BIORESORBABLE SCAFFOLDS FOR CARDIOVASCULAR TISSUE ENGINEERING

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Disclosure: No potential conflict of interest. MG and PD both contributed equally to the manuscript.
Received: 03.03.14 **Accepted:** 09.05.14
Citation: EMJ Int Cardiol. 2014;1:91-99.

ABSTRACT

Cardiovascular disease is a major cause of morbidity and mortality, especially in developed countries. Currently, when suitable autologous tissue is lacking, mostly non-degradable synthetic material or fixated xenogeneic grafts (e.g. heart valves) are used to restore, repair, or replace the injured cardiovascular tissues. However, these materials are associated with several disadvantages, such as the significant risk of thromboembolism and calcification. Bioresorbable scaffolds for tissue-engineered solutions are proposed to overcome the limitations of the current replacement materials as they provide temporary scaffolding for the *in vitro*, *in situ*, or *in vivo* formation of autologous tissue. Thereby, it is pursued that the engineered tissue mimics the composition and structure of the original tissue and has the capacity of regeneration and growth. The initial scaffold should possess strong material properties as the cardiovascular system requires an enormous strength, flexibility, and durability of the engineered structures, while on the other hand complete resorption of the scaffold material is aimed for. This review discusses the diversity of natural and synthetic bioresorbable materials that are currently investigated for their suitability as a scaffold for cardiovascular tissue engineering.

Keywords: Bioresorbable, biodegradable, tissue-engineered vascular grafts and heart valves, scaffold, starter matrix, polymers.

INTRODUCTION

Cardiovascular disease is the number one cause of death worldwide, globally claiming 17 million lives each year and accounting for 29% of all deaths. Due to an ever-ageing population and an increase of comorbidities, mortality numbers are expected to rise to about 23 million per year within the coming decades.[1] However, successful treatment of cardiovascular disease is limited in many situations by the lack of suitable autologous tissue for restoring injured cardiac muscle or serving as vascular conduits to replace or bypass diseased or occluded vessels. Despite positively influencing the field of reconstructive arterial surgery, the preparation of autologous vascular grafts increases time, cost, and the potential for morbidity to the surgical procedure.[2,3] Tissue-engineered solutions are suggested to overcome these problems with the intention to repair, replace, or regenerate injured tissues and organs (for example, the heart, lungs, liver, or bones) by engineered biological substitutes, based on cells, biocompatible scaffolds, and suitable biochemical (e.g. growth factors) and physical (e.g. cyclic mechanical loading) factors. While the engineered living substitute develops, the scaffold should degrade without leaving remnants in the body, requiring a so-called bioresorbable starter material (according to the definitions formulated by Vert et al.,[4,5] as listed in Table 1). During the last decades, tissue engineering has gained popularity also in the field of cardiovascular research, and research groups have used a variety of different approaches and methods to develop tissue-engineered heart valves (TEHVs) and tissue-engineered vascular grafts (TEVGs) that are at various stages of clinical development.

Table 1: Definitions of biodegradable, bioabsorbable, and bioresorbable materials according to Vert et al.[4,5]

Terms	Definitions	Examples
Biodegradable	Polymeric materials and solid devices that undergo dispersion as a consequence of macromolecular degradation. Although degradation products can be removed from the site of action, their degradation products will not completely be eliminated, remaining inside the human body.	Polyurethane
Bioabsorbable	Solid polymeric materials or devices that can dissolve into body fluids without breakdown of the macromolecular chain or reduction in molecular mass.	Polyethylene glycol
Bioresorbable	Solid polymers and devices that degrade into non-toxic products (low–molecular weight compounds), which will be eliminated via metabolic pathways (i.e. the citric acid cycle) or directly via renal excretion without residual side-effects.	Poly-D,L-lactide

Here we review the available bioresorbable scaffold materials that are used to develop cardiovascular substitutes with growth and regeneration capacity.

