Expert Opinion

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Cell- & Tissue-based Therapy

Biodegradable polyester elastomers in tissue engineering

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Tissue engineering often makes use of biodegradable scaffolds to guide and promote controlled cellular growth and differentiation in order to generate new tissue. There has been significant research regarding the effects of scaffold surface chemistry and degradation rate on tissue formation and the importance of these parameters is widely recognised. Nevertheless, studies describing the role of mechanical stimuli during tissue development and function suggest that the mechanical properties of the scaffold will also be important. In particular, scaffold mechanics should be taken into account if mechanical stimulation, such as cyclic strain, will be incorporated into strategies to grow improved tissues or the target tissue to be replaced has elastomeric properties. Biodegradable polyesters, such as polyglycolide, polylactide and poly(lactide-co-glycolide), although commonly used in tissue engineering, undergo plastic deformation and failure when exposed to long-term cyclic strain, limiting their use in engineering elastomeric tissues. This review will cover the latest advances in the development of biodegradable polyester elastomers for use as scaffolds to engineer tissues, such as heart valves and blood vessels.

Keywords: biodegradable elastomer, blood vessels, caprolactone, citric acid, glycerol, heart valves, polyester, polyhydroxyalkanoates, scaffolds, tissue engineering, trimethylene carbonate

Expert Opin. Biol. Ther. (2004) 4(6):801-812

1. Introduction

A major goal in the field of tissue engineering is to replace diseased or damaged tissue by combining principles and methods of chemistry, engineering and biological sciences [1]. This goal requires the use of a three dimensional scaffold for cells to grow on and differentiate properly. As many tissues in the body have elastomeric properties, successful tissue engineering of these tissues will require the development of compliant biodegradable scaffolds that can sustain and recover from multiple deformations without irritation to the surrounding tissue [2]. The development of elastomeric scaffolds is desirable because mechanical conditioning regimens have been shown to promote improved tissue formation and would allow gradual stress transfer from the degrading synthetic matrix to the newly formed tissue [3,4]. For example, cyclic mechanical strain during tissue development has been shown to increase collagen and elastin production in vascular smooth muscle cells (SMCs) and enhance the mechanical properties of SMC-containing tissue engineered constructs [3-8]. Furthermore, as vascular graft and tissue engineering scaffold materials are rigid, the development of elastomeric scaffolds may alleviate the problem of compliance mismatch between a vascular graft and a host vessel, a problem that has been linked to incomplete endothelialisation and myointimal hyperplasia at the anastomoses [9-15]. Likewise, for orthopaedic tissue engineering, several research groups have shown the importance of mechanical signals in the development of tissue-engineered cartilage [16-19].

 ISSN 1471-2598

Despite the increasing body of literature regarding the importance of mechanical stimuli during tissue development [3,5-8,16-18], there has been little research into the development of elastomeric biodegradable scaffolds. Although many new materials with elastomeric properties have been developed, relatively few have been designed for tissue engineering applications. However, within the past 5 years, investigators have recognised the need for elastomeric biodegradable scaffolds, particularly in the area of cardiovascular tissue engineering. As a result, research efforts have begun to focus on the synthesis and characterisation of materials with a wide range of elastomeric and biodegradable properties. Although polyurethanes, protein- and/or peptide-based elastomers have recently received attention for use in soft tissue engineering, this review will focus on novel biodegradable polyester elastomers that are likely to be important to the future of tissue engineering. The reader is referred to works by other researchers in the field for information on polyurethanes and peptide-based elastomers [20-28].

2. Biodegradable polyester elastomers

The most commonly used biodegradable polymers in tissue engineering are polyglycolide (PGA), polylactide (PLA) and PLA–PGA copolymers [29]. Although these polyesters have been used extensively in tissue engineering and other medical applications, their use in soft tissue engineering is limited due to their stiffness and elastic deformation characteristics. The properties of PGA, PLA, their copolymers and common soft tissues that would benefit from an elastomeric scaffold are listed in **Table 1**. The requirement and development of materials with elastomeric properties has primarily been driven by cardiovascular tissue engineering applications. Specifically, after attempting to tissue engineer a heart valve in a sheep animal model using PGA as a scaffold, Sodian and co-workers pointed out the need for a more compliant degradable scaffold in order to allow mechanical conditioning and maturation of the valve *in vitro* prior to implantation [30]. The material of choice had to withstand the extensive cyclic deformations that a heart valve cell-scaffold construct would endure prior to complete tissue formation. At that time, a relatively new company (Metabolix, Inc., Cambridge, MA, USA) was focusing on the biosynthesis of degradable polyesters, referred to as polyhydroxyalkanoates (PHAs), that would meet the purity and yield standards of the medical device industry. Depending on the monomers selected, PHA had displayed elastomeric properties, which justified its use by Sodian and co-workers in their subsequent attempt to tissue engineer a heart valve. Results were greatly improved with the use of PHAs, although the degradation timeframe of the original PHAs used was too slow. At present, mostly hydroxyalkanoates, and to a lesser extent, ε-caprolactone (CL)-based copolymers, have been used to engineer tissues with elastomeric properties. However, three recently developed biodegradable elastomers, poly(trimethylene carbonate) copolymers, poly(glycerol sebacate) (PGS) and poly(diol citrates), also show promise for potential use in soft tissue engineering.

