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Biodegradable Poly(polyol sebacate) Polymers

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Abstract

We have developed a family of synthetic biodegradable polymers that are composed of structural units endogenous to the human metabolism, designated poly(polyol sebacates) (PPS) polymers. Material properties of PPS polymers can be tuned by altering the polyol monomer and reacting stoichiometric ratio of sebacic acid. These thermoset networks exhibited tensile Young's moduli ranging from 0.37 ± 0.08 to 378 ± 33 MPa with maximum elongations at break from 10.90 ± 1.37 to $205.16 \pm 55.76\%$, and glass-transition temperatures ranged from ~ 7 to 46°C . In vitro degradation under physiological conditions was slower than in vivo degradation rates observed for some PPS polymers. PPS polymers demonstrated similar in vitro and in vivo biocompatibility compared to poly (L-lactic-co-glycolic acid) (PLGA).

1. Introduction

Biodegradable elastomers have been developed to overcome problems associated with biodegradable thermoplastic materials, such as rigid mechanical properties, bulk degradation and acidic degradation products in some cases [1–3]. Thermoset elastomers such as poly (glycerol sebacate) (PGS) have shown to primarily degrade by surface erosion [4,5], retaining their structural integrity and form stability during degradation in vivo. Therefore, biomaterials based on these elastomers hold great promise in soft tissue applications that require small features, such as gecko-inspired surgical adhesives [6], microfabricated scaffolds [7,8], cardiovascular tissue engineering applications [9,10] and small diameter nerve grafts [11]. However, current PGS elastomers cover a modest range in mechanical properties and degradation rates, limiting them to relatively short-term soft tissue applications. Recently, a versatile polymer platform composed of metabolites endogenous to the mammalian organism was described using xylitol as the central monomer, yielding hydrogels and elastomers with tunable mechanical properties and in vivo degradation rates [12].

In this study, polyols were reacted with sebacic acid, yielding a family of thermoset poly(polyol sebacate) (PPS) polymers. Three general criteria led to the selection of polyols as monomers in this design: they are (1) non-toxic, (2) multifunctional to allow the formation of randomly crosslinked networks as well as a wide range of crosslink densities, and (3) allow formation

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of hydrolyzable esters in polycondensation polymerizations. The monomers of PPS polymers have the potential to be metabolized in vivo, as sebacic acid is a metabolite in fatty acid oxidation and polyols are intermediates in mammalian carbohydrate metabolism. Polyols such as xylitol, sorbitol, mannitol and maltitol are metabolized in an insulin-independent manner [13–15]. The functionality and physical properties of the different polyols influenced polymer properties. Stoichiometry further allowed for tuning chemical, physical and mechanical properties, as well as in vitro and in vivo degradation rates. The potential use of PPS polymers in a wide variety of biomedical applications was assessed through in vitro and in vivo biocompatibility tests, and compared to PLGA.

2. Materials and Methods

2.1 Synthesis and characterization of PPS polymers

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise. Appropriate molar amounts of the polyol and sebacic acid monomer were melted in a 250 mL round bottom flask at 150 °C under a blanket of inert gas, and stirred for 2 h. Vacuum (~50 mTorr) was applied for 2 – 12 h, yielding the pre-polymers poly(xylitol sebacate) (PXS) 1:1 and PXS 1:2, poly(sorbitol sebacate) (PSS) 1:1 and PSS 1:2, poly(mannitol sebacate) (PMS) 1:1 and PMS 1:2, and poly(maltitol sebacate) (PMtS) 1:4 (Scheme 1, Table 1). The pre-polymers were sized using linear polymer standards for gel permeation chromatography (GPC) using tetrahydrofuran (THF) on Styragel columns (series of HR-4, HR-3, HR-2, and HR-1, Waters, Milford, MA, USA). ¹H-NMR spectra were obtained of all pre-polymers in (CD₃)₂NCOD, on a Varian Unity-300 NMR spectrometer. The chemical composition of the pre-polymers was determined by comparing the signal integrals of the polyol, and compared to the signal integrals of sebacic acid. The signal intensities showed peaks of –OCH₂(CH(OR))_nCH₂O– at 3.5 – 5.5 ppm from the polyol, and peaks of –COCH₂CH₂CH₂– at 1.3, 1.6 and 2.3 ppm from sebacic acid. The PPS polymers were produced by another polycondensation step using 120 – 150 °C under vacuum (~2 Pa) for 4 d (see Table 2 for specific curing conditions). Attenuated total reflectance- Fourier transform infrared spectroscopy (ATR-FTIR) analysis was performed on these polymer networks using a Nicolet Magna-IR 500 spectrophotometer. The wettability of PPS polymers was determined by contact-angle measurements, and the hydration of these polymers, determined by mass differential after 24 h in ddH₂O at 37 °C. The water-in-air contact angle of polymer films was measured using the sessile-drop method and VCA2000 image analysis software (*n* = 10). Tensile tests were performed on hydrated (ddH₂O at 37 °C >24 h) dog-bone shaped polymer strips (*n* = 4) and conducted on an Instron 5542 (according to ASTM standard D412-98a) using a 50 or a 500 N load cell equipped with Merlin software. Glass transition temperatures (*T_g*) and other potential phase transitions were measured within the temperature range of –90 °C and 250 °C with a heating/cooling rate of 10 °C per minute using a Q1000 DSC equipped with Advantage Software v2.5 (TA Instruments, Newcastle, DE USA) and analyzed with Universal Analysis Software v4.3A (TA Instruments). The mass densities were measured using a pycnometer (Humboldt, MFG. CO), and crosslink density (*n*) as well as the relative molecular mass between crosslinks (*M_c*) were calculated from the following equations for an ideal elastomer [16], where *E₀* is the Young's modulus, *R* is the universal gas constant, *T* is the temperature and *ρ* is the mass density:

$$n = \frac{E_0}{3RT} = \frac{\rho}{M_c} \quad \text{Eqn. 1}$$

2.2 In vitro degradation of PPS polymers

Degradation rates via hydrolysis were observed of sol-free PPS samples ($n = 4$) continuously agitated at 37 °C in 20 mL PBS containing sodium azide (0.05 % w/v), or in 20 mL of 0.1 M NaOH at 37 °C as previously reported [17]. At designated time points, samples were removed, washed in ddH₂O, incubated in ethanol overnight, dried at 90 °C for 1 d and weighed again to determine mass loss. The mass loss was calculated from dry weight at t (M_t) and compared to the dry weight at the start of the study (M_o) using the following equation:

$$M_{Loss} = \frac{M_o - M_t}{M_o} \times 100\% \quad \text{Eqn. 2}$$

2.3 In vitro biocompatibility of PPS polymers

Glass Petri dishes (60 mm diameter, Fisher Scientific) contained 3 g of cured elastomers (120 °C, 140 mTorr for 4 d). Petri dishes prepared with a 1.5% w/v PLGA (65/35, high M_w , Lakeshore Biomedical, Birmingham, AL, USA) solution in dichloromethane at 60 uL/cm² and subsequent solvent evaporation served as control. Washes with sterile PBS were done and before the polymer loaded dishes were autoclaved. Primary human foreskin fibroblasts (HFFs) (ATCC, Manassas, VA, USA) were cultured in high glucose Dulbecco's Minimal Essential Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (Invitrogen), 100 µg/mL streptomycin (Invitrogen), and 100 U/mL penicillin (Invitrogen). Cells between passage three and six were harvested using trypsin 0.025%/EDTA 0.01% and quenched with an equal volume of medium to resuspend the cells. Additional cell systems were chosen from tissues with mechanical properties that match, or fall close to the mechanical properties of specific PPS polymers tested. A human bone cell line derived from an osteosarcoma (OS) (CRL-1545, ATCC, Manassas, VA, USA) was cultured in PMtS 1:4 coated dishes. A human muscle cell line derived from a rhabdomyosarcoma (RMS) (CCL-136, ATCC, Manassas, VA, USA) was cultured in PSS 1:1 coated dishes. Bovine articular chondrocytes (BAC) were harvested from femoropatellar grooves of 2 – 4 week-old bovine calves, as previously described [18] and cultured in PMS 1:2 coated dishes. Human umbilical vein endothelial cells (HUVECs) (Cambrex, Walkersville, MD) were cultured on PXS 1:1 laden dishes, in EGM-2 media supplemented with SingleQuot Kits (Cambrex). HUVECs were used by passage five and in accordance with the manufacturer's instructions. The fibroblasts and tissue specific cells were seeded (at 7500 cells/cm²) in PLGA- or PPS-laden dishes and were allowed to grow to a confluent cell monolayer at 37 °C and 5% CO₂, whilst imaged after 4 h and every subsequent day after initial seeding. Phase micrographs of cells were taken at 10x magnification using Axiovision software (Zeiss). For cell proliferation measurements, randomly picked areas were imaged and cells were counted and averaged. The area and circularity [19] of cell populations were calculated manually using perimeter and area measurements by using Axiovision software (Zeiss). The circularity C was calculated using the following formula:

$$C = \frac{4\pi A}{P^2} \quad \text{Eqn. 3}$$

where A is the projected area of the cell and P is the perimeter of the cell. Circularity was used as an index of cell spreading. Three distinct cell populations ($n = \sim 80$ total) were measured to find cell population means.