TISSUE ENGINEERING AND SCAFFOLD REQUIREMENTS

Generally speaking, there are three distinct tissue engineering approaches, namely *in vitro*, *in vivo*, and *in situ* tissue engineering. The traditional tissue engineering approach generates constructs *in vitro* by seeding autologous cells onto 3D starter materials, so-called scaffolds. Thereafter, tissue formation is stimulated under controlled conditions in a bioreactor. For this approach, autologous cells are preferred to prevent immunogenic reaction and rejection. However, when relying on the regenerative capacity of the body in the case of *in situ* tissue engineering, long *in vitro* cell expansion and tissue culture times could be eliminated when an acellular scaffold is directly implanted to recruit endogenous cells.

In vivo tissue engineering describes the fabrication of an autologous substitute by making use of the body (e.g. peritoneal cavity) as a bioreactor.[6] In all cases the key processes during tissue formation and maturation are cell proliferation and migration, extracellular matrix (ECM) production and organisation, and scaffold degradation. Finally, the capacities of these engineered tissues to enable repair of structural injury, remodelling of the ECM, and potential growth are crucial for their long-term success. Therefore it is of utmost importance to understand the specific demands of the cardiovascular system that, due to the cyclic mechanical loading of the blood with every heart contraction, requires an enormous strength, flexibility, and durability of the tissue-engineered cardiovascular replacements.

Consequently, the scaffolds need appropriate mechanical properties to endure the cyclic stresses and strains exerted upon implantation. More specifically, scaffolds for TEHVs should contain stiffness at around 0.5 MPa, while being elastic without any tendency to deform permanently under the influence of stresses.[7] For TEVGs, the scaffold should be compliant and able to withstand the burst pressure requirements during degradation and neotissue formation.[8] These requirements to the overall mechanical behaviour are determined by the intrinsic material properties (e.g. stiffness), scaffold architecture (e.g. fibre thickness and direction), and degradation rate. Moreover, due to intense contact with blood in cardiovascular applications, it is desirable to use a thromboresistance material or provide an endothelial surface layer.[9,10]

In general, the scaffold microstructure (i.e. pore size and fibre thickness) primarily determines cell infiltration, which is one of the prerequisites for successful tissue regeneration.[11] Additionally, it can affect cell phenotype and influence the behaviour of the infiltrating cells regarding cell adhesion, spreading, and proliferation.[12] The microstructure is also used to attach different bioactive molecules and signals that improve specific cell function, such as pro-angiogenic signals,[13] or to promote

recruitment of specific cell types via chemotaxis. The mechanical properties of the scaffold provide an important stimulus to the cells for ECM production and remodelling, as the cells experience different local stresses and strains depending on the scaffold stiffness. On the other hand, these mechanical cues, as a result of scaffold stiffness, can modulate the differentiation of cells into pathological phenotypes, e.g. osteoblastic or myofibroblastic differentiation.[14] Although the optimal cell type for preseeding the scaffold (or to attract *in vivo*) is not yet defined, an elaborative amount of information is available in literature[15,16] but is considered beyond the scope of this review.

To conclude, the initial scaffold for cardiovascular tissue engineering should ideally meet the following requirements: (1) be biocompatible and thromboresistant; (2) be able to support cell infiltration, growth, and cell-to-cell interaction; (3) start with sufficient mechanical properties and degrade at a rate in relation to new tissue formation; (4) have optimum architectural properties of pore size, porosity, and permeability in order to allow diffusion of nutrients and metabolic waste products; and last, but not least, (5) be bioresorbable.[15]