2.1 Polyhydroxyalkanoates

The most extensively studied polyester elastomers for tissue engineering are PHAs. PHAs, biodegradable polyesters that are readily produced by microbiological organisms, are becoming increasingly popular in tissue engineering because their mechanical and degradation properties can potentially be tailored to specific applications [31-38]. Although > 100 different types of PHAs can be produced from a variety of monomers, the structure of all PHAs resemble that of chemically derived polyesters and have the general structure shown in **Figure 1**. Properties of the polymer can be modulated by varying the length of the side chain and the distance between ester linkages in the polymer backbone. This modulation can be accomplished by changing the precursor compounds fed to the microorganisms to incorporate other co-monomers or by varying the activity levels of specific pathway enzymes within the cells to change the molecular weight [39]. PHAs with short side chains tend to be hard and crystalline materials, whereas PHAs with long side chains are elastomeric. PHAs have been evaluated for several medical applications, including controlled release, manufacture of surgical sutures, wound dressings, orthopaedic uses and cardiovascular tissue engineering [30,40-44].

Of all the various types of PHAs, poly(4-hydroxybutyrate) (P4HB), its copolymers with poly(3-hydroxybutyrate) (PHB) and polyhydroxyoctanoate (PHO) are of interest in tissue engineering because of their non-toxic degradation products, stability in tissue culture media and the potential to tailor the mechanical and degradation properties to match soft tissue. Elongation at break for P4HB can exceed 1000%, while copolymers of P4HB and PHB can have elongations at break ranges of up to 1000% [39]. Mechanical properties of PHB and P4HB are shown in **Table 1**. Degradation rates of P4HB can be varied by changing the configuration of the scaffold or copolymerisation with other PHAs. For instance, when solid, 50% porous and 80% porous P4HB were implanted subcutaneously in rats, the samples lost 20, 50 and almost 100% of their original mass, respectively, over a 10-week period. This finding suggests that the degradation rate depends in part on the surface area. By combining P4HB with another PHA, poly(3-hydroxybutyrate-co-3-hydroxyvalerate), the *in vivo* degradation time can be increased to > 1 year. On degradation, the hydrolysis of P4HB yields 4HB, a natural human metabolite present in brain, heart, lung, liver, kidney and muscle [45]. This metabolite is rapidly eliminated from the body via the Krebs cycle and expired as carbon dioxide [46]. Another advantage of P4HB over commonly used α-hydroxyacids is the less acidic nature of the degradation product 4HB. Regarding PHO, although it has not been evaluated for biocompatibility and degradation, a copolymer containing hydroxyoctanoate, poly(hydroxyoctanoate-co-hydroxyhexanoate) (PHOH), has been examined at Metabolix, Inc. through subcutaneous implantation of polymer discs [35].

Table 1. Mechanical properties of biodegradable polymers and soft tissues.

Polymer	Tensile strength (MPa)	Modulus (MPa)	Elongation (%)	Degradation (months)	Ref.
PGA[a]	70	6900	< 3	1.5	[39,101]
PLLA[b]	28 – 50	1200 – 2700	6	18 – 60	[39,101]
D,L-PLA[c]	29 – 35	1900 – 2400	6	3	[39,101]
PLGA[d]	41.4 – 55.2	1.4 – 2.8	3 – 10	Variable	[101]
PCL[e]	20.7 – 34.5	0.21 – 0.34	300 – 500	> 24	[101]
Ulnar cadaveric peripheral nerve	9.8 – 21.6	-	8 – 21	-	[102]
Medial cadaveric peripheral nerve	9.8 – 30.4	-	6 – 22	-	[102]
Human bladder	0.27 ± 0.14	0.25 ± 0.18	0.69 ± 0.17*	-	[103]
Rat bladder	0.72 ± 0.21	0.76 ± 0.44	2.03 ± 0.44*	-	[103]
Pig bladder	0.32 ± 0.10	0.26 ± 0.18	1.66 ± 0.31*	-	[103]
Rat abdominal aorta	0.40 ± 0.01	0.17 ± 0.02	-	-	[104]
Porcine aortic heart valve (radial)	1.4 ± 0.2	6.4 ± 0.9	134.8 ± 27.7	-	[105]
Porcine aortic heart valve (circumferential)	8.3 ± 0.9	44.7 ± 5.3	48.7 ± 7.3	-	[105]
Bovine elastin	-	1.1	-	-	[87]
Collagen fibres	-	100	50	-	[106]
Smooth muscle relaxed	-	0.006	300	-	[106]
Smooth muscle contracted	-	0.01	300	-	[106]
PHB[f]	36	2500	3	24	[39]
P4HB[g]	50	70	~ 1000	2 – 12	[39]
PHB-4HB[h]	25.51	-	444	Variable	[107]
PHBHHx:PHB[i]	20.9 – 23.5	-	15 – 106	-	[49]
PGCL[j]	< 1	-	~ 250	> 1.5	[59]
PGS[k]	> 0.5	0.282 ± 0.0250	> 267	1	[2,88]
POC[l]	Up to 6.7 ± 1.4	0.92 – 16.4	265 ± 10	Variable	[90]
PDC[m]	Up to 3.14 ± 0.5	1.05 – 3.50	322 ± 20	Variable	Unpublished
P(TMC-DLLA) Dry (50:50)[n]	10	16	570	< 11	[66,76,77]
P(TMC-DLLA) Dry (20:80)[o]	51	1900	7	< 11	[66,76,77]
P(TMC-DLLA) Wet (50:50)[p]	11	13	900	< 11	[66,76,77]
P(TMC-DLLA) Wet (20:80)[q]	38	1100	7	< 11	[66,76,77]
P(TMC-CL) (10:90)[r]	23	140	-	> 24	[66,76,77]

