thinner-fiber scaffolds expressed proinflammatory phenotype. In vivo test results demonstrated that the macroporous grafts markedly enhanced cell infiltration and ECM secretion, confirming that the scaffold structure can regulate macrophage phenotype [147].

Bode et al also produced an electrospun PCL graft, which could reduce or prevent folding due to their higher flexibility. In order to improve the mechanical behavior of the grafts, various electrospinning collectors were designed using different patterns. Subsequently, the grafts were examined for scaffold morphology, mechanical stregth and flexibility [148].

Another synthetic polymer that has been applied in vascular TE in recent years is PU, because it presents a mechanical behavior similar to blood vessels. Thermoplastic polyurethane (TPU) is a class of PU that presents good processability, high tenacity, and excellent abrasion and tear resistance. Jung et al fabricated tubular scaffolds of TPU/graphene oxide (GO) via electrospinning and plasma treatment. Graphene and its derivatives have attracted considerable attention as potential biomaterials because they have a large number of hydrophilic groups on their surface. Results of mechanical and surface properties tests, as well as in vitro culture with mouse fibroblast (3T3) and human umbilical vein endothelial cells (HUVECs), suggested the scaffolds have potential to be used as small diameter vascular grafts. Also, the researchers submitted the tubular scaffolds to cyclical tensile, suture retention and burst pressure tests, concluding that they meet the requirements for a TEVG [149].

Johnson et al proposed a production method capable of producing TEVG from fully synthetic, resorbable polymers that meet basic minimum mechanical requirements for potential vascular grafts, and have compliance similar to that of the intended vasculature being replaced. Authors investigated several polymers which have FDA approval for medical use (PCL, PCL/CS blend, PDO, PGA, PLCL, PLGA and PLLA) and all of that met the minimum mechanical requirements for compliance, burst pressure, and suture retention strength required by TEVGs. The authors acknowledge that, although these results are promising, is still necessary to know the degradation rate and remodeling profile for each of these TEVGs since these parameters influence significantly the performance and requirements for use in the clinic [150].

In 1998, Shinoka's group was the first to describe the successful use of a TEVG as a pulmonary artery (PA) interposition graft in vivo in a lamb model. For this, they seeded either autologous arterial or venous cells onto a tubular biodegradable scaffold of polyglactinwoven mesh sealed with a nonwoven PGA mesh and implanted it as interposition grafts replacing 2 cm sections of the main PA in juvenile lambs. According to authors, 6 months after implantation, all seeded scaffolds were patent and demonstrated nonaneurysmal growth in diameter, endothelial lining and overall morphology resembling native PA [152].

Table 3: Summary of main publications regarding fibrous scaffolds for TEVGs.

Biomaterial of tubular scaffold/Fabrication technique	Latest stage*	Replaced vessel/Animal model	Ref.
Synthetic polymer scaffolds			
Electrospun PLLA/PCL (75:25) blend aligned nanofibrous	*In vitro* tests with human coronary artery smooth muscle cells	-	C. Y. Xu, R. Inai et al. [146]
Electrospun PCL	Scaffold characterization	-	Z. Wang et al. [147, 148]
Electrospun TPU/graphene oxide composite plasma treated	Biomaterial characterization and *in vitro* biocompatibility tests with fibroblast (3T3) and human umbilical vein endothelial cells (HUVECs)	-	X. Jing, H. Y. Mi et al. [149]
Electrospun PCL, PCL/CS blend, PDO, PGA, PLCL, PLGA and PLLA	Performance tests	-	J. Johnson et al. [150]
Electrospun PCL/CS blend	*In vivo* – small and large animal models	Unseeded scaffold implanted in the infrarenal abdominal aorta in mice and in the carotid artery interposition conduit in sheep	T. Fukunishi et al. [151]
Polyglactin-woven mesh sealed with nonwoven polyglycolic acid (PGA) mesh	*In vitro* incubation and *In vivo* tests	Implantation of TEVG seeded with or without autologous cells in a sheep model (pulmonary artery)	T. Shinoka, J. T. Patterson et al. [152, 153]
50:50 copolymer of l-lactide and ε-caprolactone reinforced with nonwoven PGA fiber fabric	*In vivo* tests	Intrathoracic inferior vena cava (IVC) interposition grafts in a dog model	J. T. Patterson, M. Watanabe et al. [153, 154]
50:50 copolymer of l-lactide and ε-caprolactone reinforced with woven PGA fibers	Clinical test (Japan)	Scaffold seeded with autologous vascular cells was implanted in a 4 years old child (pulmonary artery)	T. Shin'oka et al. [155]
50:50 copolymer of l-lactide and ε-caprolactone reinforced with nonwoven PGA or PLLA fiber fabric	Clinical trial (Japan)	Scaffolds seeded with autologous bone marrow cells (BMCs) were implanted: 23 patients had a tube graft as an extracardiac total cavopulmonary connection (TCPC), while the other 19 patients had a sheet-type patch used for repair of congenital cardiac defects	T. Shin'oka et al. [156]
Polyesters based on PLLA, PGA and PCL	Clinical trial (USA)	Scaffolds seeded with autologous bone marrow (BMCs)	G. Vogel et al. [157]
Natural polymer scaffolds			
Collagen	Biomaterial characterization and *in vitro* tests with fibroblasts, porcine endothelial and smooth muscle cells (SMCs and ECs)	-	W. He, Z. Ma, V. H. Barocas, N. L'Heureux et al. [158–160]