BIORESORBABLE SCAFFOLD MATERIALS

Roughly, the bioresorbable materials can be divided into natural-based and synthetic polymers. For natural polymers, no toxic degradation or sustained inflammatory reactions are expected as they can be produced from biological sources, such as collagen, gelatin, fibrin, hyaluronic acid, alginate, and decellularised matrices.[17] In contrast, the bulk degradation of several synthetic polymers, such as polyglycolic acid (PGA), polylactic acid (PLA), polyhydroxyalkanoate, and copolymers, leads to a decrease in the pH within the polymeric matrix and might result in local inflammation.[18] The degradation of implants is of special interest for the medical industry as no further surgical procedure is required to remove the implant. Such biomaterials should have degradation and resorption rates which are compatible with the formation of the neotissue; there must be a balance between scaffold degradation and ECM production.[19,20] The bioresorbable polymers should maintain mechanical features and secure their function during tissue formation as unexpected faster material degradation can result in mechanical instability and vessel rupture or valve failure. However, after healing of tissue, the implanted scaffold must be completely degraded and resorbed in order to avoid side-effects.[19,20] Prolonged macrophage activity due to the presence of synthetic scaffold remnants may lead to excessive chronic inflammation resulting in fibrosis, calcification, and/or degeneration of the cardiovascular implants.[18] Different factors influence the degradation kinetics, including hydrophobicity, crystallinity, configurational structure, molar mass, stress and strain, polydispersity, chain orientation, and site of implantation.[21-24]

There are four main degradation mechanisms for polymers: (1) hydrolysis; (2) oxidation (due to oxidants produced by tissues); (3) enzymatic degradation; and (4) physical degradation.[25] Degradation via hydrolysis has been studied intensively, in particular for bioresorbable polymers. The chemical properties enable hydrolytic degradation as a consequence of ester bond hydrolysis. After degradation, the monomeric components are removed by the natural (metabolic) pathways of the human body. Table 2 displays the degradation time and products of the most important natural-based and synthetic polymers, such as PLA and poly-ε-caprolactone (PCL), which have been used in several cardiovascular tissue engineering applications.[27-29]

SYNTHETIC POLYMERS

Synthetic bioresorbable polymers have found extensive applications in tissue engineering, mainly due to their variety of advantages compared to natural scaffold materials, including: more predictable mechanical and physical properties such as durability, degradation rate, strength, and elastic modulus. Furthermore, these materials are less expensive, better to reproduce, and may be stored over longer time periods, which make them interesting raw materials for scaffold fabrication.

Aliphatic Polyesters

Aliphatic polyesters have been known and studied since the 1930s[30] and their success in tissue engineering relies mainly on their degradability, biocompatibility, good processability, and mechanical features. Currently, the most widely investigated and most commonly used biomedical aliphatic polyesters are PGA, PLA, and PCL.

PGA is a rigid thermoplastic material with high crystallinity and is not soluble in most organic solvents. PGA is usually synthesised by ring-

Table 2: Bioresorbable polymers and their degradation time and degradation products.

Polymer	Abbreviation	Approximate Degradation Time	Degradation Products	Reference
Polyglycolic acid	PGA	6-12 months	Glycolic acid	107
Poly-L-lactic acid	PLLA	>24 months	L-lactic acid	107
Poly-D,L-lactic acid	PDLLA	12-16 months	D,L-lactic acid	107
Poly-D,L-lactide-co-glycolide 50:50	PDLGA 50:50	2 months	D,L-lactic acid Glycolic acid	107
Poly-D,L-lactide-co-glycolide 15:85	PDLGA 15:85	5 months	D,L-lactic acid Glycolic acid	107
Polycaprolactone	PCL	>24 months	Caproic acid	107
Poly-4-hydroxybutyrate	P4HB	2-12 months	4-hydroxbutyrate	108
Collagen		~2 weeks	Amino acids	109
Fibrin		few days	Fibrin degradation products (FDP)	110

opening polymerisation of glycolide, the cyclic dimer of glycolic acid.[31] Similarly, lactic acid is polymerised to synthesise PLA. Lactic acid, which is normally produced by muscular contraction, can be eliminated through the citric acid cycle, whereas glycolic acid may be eliminated directly in urine or may be converted to enter the citric acid cycle via pyruvic acid.[32] Due to the chiral nature of PLA, distinct forms exist, namely poly-L-lactide (PLLA), poly-D-lactide (PDLA), and racemic polylactide (PDLLA). The characteristics of PLA are highly affected by the stereo-isomeric L/D ratio of lactate units. Generally, an increased stereo-isomeric ratio decreases the crystallinity, whereby the degradation is enhanced. For example, degradation of PLA is faster than PDLA due to the lower crystallinity of PLA.