*Maximum strain calculated as the displacement of the specimen divided by the initial gauge length in mm/mm.

[a–e]PGA: Poly(glycolic acid); PLLA: Poly(L-lactic acid); D,L-PLA: Poly(D,L-lactic acid); PLGA: Poly(lactic-co-glycolic acid); PCL: Poly(ε-caprolactone). Properties depend on molecular weight, crystallinity, porosity, etc.

[f]PHB: Poly(3-hydroxybutyrate).

[g]P4HB: Poly(4-hydroxybutyrate).

[h]PHB-4HB: Poly(3-hydroxybutyrate-co-4-hydroxybutyrate) containing 16% 4-hydroxybutyrate.

[i]PHBHHx:PHB: Poly(hydroxybutyrate-co-hydroxyhexanoate) blended with poly(3-hydroxybutyrate). Properties are presented for blends ranging 40 – 60% PHBHHx.

[j]PGCL: Poly(glycolide-co-caprolactone) 1:1 mole ratio polymerised at 170°C for 20 h in the presence of catalyst.

[k]PGS: Poly(glycerol sebacate) 1:1 mole ratio polymerised for 77 h at 120°C.

[l]POC: Poly(1,8-octanediol-co-citric acid) 1:1 mole ratio polymerised at temperatures ranging 80 – 120°C for times ranging 1 – 4 days.

[m]PDC: Poly(1,10-decanediol-co-citric acid) 1:1 mole ratio polymerised at temperatures ranging 80 – 120°C for times ranging 1 – 4 days.

[n–q]P(TMC-DLLA): Poly(trimethylene carbonate-co-D,L-lactic acid) Mole% shown in parenthesis with polymerisations at 130°C for 3 days.

[r]P(TMC-CL): Poly(trimethylene carbonate-co-caprolactone) Mole% shown in parenthesis with polymerisations at 130°C for 3 days.

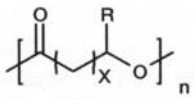

Figure 1. General chemical formula for polyhydroxyalkanoates. PHB: R = Methyl, X = 1; P4HB: R = Hydrogen, X = 2; PHO: R = Pentyl, X = 1; PHHx: R = Propyl, X = 1.
P4HB: Poly-4-hydroxybutyrate; PHB: Poly(3-hydroxybutyrate); PHHx: Polyhydroxyhexanoate; PHO: Polyhydroxyoctanoate.

Polymer degradation was very slow (> 52 weeks), with loose connective tissue containing 3 – 6 fibroblast cell layers and collagen surrounding the implant. However, the reaction was considered to be mild by the investigators. Nevertheless, as described below, PHO has been used in several tissue engineering applications with little inflammatory response.

Early attempts to tissue engineer valve leaflets and entire heart valves used polyhydroxyoctanoate [30,41]. However, because the PHO did not completely degrade during the time course of the study, a mild chronic inflammatory response was observed. Hoerstrup and co-workers used P4HB that had been solvent coated onto non-woven PGA meshes to increase the degradation rate of the scaffold and eliminate the inflammatory response caused by residual polymer [42,43]. Autologous vascular cells from a sheep were seeded onto the material and *in vitro* conditioning was performed for 2 weeks in a pulsatile flow bioreactor. After conditioning, the cell/scaffold construct was implanted in the place of the native pulmonary valve and functioned without stenosis, thrombus formation or aneurysm for 20 weeks. Analysis of the construct showed that the PGA core degraded completely within 4 weeks, whereas the P4HB totally degraded after 8 weeks, leaving behind a layered leaflet structure. At the end of 20 weeks, the implanted tissue-engineered heart valve had greater amounts of glycosaminoglycan (130%), collagen (150%) and cell content as measured by DNA (150%), when compared with native tissue [42]. In addition, elastin could be detected histologically at 6 weeks postimplantation. Mechanical testing revealed a higher tensile strength (130%) and a stress-strain curve similar to that of native pulmonary valve tissue. However, at 16 and 20 weeks postimplantation, a mild-to-moderate valve regurgitation was observed.