Silk fibroin	Biomaterial characterization: mechanical strength and thrombogenic potential *In vitro* and *in vivo* tests	*In vitro* test with endothelial and smooth muscle cells (SMCs) *In vivo* test abdominal aortas of Sprague-Dawley rats	M. Lovett, S. Enomoto, L. Soffer et al. [161–163]
Chitosan	Mechanical performance and *In vitro* tests using rabbit vascular SMCs	-	L. Zhang, C. Deng et al. [164, 165]
Hybrid/Other			
Electrospun P(LA-CL) 70:30 copolymer tubular nanofiber scaffolds	Static culture *in vitro* and *in vivo* surgical process structure integrity tests	Replacement of rabbit´s inferior superficial epigastric veins of	W. He, Z. Ma, W. He et al. [158, 166]
Electrospun PHBV/PCL blendbiofunctionalised with RGD	Morphological, physico mechanical characterization *In vivo* implant	Wistar rats abdominal aorta	L. V. Antonova et al. [167, 168]
Decellularized rat aortic vessel, PCL electrospun fibers and heparin	*In vivo* implant	Rats abdominal aorta interposition grafts	W. Gong et al. [169]
Smooth muscle cell sheet -electrospun PCL/ Collagen blend scaffolds	*In vitro* evaluations of the cell sheet-electrospun scaffolds	-	H. Ahn et al. [170]

*Current stage of the research – considering they all begin with material characterization (mechanical, chemical, etc.), go through *in vitro* assessments, *in vivo* implantation and eventually clinical trials.

Posteriorly, the group reported the use of a biodegradable scaffold composed of a 50:50 copolymer of PLLA and PCL, reinforced with nonwoven PGA fiber fabric. The lumen was seeded with a mixed cell populations obtained from explanted segments of saphenous vein and the construct was implanted as intrathoracic inferior vena cava (IVC) interposition grafts in a dog model. The TEVGs were harvested over a 6-month time course and showed no evidence of stenosis, dilatation or thromboembolic complications, even without anticoagulation therapy [154]. These results provided the basis for the first clinical trial of synthetic TEVG that was also conducted by Shinoka´s group starting in Japan (Tokyo Women's Medical University), in 1999 [155]. The patient, a 4-year-old girl who had undergone a PA angioplasty and Fontan procedure at 3 years of age received a scaffold implant fabricated from a 50:50 copolymer of l-lactide and ε- caprolactone reinforced with woven PGA (PGA-P(CL–LA)) fibers seeded with autologous vascular cells. No postoperative complications occurred, and 7 months after implantation, the patient was doing well, without evidence of graft occlusion or aneurysmal changes on chest radiography.

Finally, in 2005, the group published their midterm clinical results of TEVGs performed between 2001 and 2004 in Japan (Tokyo Women's Medical University). In these clinical trials the same approach was used, scaffolds of 50:50 copolymer P(CL-LA) reinforced with nonwoven PGA or PLLA fiber fabric seeded with BMCs, enriched for the mononuclear cell populations were implanted in humans. Twenty-three patients had a tube graft as an extracardiac total cavopulmonary connection (TCPC) implant, while the other 19 patients had a sheet-type patch used for repair of congenital cardiac defects [156]. According to authors, the mean follow-up after surgery was 16.7 months on average. There were no complications such as thrombosis, stenosis, or obstruction of the tissue-engineered autografts, neither evidence of aneurysm formation or calcification.