PCL is a semicrystalline, aliphatic polyester which is synthesised by ring-opening polymerisation of ε-caprolactone.[33,34] It displays good mechanical characteristics, such as high elongation and strength. PCL degrades very slowly *in vivo* by enzymatic action and by hydrolysis (Table 2).[33] All three polyesters are FDA approved polymers for clinical use.[35]

The feasibility of creating autologous TEVGs was first demonstrated in 1998 by seeding vascular cells onto PGA scaffolds.[36,37] After *in vitro* culture and biomimetic perfusion, these grafts were implanted into the right saphenous artery of miniature swine and demonstrated to stay patent up to 24 days.[36] However, the relatively stiff nature of the PGA fibres was not optimal and resulted in poor compliance match and poor surgical handling qualities.[37] Despite PGA being bioresorbable, breakdown products are acidic, which could induce an inflammatory response. Furthermore, PGA degrades faster than PLA, resulting in lower mechanical properties of TEVGs.[36] Other studies used PGA-PLLA scaffolds for microvessels in mice[38] or investigated TEVG scaffolds composed of polyglycolide knitted fibre and an L-lactide and ε-caprolactone copolymer sponge in a canine inferior vena cava model.[39] Surgical handling characteristics were improved after the introduction of a more elastic hybrid polymeric scaffold, fabricated from either PGA or PLA fibre-based mesh coated with a 50:50 copolymer of L-lactide and ε-caprolactone (PCLA/PGA or PCLA/PLA), due to an improved compliance match between vessel and conduit.[40] In 1998, Shinòka et al.[37] reported surgical implantation of TEVGs in lambs, based on autologous myofibroblasts and endothelial cells seeded onto PGA scaffolds. This study demonstrated the first vascular graft using autologous cells that yielded a viable structure.[37]

Polyhydroxyalkanoates (PHA)

Another group of polyesters that appeared to be convenient for tissue engineering is the PHA family, which is built from hydroxyacids produced by microorganisms under unbalanced growth conditions.[41,42] This group is generally bioresorbable and thermoprocessable and includes

poly-3-hydroxybutyrate (P3HB), copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (PHBV), poly-4-hydroxybutyrate (P4HB), copolymers of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx), and poly-3-hydroxyoctanoate (PHO). A disadvantage of some PHA polymers, however, is their limited availability and the time-consuming production by bacteria. In order to adjust mechanical features or biocompatibility PHA polymers can be either blended, surface modified, or composed with other polymers, enzymes, or inorganic materials.[43] Additionally, their degradation rate can be tailored by varying their copolymer ratio. For example in 1999, TEVGs based on PGA-PHA scaffolds were implanted in the abdominal aorta of lambs and successfully followed up to 5 months *in vivo*. Overtime, the mechanical properties of these preseeded TEVGs changed towards those of native blood vessels.[44] For both heart valve and vascular tissue engineering the use of PGA coated with P4HB, meaning the combination of the thermoplastic characteristic of P4HB and high porosity of PGA, has been investigated intensively, presenting promising results in *in vitro* and preclinical studies.[45-50] Hoerstrup et al.[51] provided, in 2006, the first evidence of growth of living, functional pulmonary arteries engineered from vascular cells seeded on PGA/P4HB scaffolds in a growing lamb model. The evidence of the growth and remodelling capacity of these implants proved their potential also for use in paediatric applications.

NATURAL POLYMERS

Whereas synthetic materials have performed better in durability and strength, they pale in comparison with the functional capabilities of natural tissues. A good alternative to the synthetic polymers are natural polymers which possess biologically recognisable side groups. The category of natural-based materials for scaffolds includes polysaccharides (aliginate, chitin/chitosan, and hyaluronic acid derivate), proteins (soy, collagen, fibrin, gels, and silk), or decellularised ECM. Natural polymers are used as pure materials or in combination with synthetic polymers or inorganic substances to produce scaffolds.[52-54] The natural polymers mostly used for cardiovascular tissue engineering are collagen, fibrin, and decellularised ECM.