P4HBs have also been used for vessel augmentation. Stock and co-workers used porous P4HBs that were seeded with autologous sheep endothelial, smooth muscle and fibroblast cells as a pulmonary artery patch [44]. Examination of the cell-seeded implants through histology at 24 weeks showed large amounts of collagen and proteoglycans in an organised fibrous tissue, with endothelial cells lining the internal surface. However, remnants of the polymer, deep below the surface, were observed, along with a slight inflammatory response. A control patch, which consisted of no cell-seeding, showed mild bulging and less tissue regeneration at 20 weeks.

Other experiments with tissue engineering of blood vessels include the use of a PGA (inner layer)/PHO (outer layer) composite for tissue engineering of the abdominal aorta in a lamb model [47]. Composite scaffolds were seeded with autologous cells and implanted for up to 5 months. Unseeded vessels were used as a control. Unlike the control vessels, the tissue engineered constructs remained patent the entire time. After 5 months of implantation, total collagen content was 99.6% of native, cell content (via DNA quantitation) was 150% and elastic fibres were observed (but not quantified) within the medial layer. Mechanical tests on the explanted constructs revealed a stress-strain curve that approached that of native tissue. Furthermore, endothelial cells were observed on the luminal surface. In a later experiment involving the pulmonary artery in sheep, a PHO/PGA scaffold was seeded with autologous cells and implanted [48]. After 169 days, levels of collagen, elastin and proteoglycans were all greater than that in a native vessel. However, the elastin and proteoglycan contents appeared to show a decreasing trend towards that of normal tissue at the end of the study. These results support the potential for *in vivo* tissue engineering provided that scaffolds with suitable mechanical properties for implantation can be developed.

More recently, Zhao *et al.* have experimented with blends of PHB and poly(hydroxybutyrate-co-hydroxyhexanoate) (PHBHHx) [49]. Elongations at break in the range of 15 – 106% were observed, with contents of PHBHHx ranging from 40 – 60%. This increase in PHBHHx content was accompanied by a decrease in tensile strength from 23.5 to 20.9 MPa (**Table 1**). Moreover, rabbit chondrocytes attached, proliferated and produced extracellular matrix on the 60% PHBHHx scaffolds. In later experiments it was shown that cell proliferation was greater on blends of 2:1 PHBHHx:PHB than on pure PHB [50]. Although there have been no reports on the biodegradability of PHBHHx *in vivo*, it is believed that the amorphous PHBHHx will be more susceptible to hydrolysis or enzymatic degradation than the crystalline PHB, much like other blends of amorphous PHAs with PHB [51].

2.2 Poly(ε-caprolactone) copolymers with glycolide or lactide

As noted above, PLLA and PGA are commonly used polymers in tissue engineering, but their rigid and plastic deformation characteristics render them unsuitable for soft tissue engineering applications. Poly(ε-caprolactone) (PCL), although less widely used in the medical and tissue engineering fields, is FDA approved for use in several medical and drug delivery devices, and there is a large amount of literature documenting its fate *in vivo* [52-54]. PCL is a flexible semicrystalline linear aliphatic polyester whose degradation products are either metabolised via the tricarboxylic acid cycle or eliminated through renal excretion [54,55]. The total degradation time is typically 2 – 3 years, a timescale that is likely to be too slow for most tissue engineering applications, thus requiring strategies for improvement. To provide better control over the

Figure 2. Synthesis of poly(glycolide-co-caprolactone).

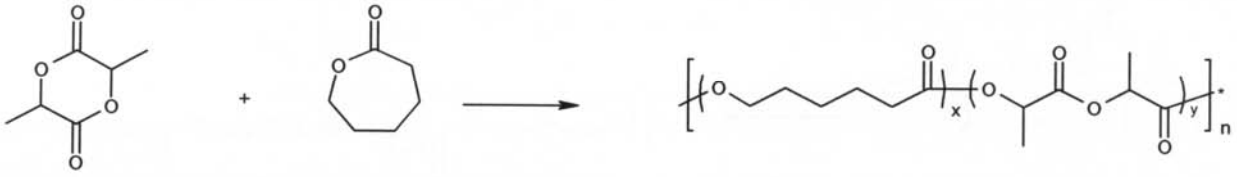

Figure 3. Synthesis of poly(lactide-co-caprolactone).

degradation and mechanical properties without sacrificing biocompatibility, PCL-based materials have been copolymerised or blended with other hydroxyacids or polymers thereof [56-61]. In particular, copolymers of PCL with glycolide or D,L-lactide (DLLA) are elastomeric materials. The reaction schemes for various PCL based copolymers are shown in **Figures 2** and **3**.