These results encouraged Shinoka's group to continue dedicated to study and optimize the TEVG and the obtained results was reviewed by Patterson et al [153]. Although their investigation clearly demonstrated the feasibility of using TEVGs seeded with autologous cells in congenital heart surgery, they believe that the clinical utility of that technology is limited by the prolonged period of time required to expand the cells in culture and the understanding about the mechanisms that guide the neovessel development. For this reason, they began to explore the potential of using alternative cell types and cell sources for constructing TEVGs [172-174], as well as the cell-free TEGVs approaches [175].

In August 2011, it was started the first clinical trial in USA, after U.S. Food and Drug Administration-approved human clinical trial in the U.S. investigating TEVG use in children with congenital heart defects. This trial has been performed at Yale University and is titled "A Pilot Study Investigating the Clinical Use of Tissue Engineered Vascular Grafts in Congenital Heart Surgery" [157, 171, 176].

Natural polymer scaffolds

Different naturally derived polymers have also been tried as raw material in fabricating fibrous TEVG. Although they present advantages such cell adhesion and biocompatibility, their use remain limited due poor mechanical strength presented by them [177]. Besides that, the fabrication of nanofibers from natural polymers by using electrospinning is not trivial. Even so, some natural polymers can be highlight: collagen, silk fibroin and chitosan [158, 160-165] .

Weinberg and Bell started the use of collagen in TEVG scaffolds and since then, the performance of collagen-based scaffolds has been improved by modifying fiber morphology and arrangement, crosslinking approaches and using specific molding techniques [159, 160]. The addition of elastin fibers to collagen was also tried to form a hybrid scaffold with adequate mechanical properties of TEVGs,

however, the ultimate tensile strengths and burst pressures of these constructs remained lower than those of the target tissue, the native vessels [178].

Another interesting material that have been used as raw material for TEVGs fabrication is silk fibroin (SF) since it is bioresorbable and present good mechanical strenght [161]. Enomoto et al fabricated SF scaffolds by electrospinning technique which showed acceptable biocompatibility when tested in vitro and reached elevated rates of patency in rat abdominal aorta replacements when compared with PTFE grafts [161, 163, 179]. The mechanical properties of SF scaffolds can be improved to attain similar values of native vessels by fiber alignment techniques during the manufacturing process. Mantovani and colleagues fabricated a scaffold composed by SF and collagen using electrospinning technique, but although the composed scaffold was shown to have superior strength compared with pure SF, it remained less strenght than natural vessels [179].

Chitosan (CS) has also been studied for the development of TEVG scaffolds. CS is the most important derivative of chitin, a linear polysaccharide that is commonly found in the shells of marine crustaceans and cell walls of fungi. CS scaffolds were prepared by Zhang et al and showed adequated values of burst pressure and suture retention strength as well as acceptable cell adhesion and proliferation when tested in vitro with rabbit vascular SMCs culture, but there is still the necessity of in vivo tests to prove the potential of this biomaterial in TEVGs use [164], [165].

In Summary, while there are promising results being observed with the use of natural polymers in TEVGs, the viability of their uses requires the development of strategies to improve mechanical performance and therefore a strategy used has been the use of natural and synthetic polymers to produce hybrid TEVGs.

Hybrid approachess

Recent studies have investigated materials fabricated by combining both synthetic polymers and natural proteins. These hybrid scaffold materials show potential for the next generation of small diameter arterial TEVGs because of their fast degradation and hydrophilic characteristics that promote better cell infiltration, proliferation and neotissue formation [151].