Collagen

Collagen molecules have a triple-helical structure and provide high tensile strength due to the arrangement of triple helices in fibrils. This biopolymer is the major protein component of the ECM and plays a dominant role in maintaining the biologic and structural integrity. There are four main collagen Types (I, II, III, and V) that make up the essential part of collagen in bone, cartilage, tendon, skin, and muscle.[55,56] The most explored collagen for biomedical applications is collagen Type I. Collagen scaffolds have been investigated for blood vessels, heart valves, and ligaments[57] in a variety of formats including porous sponges,[60] gels, sheets,[61,62] and foams.[63] The possible degradation by human collagenases makes collagen an ideal scaffold for tissue engineering and could potentially lead to the restoration of tissue structure and functionality.[58] The degradation rate often needs to be regulated using diverse methods such as crosslinking techniques.[59] The feasibility of TEVGs made of collagen and cells was first demonstrated in 1986 by Weinberg and Bell.[64] They generated cultures of bovine endothelial cells, smooth muscle cells (SMCs), and fibroblasts in layers of collagen gel. Later, a polyethylene terephthalate (PET) mesh was added to enhance the burst pressure.[64] Since, several studies have been conducted to improve the strength of collagen-based constructs by incorporating cells, matrix components, undegradable or degradable meshes,[69-71] and intracellular biomolecules by glycation[65-68] or dynamic mechanical stimulation.[72] Furthermore, cross-linking with chemicals makes collagen stronger and modifies its degradation rate.[73] A distinctive drawback, however, is the low availability of homologous (human) collagen.

Fibrin

Fibrin is a biopolymer of the monomer fibrinogen, i.e. the end-product of the coagulation cascade following the conversion of fibrinogen in the presence of thrombin and calcium.[74] Fibrinogen is a soluble plasma glycoprotein, which is produced by the liver. Fibrin and fibrinogen play essential roles in blood clotting, fibrinolysis, cellular and matrix interactions, the inflammatory response, wound healing, and neoplasia.[74] Fibrin is used as a naturally-occurring, autologous scaffold without the potential risk of a foreign body reaction.[75] Based on the autologous and bioresorbable properties, many applications for tissue engineering have been established in combination with cells,

growth factors, or drugs.[76-78] The most broadly used forms of fibrin scaffolds are fibrin hydrogels, fibrin glue, and fibrin microbeads.[79] Interestingly, fibrin gels can stimulate SMCs to synthesise elastin, which is an important component of arteries.[81] Fibrin vascular constructs are weaker and more extensible than collagen-based constructs. Therefore mechanical properties can be improved by fibrin-collagen composites presenting higher strength than collagen alone, but also more gel compaction.[80] Next to the shrinkage of the gel and low mechanical stiffness, its rapid degradation before proper tissue formation is another major disadvantage of fibrin hydrogels.[76,82] On the other hand, degradation within several days by cell-associated enzymatic activities can be utilised for the controlled release of growth factors.[83] Moreover, by adding aprotinin (for example), which restricts or even stops fibrinolyse, the degradation can be controlled.[82] Aprotinin is a monomeric serine protease inhibitor found to effectively inhibit the activity of several proteases, including plasmin, trypsin, chymotrypsin, and kallikrein.[84]

Decellularised ECM

Besides using proteins, such as fibrin or collagen, the complete natural ECM, i.e. decellularised xeno or homo-grafts, has also been discovered as an appropriate scaffold for tissue engineering. The ECM works as a supporting material and regulator of cellular functions including cell survival, proliferation, morphogenesis, and differentiation.[85] However, xenografts are associated with the risk of immunogenic reactions or disease transmission, and the availability of homografts is limited.[86] To eliminate immune reactions the xenogenic tissues can be decellularised, which is generally performed by perfusion of the tissue with various detergents or enzymatically, aiming at the removal of all cellular and nuclear matter while reducing any effects on the integrity and structure of the remaining ECM.[87-89] For this purpose, many organs and tissues, such as vessel, heart valves, and pericardium from humans and animals (e.g. sheep, pigs, and rabbits) have been studied.[90-93] Moreover, *in vivo* complete cellular ingrowth was proved in animal models.[94,95]