2.4 In vivo biocompatibility of PPS polymers

PPS discs $d = 10$ mm, $h = 1$ mm were implanted. Comparable PLGA pellets were melt-pressed (0.3g, 172 °C, 5000 MPa) into a mold ($d = 10$ mm, $h = 1$ mm) using a Carver Hydraulic Unit

Model #3912-ASTM (Carver, Inc. Wabash, IN). Two female Lewis rats (Charles River Laboratories, Wilmington, MA) weighing 200–250 grams had access to water and food *ad libitum*. Animals were cared for according to the protocols of the Committee on Animal Care of MIT in conformity with the NIH guidelines (NIH publication #85–23, revised 1985). The animals were anaesthetized using continuous 2% isoflurane/O₂ inhalation. The implants were introduced by three small midline dorsal incisions, and five polymer formulations were placed in subcutaneous pockets created by blunt lateral dissection. The skin was closed with staples. Rats were sacrificed, the implants were resected *en bloc* with surrounding tissue and fixed in formalin-free fixative (Accustain). These specimens were embedded in paraffin after a series of dehydration steps in ethanol and xylene. Sequential sections (8–15 µm) were stained with hematoxylin and eosine (H&E) and histology was evaluated by a medical doctor (JPB). Throughout the study, all rats remained in good general health as assessed by their weight gain.

2.5 Statistical analysis

Two-tailed student's t-tests with unequal variances were performed to determine statistical significance (Microsoft Excel, Redmond, WA USA). Two-way ANOVA tests were performed where appropriate (GraphPad Prism 4.02, GraphPad Software, San Diego, CA USA). Bonferroni multiple comparison post-tests were used to determine significance between specific treatments. All tabulated and graphical data is reported as mean ± S.D. Significance levels were set at $p < 0.05$ (indicated with *).

3. Results

3.1 Pre-polymer synthesis and characterization

All pre-polymers were prepared through bulk polycondensation reactions of the polyol and sebacic acid monomers (Scheme 1). The following stoichiometric ratios were prepared: PXS 1:1 and 1:2, PSS 1:1 and 1:2, PMS 1:1 and 1:2, and PMtS 1:4. Figure 1A shows a typical ¹H-NMR spectrum of a PPS pre-polymer. The chemical composition of the pre-polymers was determined by calculating the ratios of the signal integrals of the polyol to sebacic acid. ¹H-NMR revealed polymer compositions that are summarized in Table 1. In addition, weight average molecular weight (M_w), number average molecular weight (M_n) and polydispersity index (*PDI*) for all PPS pre-polymers were determined by GPC, and are shown in Table 1. No distinct, broad peaks associated with a melting temperature (T_m) could be detected with DSC for the pre-polymers, most likely due to the polydispersity of the pre-polymers. However, different temperatures were used to melt process these pre-polymers, and are listed in Table 1 as well. All polymers are clear, viscous liquids at 130 °C, and waxy, opaque materials at room temperature. The pre-polymers are soluble in common solvents such as ethanol, acetone, dimethyl sulfoxide, tetrahydrofuran, and dimethylformamide.

3.2 Characterization and physical properties of PPS polymers

The pre-polymers were thermally cured into thermoset networks under different curing conditions, as summarized in Table 2. FT-IR of the cured polymers confirmed ester bond formation in all polymers, with a stretch at 1,740 cm⁻¹ which corresponds to the ester linkages. A broad stretch was also observed at approximately 3,448 cm⁻¹ which corresponds to hydrogen bonded hydroxyl groups. The FT-IR spectrum of PMtS 1:4 illustrated an additional stretch at 1,050 cm⁻¹ compared to the spectra of the other PPS polymers, which is associated with the vibration of the ether bond within the maltitol monomer (Figure 1B).

The thermal properties of the PPS polymers were revealed by DSC: PXS 1:1, PSS 1:1 and PMS 1:1 had glass-transition temperatures below room temperature. PXS 1:2, PSS 1:2 and PMS 1:2 had glass-transition temperatures higher than the 1:1 stoichiometries, but still remained below 37 °C, indicating that these PPSs are rubbery at physiological temperatures.

PMtS 1:4 showed the highest glass transition temperature at 45 °C, demonstrating that this polymer is glassy at 37 °C (Table 2).

Increasing the sebacic acid monomer feed ratio resulted in a higher crosslink density and in lesser wettability of the polymers, as demonstrated by weight differential at equilibrium hydration, as well as contact angle measurements, as summarized in Table 2.