Lee and co-workers have synthesised a poly(glycolide-co-caprolactone) (PGCL) copolymer that has shown potential for soft tissue engineering [59]. Porous scaffolds were fabricated via solvent casting and salt leaching. Unlike poly(lactic-co-glycolic acid) (PLGA) scaffolds that break at elongations of 20%, PGCL scaffolds can be elongated up to 250% (**Table 1**). At applied strains of 120%, recovery from extension was 98% for PGCL, whereas PLGA scaffolds underwent plastic deformation and could only show 85% recovery at 3% strain. Under cyclic loading, PGCL scaffolds remained elastic, with a permanent deformation of < 4% of the applied strain magnitude (applied strains of 5 – 20%, frequencies of 0.1 and 1 Hz, 6 days). Similar results were obtained for PGCL scaffolds incubated in phosphate buffered saline (PBS) as they displayed permanent deformation of < 5% of the applied strain (10%) under cyclic loading. Results from degradation experiments showed that after incubation in PBS for 2 weeks, PGCL scaffolds lost 3% of their initial mass and after 6 weeks lost 50%. Although the degradation products were not characterised, as degradation occurs through hydrolysis of the ester bonds, the degradation products should contain short chain oligomers and the corresponding monomer hydroxy acids (glycolic acid and 6-hydroxyhexanoic acid). Regarding tissue engineering applications, Lee and co-workers confirmed the ability of rat aortic smooth muscle cells to attach and proliferate on the surface. The seeded scaffolds were also implanted subcutaneously in nude mice and evaluated histologically and immunohistochemically. On explantation, the cells were differentiated based on the presence of α-smooth muscle actin.

Copolymers of CL and lactide have also been proposed for tissue engineering applications. However, biocompatibility, biodegradation and mechanical properties of the resulting copolymers have not been thoroughly investigated. Matsumura and co-workers recently used poly(lactide-co-caprolactone) (PLACL) scaffolds for tissue engineering of vascular autografts [62,63]. In these procedures, autologous vascular cells or bone marrow were seeded onto 50:50 PLACL scaffold reinforced by a PLLA mesh. In particular, bone marrow-seeded grafts were implanted into the inferior vena cava of adult beagles [62,64]. The implanted grafts functioned without occlusion or stenosis and cells displayed both endothelial and smooth muscle specific markers. The use of PLACL 50:50 grafts was also documented for cardiovascular tissue engineering in humans [63,65]. A PLACL scaffold was seeded with autologous cells and implanted into the pulmonary artery of a 4-year-old girl. Postoperative examinations 7 months after transplantation revealed no dilation or rupture of the grafts or complications related to the tissue engineered autografts. However, because these studies were performed in humans, the histogenesis of the engineered grafts is difficult to document and understand. In addition, there is little evidence that the mechanical properties and compliance values of PLACL-based tubular scaffolds are similar to those of native tissue.

2.3 Poly(1,3-trimethylene carbonate) copolymers

Pego and colleagues have recently investigated trimethylene carbonate-based materials as biodegradable elastomeric scaffolds for tissue engineering of nerve and heart tissues [66-70]. The basis for these copolymers is poly(1,3-trimethylene carbonate) (PTMC), a rubbery amorphous polymer that is an elastomeric aliphatic polycarbonate [71-74]. Although PTMC is rubbery by itself, it is not ideal for biomedical applications as high molecular weights are required for suitable mechanical properties. The material also endures significant creep and does not recover well from deformation unless crosslinked via gamma radiation or some other mechanism [75]. In addition,

Figure 4. Synthesis of poly(trimethylene carbonate-co-D,L-lactide).

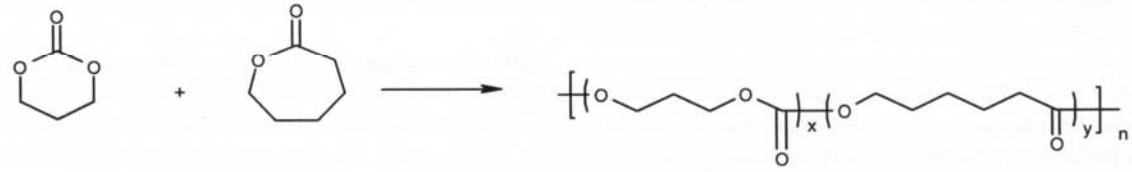

Figure 5. Synthesis of poly(trimethylene carbonate-co-ε-caprolactone).

the degradation rates of PTMC are difficult to predict. For example, although high molecular weight PTMC (M_w ~ 500,000 – 600,000 g/mol) degrades very slowly (> 2 years) when tested *in vitro* [66,68], when implanted *in vivo* it degrades within 3 – 4 weeks, the faster degradation most likely being due to enzymatic activity [76]. In order to obtain better control of the degradation rate and mechanical properties, trimethylene carbonate has been copolymerised with either DLLA or CL [66,67,77].