He et al performed studies with collagen-coated PLLA-co-PCL nanofiber meshes seeded with human coronary artery endothelial cells (HCAECs). The PLLA-co-PCL meshes were electrospun, followed by plasma treatment and collagen coating. The presence of collagen on the scaffold´s surface enhanced the spreading, viability and attachment of HCAECs that maintained the phenotype, leading authors to believe in the potential of the construct for a TEVG [180]. Following this study, the group fabricated tubular nanofiber scaffolds using the same biomaterials and techniques, also seeding an EC layer in the scaffold´s lumen, and the resulting constructs were implanted in rabbits. Results showed that cells were evenly distributed throughout the lumen, and that the scaffolds could sustain the suturing during the implantation process with no signs of blood leaking, kept the structure integrity and showed patency for 7 weeks [166].

Studies performed by Antonova and colleagues in 2015-2016 revealed that the combination of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) with PCL improves biocompatibility of electrospun small-caliber TEVGs. PHBV/PCL grafts were submitted to biofunctionalization with arginine-glycine-aspartic acid (RGD) peptides to improve the biophysical properties and promote endothelialization. The scaffolds were tested in vitro and in vivo, and the results showed that both PHBV/PCL and PCL biofunctionalized grafts had the endothelization improved but the durability was decreased [167, 168].

Interesting approach was adopted by Gong's research group who generated a hybrid TEVG containing a decellularized rat aortic vessel and PCL electrospun fibers, enhance the biomechanics of the natural acellular matrix. The intimal surfaces of the hybrid TEVGs were coated with heparin and tested in vivo using a rat model. The hybrid scaffold performance in long-term applications was compared with controls, decellularized vessel without PCL fiber reinforcement. The results demonstrate that PCL reinforced the decellularized vessels, and the final structure could resist to biomechanical demand, presenting a much better long-term performance compared to the decellularized vessels alone [169].

Ahn et al developed a novel method to combine cell sheet engineering and electrospinning technology. The TEVGs was fabricated by wrapping an electrospun fibrous mesh around pre-fabricated SMC sheets, and the construct was conditioned in a pulsatile perfusion bioreactor. The authors believe that this strategy would provide fully cellularized and matured vascular constructs that display prolonged biological and biomechanical stability when exposed to vascular physiological conditions in vivo since in vitro tests results showed the formation of a mature smooth muscle layer showing cell-to-cell junction and contractile proteins [170].

According to Fukunishi et al, the use of slow degrading materials with mechanical properties adequated for TEVGs results in limited host cell infiltration, and consequently, poor remodeling, stenosis, and calcification. Considering that, they created a TEVG using fast degra--ding material, a nanofiber blend of PCL and CS. Since the degradation rate of PCL is slow, the authors added the biopolymer CS, that present higher degradation rate and investigated the feasibility of unseeded TEVGs in small and large animal models over a 6 month time course. Their results in a sheep model of carotid artery (CA) interposition grafting demonstrated the PCL/CS nanofiber scaffolds promoted excellent cellular infiltration and neotissue formation without calcification or aneurysm; also the mechanical properties of TEVGs were comparable to native CA. The authors concluded that the modulation of host macrophage infiltration into the scaffold is a key to reducing excessive neotissue formation and stenosis of implanted TEVGs [151, 181].

Challenges and Future Perspectives

Despite the relevant results of TE techniques in cardiovascular regenerative medicine and the great progress seen in the last decades, few patients have been truly benefited from this technology. To be clinically embraced, TE must still be pushed further, and try to provide less invasive procedures, off-the-shelf availability and cost-effective logistics it should be interesting not only to physicians and patients, but also for manufacturers and, in some cases, for the government.

The authors of this review, who are mostly engineers, strongly believe in the importance of technological aspects, such as the selection of biomaterial and manufacturing techniques, to obtain adequate microstructure, to achieve reproducibility and to scale up production. Nevertheless, these features cannot be achieved without: (a) developing in silico models to allow a more efficient selection of biomaterials, as well as to better understand the influence of manufac-turing parameters over the resulting microstructure; (b) evaluating the behavior of the tissue-engineered constructs over time, considering that the biomaterial is gradually reabsorbed as the neo-tissue is formed and (c) developing standardized procedures for evaluating such implantable

results showed that both PHBV/PCL and PCL biofunctionalized graftsdevices over time in terms of functional performance, since the curr-ently available protocols are focused on the evaluation of permanent devices.

To progress in all those directions, a multidisciplinary effort of biologists, engineers, and clinicians must take place. Unfortunately, this is not common practice in several countries, where research done in universities is often too compartmentalized and rarely reaches the outside world.

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