3.3 Mechanical properties of PPS polymers

All hydrated PPS polymers, with the exception of PMtS 1:4, showed stress versus strain plots that are typical of hydrated high – and low modulus PPS elastomers above their T_g s. Figure 2A and B show representative stress versus strain plots for the PPS polymers studied here. The average tensile Young's modulus, ultimate tensile strength (UTS) and elongation at break for the PPS polymers are summarized in Table 2. PMtS 1:4 was observed to be the stiffest material and revealed a tensile Young's modulus of 378 ± 33.0 MPa, UTS of 17.6 ± 1.30 MPa, and average elongation at break of 10.90 ± 1.37 % (Table 2, Figure 2A). PSS 1:1 was observed to be the softest material with a tensile Young's modulus of 0.37 ± 0.08 MPa, UTS of 0.57 ± 0.15 MPa and elongation at break of 192.24 ± 60.12 %. In addition, limited hysteresis was seen after 1000 cyclic compression cycles up to 50 N for the PXS 1:1 elastomer as shown in Figure 2C. Also, PPS pre-polymers are miscible and allowed for formation of co-polymers. As an example, three PXS 1:1 and PMtS 1:4 co-polymers were produced. Representative stress versus strain plots for different PXS 1:1/PMtS 1:4 w/w ratios are shown in Figure 2D: the average tensile Young's moduli of these PXS 1:1/PMtS 1:4 co-polymers were 7.25 ± 0.47 MPa, 3.94 ± 0.32 MPa and 1.61 ± 0.22 MPa for the 25/75, 50/50 and 75/25 PXS 1:1/PMtS 1:4 w/w ratios respectively.

3.4 In vitro degradation of PPS polymers

Biodegradable polyesters can degrade through hydrolysis. Therefore, the in vitro degradation under physiological conditions was investigated. Mass loss was detected for all PPS polymers (Figure 3A). After 105 d, PXS 1:1 and 1:2 revealed a mass loss of 1.78 ± 0.30 % and 1.88 ± 0.22 % respectively. PXS 1:1 did not reveal a similar mass loss profile as PSS 1:1 (15.66 ± 1.75 %) and PMS 1:1 (21.90 ± 6.99 %). PSS 1:1 and PMS 1:1 had degraded more than their corresponding 1:2 stoichiometries: PSS 1:2 had degraded 5.57 ± 1.00 % of their original mass, and PMS 1:2 degraded 9.00 ± 0.54 % at this time. PMtS 1:4 showed the least mass loss of 0.76 ± 0.30 % (Figure 3A). Although degradation under physiological conditions was observed for all PPS polymers, an additional in vitro degradation study in high pH (0.1 N NaOH) was performed as previously described [17]. Again, all polymers revealed a mass loss over a course of 50 h, and showed resemblance to what was found in the previous degradation study: at 50 h, PMtS 1:4 revealed a mass loss of 4.07 ± 2.80 %, and PXS 1:1 and 1:2 a mass loss of 4.40 ± 0.33 % and 4.24 ± 0.52 % respectively. PSS 1:1 and PMS 1:1 had fully degraded under 20 h. Also, in concert with their degradation in PBS, PSS 1:2 and PMS 1:2 degraded at a slower rate than the 1:1 stoichiometric ratios. After 50 h, 74.10 ± 0.41 % of the original mass of PSS 1:2, and 13 ± 7.45 % of PMS 1:2 remained (Figure 3B).

3.5 In vitro biocompatibility

The biocompatibility of PXS 1:1 and PXS 1:2 elastomers has been reported elsewhere [12, 20]. We therefore conducted an initial in vitro biocompatibility of the other PPS polymers with primary HFFs. Fibroblasts are important regulators of the wound-healing process in vivo. The initial attachment and subsequent proliferation into a confluent cell monolayer was compared to PLGA (Figure 4). HFFs readily attached on all but PSS 1:1 and PMS 1:1 polymers. A confluent cell layer was achieved after 5 d for all substrates, except PSS 1:2 (achieved at 7 d, data not shown). Sporadic attachment was seen on PSS 1:1 and PMS 1:1 substrates (Figure

4A, B, D). Fewer cells attached to PSS 1:1 and PMS 1:1, which did not spread nor proliferated into a confluent monolayer.