TMC (trimethylene carbonate)–DLLA (D,L-lactide) copolymers range from hard and brittle to rubbery and soft as the percentage of DLLA in the polymer decreases. The reaction of TMC with DLLA is shown in **Figure 4**. In particular, copolymers with 20 and 50 mol% TMC are of interest in soft tissue engineering. The 50% TMC–DLLA copolymers are highly flexible and strong with an elongation at break of 570% and an ultimate tensile strength of 10 MPa, whereas the 20% materials display rubbery behaviour only when incubated in PBS [77]. Mechanical properties of various TMC copolymers are summarised in **Table 1**. *In vitro* degradation was examined by incubating samples in PBS for up to 2 years [78]. Although the degradation products were not examined, one would expect that hydrolysis of the ester bonds will yield short chain oligomers and the original monomers. All poly(TMC–DLLA) copolymers degrade *in vitro* in < 2 years. Poly(TMC–DLLA) (50:50) degrades the fastest, with the polymer breaking into small pieces after 3 months, whereas poly(TMC–DLLA) (20:80) becomes brittle, but remains intact after 4 months. Both polymers have been shown to undergo total mass loss after 11 months [78]. The *in vivo* degradation and tissue response of TMC–DLLA was investigated by implanting a 52 mol% DLLA copolymer subcutaneously in rats for up to 1 year [76]. The TMC–DLLA copolymer became smaller and lost 96% of its total mass over the time course of this study. Histological results showed a normal inflammatory response and foreign body reaction, similar to that observed following implantation of other common biodegradable polymers. However, in the later stages of the study, the TMC–DLLA polymer showed a second inflammatory reaction triggered by the cellular removal of residual polymer. The potential for heart tissue engineering was demonstrated by the ability of rat cardiomyocytes to attach to TMC–DLLA films [68].

Whereas TMC–DLLA properties can range from hard and brittle to rubbery and soft, TMC–CL-based materials are all flexible with higher CL content improving strength [66,67]. The reaction scheme for TMC–CL is shown in **Figure 5**. Of interest in tissue engineering are TMC–CL copolymers containing 10 mol% TMC. Copolymers with increased percentages of TMC are semicrystalline and, thus, not desirable for tissue engineering, as crystalline debris formed during degradation may cause chronic inflammatory responses [79]. The mechanical properties of TMC–CL (10:90) are shown in **Table 1**. At equilibrium water uptake, the mechanical properties of TMC–CL copolymers are not significantly different from those in the dry state. In contrast to TMC–DLLA (50:50 and 20:80), *in vitro* degradation of TMC–CL copolymers is very slow, with little mass loss over the period of 1 year. However, mechanical properties were maintained over the course of the year [78]. Once again, degradation of this copolymer should yield short chain oligomers and the resulting monomers. TMC copolymers containing 89% CL were implanted subcutaneously to examine *in vivo* degradation and biocompatibility [76]. Like the *in vitro* studies, degradation was slow with no change in shape or size of the implant observed after 1 year. Histological results were similar to TMC–DLLA or other commonly used biodegradable polymers. As the TMC–CL copolymer did not degrade, a mature fibrous capsule remained throughout the year.

Poly(TMC–CL) was examined for use in an artificial nerve graft [66,67]. The TMC–CL copolymers were processed into two-ply porous tubes using salt leaching to obtain the inner part and fibre winding to obtain the outer layer. Schwann cells (human cell lines NCN61 and NCN68), which can enhance the rate of nerve regeneration, attached and proliferated on all

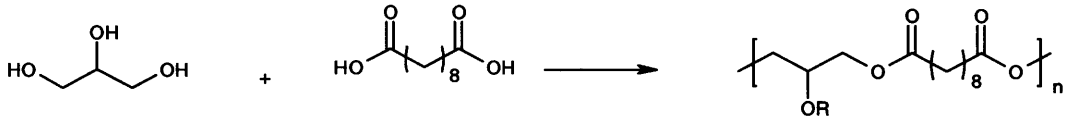

Figure 6. Reaction scheme for poly(glycerol sebacate).

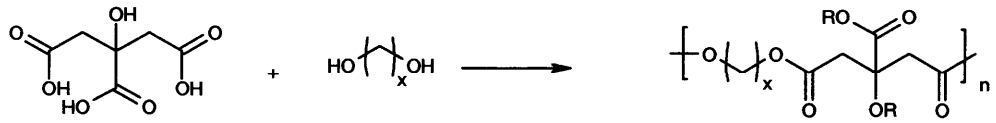

Figure 7. Synthesis of poly(diol citrates).

surfaces to various degrees, with the TMC-rich surface having the best cell affinity [80]. The growth rate was similar on all surfaces despite the preferential initial adhesion on TMC-rich surfaces. It remains to be seen if these materials will be useful as *in vivo* studies, which will confirm any ability of these materials to support nerve or heart regeneration.

2.4 Poly(glycerol sebacate)

Although polymers from glycerol and sebacic acid have not been used in a tissue engineering setting to date, their ease of synthesis, mechanical properties, biocompatibility and biodegradation make them excellent candidate materials for soft tissue engineering. Materials made from glycerol and sebacic acid were first synthesised by Nagata and co-workers in an effort to create environmentally friendly plastics [81,82]. With a 2:3 mole ratio of glycerol to sebacic acid, this material was highly crosslinked and rigid. In 2002, Wang and co-workers created a novel elastomeric material using a different synthesis method and a 1:1 monomer mole ratio for potential use in the engineering of soft tissues [2]. This elastomer, referred to as poly(glycerol-co-sebacic acid) or poly(glycerol sebacate) (PGS), is composed of naturally-occurring metabolic products (Figure 6). Glycerol is the basic building block for lipids whereas sebacic acid is the natural metabolic intermediate in β-oxidation of medium- to long-chain fatty acids [83-85]. In addition, glycerol and copolymers containing sebacic acid have been approved by the FDA for use in medical applications [86].