CLINICAL APPLICATIONS

Next to the elimination of possible immune rejection and the requirement of lifelong anticoagulation therapy, the major advantage of autologous tissue-engineered vessels or valves compared to current replacements is their ability to grow, repair, and remodel. However, despite the variety of accepted biomaterials for clinical applications and successful *in vitro* studies, just a few reports describe the effective transplantation of cardiovascular tissue engineering into clinics. In 2001, Shinòka's group[96] performed the first clinical study using vascular cell-seeded biodegradable polymer scaffolds (PGA or PLLA 50:50 copolymer ε-caprolactone) in the high-flow low-pressure pulmonary venous system of paediatric and young patients. 7 months after implantation, TEVGs were still functional, without complications or aneurysm.[96] Nevertheless, few patients showed TEVG stenosis, which was successfully treated with angioplasty.[97] L`Heureux`s group[98] treated haemodialysis patients with end-stage renal disease and haemodialysis failure with complete autologous *in vitro* grown TEVGs.[98] However, three out of ten TEVGs failed due to thrombosis or aneurysm, attributed to a low postoperative flow rate and diffuse dilatation. The clinical translation of autologous living valves, created according to the classical tissue-engineering paradigm, is, among others, limited by cell-mediated contraction of the valve leaflets caused by traction forces exerted by the cells. Decellularisation of the TEHVs before implantation can prevent this retraction, meanwhile enabling off-the-shelf availability.[50,99] Although preclinical trials have been performed for this promising approach,[49,50,100] more sophisticated studies should be performed to support future clinical studies.

The first clinical implementation of valves was performed with decellularised pulmonary allografts, reseeded with autologous endothelial progenitor cells, showing improved freedom from reintervention in contrast to conventional homografts and xenografts.[100] Additionally, decellularised allografts, reseeded with autologous vascular endothelial cells, demonstrated uncompromised follow-up of 10 years with excellent haemodynamic performance.[101] Nevertheless, the need for valve replacements exceeds the supply of donor valves. Therefore, also the largely available decellularised xenogenic pulmonary valves, reseeded with autologous vascular endothelial cells, have been introduced for right ventricular outflow tract reconstruction, with excellent early and midterm results.[101] Moreover, the short and mid-term performance of non-seeded decellularised xenograft valves in children and patients with congenital heart disease were recently reported to meet the performance of other currently available

implants.[102] However, cellular infiltration is sparse in humans.[102-104,106]

CONCLUSION

This review displays the significant progress of the applications of bioresorbable materials in the field of cardiovascular tissue engineering. As the scaffold plays a crucial role in the successful design of tissue-engineered constructs, the choice of material directly influences the outcome. Natural-based polymers display no toxic degradation or sustained inflammatory reactions and can be produced from biological sources. On the other hand, synthetic polymers present a higher strength and durability compared to natural polymers, but the bulk degradation might result in local inflammation. Composite biomaterials have the potential to overcome the current predicament of having to choose between either synthetics or natural tissues. Therefore, despite a variety of materials having been validated, future effort should focus on perfecting composite materials to take full advantage of the best properties. Moreover, optimising methods to extract natural polymers from human (cell) sources will enable the production of non-genoxenic (composite) grafts. In the coming decades research will most likely focus on intelligent, off-the-shelf scaffolds that make use of the regenerative capacity of the human body by attracting endogenous cells. Obviously, long-term (pre-clinical) studies are compulsory to evaluate the remodelling of these optimised materials towards native-like tissues. Nevertheless, bioresorbable scaffolds have large potential to eventually replace the use of the current synthetic and fixed-biological grafts in clinical practice.

Acknowledgements

The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n^0. 242008 and grant agreement n^0. 626976.

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