PGS was synthesised through polycondensation of equimolar amounts of the two monomers at 120°C for a period of 3 days. Tensile tests show a stress strain curve similar to that of vulcanised rubber with a Young's modulus of 0.282 ± 0.0250 MPa and a tensile strain of at least 267 ± 59.4%. Although the sample broke from the grips during testing, the reported ultimate tensile strength was at least 0.5 MPa. The mechanical properties of PGS compare favourably with soft tissues as the Young's modulus of bovine elastin is 1.1 MPa and the maximum tensile strain of arteries and veins is ~ 260% [2,87]. The mechanical properties of PGS along with common soft tissues are summarised in Table 1. Although PGS is a thermoset copolymer, the prepolymer can be processed into various shapes due to its solubility in common organic solvents such as 1,3-dioxolane, tetrahydrofuran, isopropanol, ethanol and *N,N*-dimethylformamide. This solubility allows porous scaffolds and complex shapes to be fabricated using solvent casting and salt leaching methods.

In vivo biocompatibility was assessed via subcutaneous implantation in Sprague–Dawley rats. PLGA implants were used as a control polymer. A fibrous capsule ~ 140 µm thick developed at 14 days postimplantation around the PLGA and remained throughout the duration of the study. In contrast, a vascularised fibrous capsule 45 µm thick appeared around PGS at 35 days, with the implant completely absorbed with no signs of granulation or scar formation after 60 days. *In vitro* biocompatibility was assessed through culture of cells on the surface of the polymer. NIH 3T3 fibroblasts attached, showed normal morphology and had growth rates as good as those on PLGA and tissue culture polystyrene [2].

Degradation rates for the copolymer were measured *in vivo* and *in vitro*. When incubated in PBS at 37°C for 60 days, PGS lost 17 ± 6% of its mass. As noted above, during the same time period *in vivo*, the copolymer was completely absorbed. This quicker degradation may be caused by biological enzymes attacking polyester bonds and catalysing the degradation process, likely resulting in low molecular weight oligomers and the original monomers. In subsequent *in vivo* experiments it was found that the mechanical properties (measured as compression modulus) decreased linearly and in parallel with the degradation of PGS, suggesting surface erosion as the main mechanism of degradation [88]. Surface eroding materials are advantageous because the rate of degradation is predictable [89]. In contrast to PGS, PLGA lost its shape and most of its mechanical properties within 7 days of implantation.

2.5 Poly(diol citrates)

Recent work in the authors' laboratory has led to the development of a new family of biodegradable polyester elastomers based on polymerisation of a linear diol with citric acid (Figure 7) [90]. In particular, elastomers with varying mechanical and degradation properties have been synthesised by reacting citric acid with various linear aliphatic diols (3 – 16 carbons) and polyether diols such as polyethylene oxide. So far, the most studied copolymers have been poly(1,8-octanediol-co-citrate)

(POC) and poly(1,10-decanediol citrate) (PDC). The diols were chosen to enable ester bond formation and facilitate degradation through hydrolysis, thus yielding the original monomers. 1,8-Octanediol is the largest aliphatic diol that is water-soluble with no reported toxicity. This property should be beneficial to the degradation process because the formation of insoluble complexes is avoided or minimised. In contrast, 1,10-decanediol is significantly less soluble in water and is expected to confer different mechanical and biodegradation properties to the resulting copolymer. Citric acid was chosen as a multifunctional monomer to enable network formation, and because it is a non-toxic metabolic product of the body (Krebs or citric acid cycle), it is readily available, inexpensive, FDA-approved for use in humans and can participate in hydrogen bonding interactions within a polyester network.

Synthesis of this family of copolymers is simple and can be conducted under very mild conditions. A prepolymer solution can be created by melting equimolar amounts of citric acid and the diol while stirring under a flow of nitrogen gas, followed by further polymerisation for 1 hour at a lower temperature. Like PGS, the resulting prepolymer solution can be dissolved in several solvents including ethanol, acetone, 1,4-dioxane and 1,3-dioxolane. The prepolymer can be purified via precipitation in purified water followed by freeze-drying, or used as is for further postpolymerisation. In the authors' experiments, the prepolymer has been crosslinked at 120, 80, 60 and even 37°C, with and without vacuum, to create copolymers with various degrees of crosslinking.

Mechanical properties of poly(diol citrates) can be modulated by controlling synthesis conditions such as crosslinking temperature and time, vacuum and initial monomer molar ratio [90]. For POC and PDC, maximum elongations at break are 265 ± 10 and 322 ± 20%, respectively. As with PGS, these values are comparable with that of arteries and veins [91]. Ultimate tensile strengths for POC can reach 6.7 ± 1.4 MPa and the Young's moduli range from 0.92 ± 0.02 to 16.4 ± 3.4 MPa (the lowest modulus in the range being similar to that of bovine elastin, 1.1 MPa). For PDC, ultimate tensile strengths were as high as 3.14 ± 0.5 MPa and the Young's modulus ranged from 1.05 ± 0.05 to 3.50 ± 0.06 MPa. Properties of POC and PDC are shown in **Table 1**. As these polymers are being targeted for small-diameter blood vessel tissue engineering, an important design criteria is the burst pressure of the scaffold. Burst pressures of solid POC tubes (3.5 mm internal diameter, ~ 150 μm wall thickness) are ~ 1300 mmHg [90]. This burst pressure is similar to that of native small diameter arteries (~ 2000 mmHg, ~ 300 μm wall thickness) and human saphenous vein (1680 ± 307 mmHg), the most common vessel for bypass grafting [3].

In addition to modulating mechanical properties, changing the postpolymerisation conditions or molar ratio of the monomers can also modulate the degradation rates of poly(diol citrates). Degradation has been tested through hydrolysis in PBS as well as enzyme catalysed hydrolysis in *Rhizopus delemar* lipase in PBS. After 1 week in the above lipase solution with agitation, a PDC polymer that had been polymerised at 80°C for 2 days had lost 19 ± 2.1% of its initial mass. In contrast, polymers incubated in PBS alone under agitation lost only 13 ± 1.4% of initial mass. By increasing the postpolymerisation time for an additional 2 days at 120°C under vacuum, the amount of mass lost decreased to 2.5 ± 0.5% with the above lipase solution and 1.75 ± 0.6% with PBS alone. Changing the monomer composition of POC while maintaining the same postpolymerisation conditions can also help control degradation properties while still maintaining strength. For example, the degradation rate can be decreased through the addition of excess 1,8-octanediol or increased with a slight excess of citric acid [90]. Despite the stoichiometric imbalance in the ratio of hydroxyl to carboxyl groups caused by increasing the content of one of the monomers, the materials maintain high strength (tensile stress = 2.90 ± 0.40 MPa, Young's modulus = 3.98 ± 0.09 MPa for 1.2:1.0 octanediol:citric acid, tensile stress = 3.10 ± 0.22 MPa, Young's modulus = 3.24 ± 0.26 MPa for 1.0:1.2 octanediol:citric acid) as compared with the original 1:1 monomer ratio.

In vitro cell culture results show that human aortic smooth muscle and endothelial cells cultured in low serum media can attach, proliferate and achieve confluence without surface modification. Furthermore, the proliferation rate on POC was similar to that on PLLA films [90]. The ability of endothelial cells to attach and proliferate on poly(diol citrates) is important, as a confluent, adherent and functional endothelium is vital to the long-term success of a vascular graft [92,93]. *In vivo* biocompatibility was assessed through subcutaneous implantation of polymer discs in Sprague–Dawley rats. Based on histology, there were no detectable signs of a chronic inflammatory response and the thickness of the fibrous capsule was ~ 45 μm for POC and 60 μm for PDC after 4 months. This thickness is similar to that reported for PGS and smaller than that reported for a commonly used copolymer in tissue engineering, PLGA (~ 140 μm) [2].

3. Expert opinion and conclusion

Tissue engineering research is moving into several directions in an attempt to meet the goal of controlled tissue neoformation. One of these directions will involve studying the role of scaffold mechanics and cell mechanotransduction in tissue development. Recent research has shown that the mechanical properties of the biomaterial or substrate on which cells proliferate can influence their phenotype and differentiation; therefore, potentially affecting tissue function [94,95]. Many groups have also demonstrated how mechanical conditioning regimens that involve cyclic strain of the cell-scaffold construct *in vitro* can influence the quality of the resulting tissue [4-6,8,96-100].

Recent interest in understanding how scaffold mechanics can affect cell processes within the context of tissue engineering has prompted the development of biodegradable materials with elastomeric properties. Soft tissues, such as heart, blood

vessels, heart valves, nerve, bladder and the gastrointestinal tract, should benefit from the use of biodegradable scaffolds with mechanical properties that are similar to those of native tissue. The biodegradable polyester elastomers discussed in this review are likely to become part of the available tools that tissue engineers use to design better scaffolds to promote tissue formation. These materials can be synthesised via bacterial fermentations, as in the case of PHAs or traditional chemical synthesis methods that employ biocompatible monomers. The synthesis methods may employ catalysts to generate linear copolymers based on trimethylene carbonate and/or CL. Biodegradable polyester elastomers may also be synthesised without the use of exogenous catalysts to generate crosslinked copolymers, as in the case with PGS and poly(diol citrates). The use and evaluation of these materials in tissue engineering will ultimately determine the benefits of matching the mechanical properties of the scaffold to those of the tissue it is meant to reconstruct.

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