3.6 In vivo biocompatibility and degradation

The in vivo biocompatibility of PPS polymers was evaluated via subcutaneous implantation in rats. After 10 d in vivo, the acute inflammatory response was mild for all implanted polymers. The surrounding tissues did not show necrosis, nor an abundant perivascular infiltration of mononuclear cells. In addition, the fibrous capsules surrounding the PPS polymers were thin (Figure 5A–E). The assessment of the chronic inflammatory response at 12 weeks implantation revealed thicker fibrous capsules in comparison to the acute inflammatory response at 10 d (Figure 6). The fibrous capsule formation surrounding PPS polymers however, seemed similar, or less to the prevalently used PLGA at 12 weeks (Figure 6E). At this point, the foreign body response was still mild, as suggested by the thicknesses of these capsules and the presence of few visible vessels within the capsules (Figure 6A–D). The PSS 1:1 elastomer appeared to have fully degraded at this time, without detectable trace despite repetitive sectioning of the implantation area.

3.5 Initial in vitro biocompatibility analysis of PPS for tissue specific applications

In vitro attachment and subsequent proliferation into a confluent cell monolayer of tissue specific cell lines and primary cells were assessed by light microscopy (Figure 7). PMtS 1:4 revealed similar attachment and growth rate of a human osteosarcoma (OS) cell line (derived from bone) compared to PLGA. In addition, cell morphology, as assessed by circularity and cell area, was not significantly different for OS cells that were cultured on PLGA (Figure 7A i–v). A difference in cell morphology was found however, for a rhabdomyosarcoma (RMS) cell line (human muscle origin) that was cultured on PSS 1:2 substrates, and compared to PLGA (Figure 7B i). RMS cells revealed more spindle-like morphology before a confluent cell layer was achieved, as shown in Figure 7B ii–v: cell circularity was significantly less for cells cultured on PSS 1:2 ($p < 0.05$). In addition, RMS cells spread more, resulting in a larger cell area ($p < 0.05$) (Figure 7B iii). However, initial cellular attachment and subsequent cell numbers during proliferation were not different from PLGA. Cell numbers however, did reveal significant difference for primary BACs after 4 and 6 d culture on PMS 1:2 as compared to PLGA (Figure 7C i). A difference in chondrocyte morphology was also noted. Although cell area was greater for BACs cultured on PMS 1:2 ($p < 0.05$), cell circularity was not significantly different ($p > 0.05$) (Figure 7C ii–v). HUVECs cultured on PXS 1:1 exhibited attachment, growth rates and cell morphology that were comparable to PLGA substrates (Figure 7D i–v).

4. Discussion

The synthesis of PPS polymers is straightforward and does not require the use of organic solvents or cytotoxic additives. PPS polymers were produced in sub-kilogram quantities and are inexpensive. The first polycondensation step yields PPS pre-polymers that allowed processing into various scaffold geometries, after which the second polycondensation step cures the pre-polymers into a set crosslinked polymer network of desired shape. Curing conditions can be adjusted to modify crosslink densities within a modest range [12,21]. However, adjusting stoichiometry allowed for a much wider range of crosslink densities and subsequent polymer properties (Table 2). The reacting stoichiometry of sebacic acid to polyol was chosen such that the number of hydroxyl functionalities of the polyol was always greater than the number of carboxylic functionalities by 1 or 2, to ensure step-growth polymerization whilst exposing free hydroxyl groups in the polymer backbone. These free hydroxyl groups may contribute in intra-network hydrogen bond formation and may be available for functionalization chemistries, as previously shown [1,12].

Of the PPS polymers, PXS and PSS elastomers revealed comparable physical and mechanical properties, most likely because their polyol monomers have T_m s close to each other (95 and 97 °C respectively) and have similar water solubility (Table 2). Using mannitol as a monomer, polymers were produced exhibiting higher tensile Young's moduli and T_g s than PSS elastomers (Figure 2), as well as higher contact angles (Table 2) although mannitol is a stereoisomer of sorbitol. Mannitol has a higher T_m (165 °C) and a lower water solubility, which may explain the differences observed between PSS and PMS elastomers. Maltitol-based polymers (PMtS 1:4) on the other hand, showed similar contact angles to PXS 1:2 and PSS 1:2 stoichiometries, which can be rationalized if PMtS 1:4 is essentially viewed as a glucose:sorbitol:sebacate 1:1:4 polymer. However maltitol, exposing nine hydroxyl groups, allowed for a higher sebacic acid monomer feed ratio. This resulted in a higher degree of crosslinking, and therefore in a glassy polymer with a T_g above ambient and physiological temperatures, also resulting in a limited water-uptake (Figure 2, Table 2). The tensile Young's moduli of PMtS 1:4 polymers are comparable to trabecular bone (50 – 100 MPa) and may potentially be developed for bone tissue engineering or osteosynthesis applications [22]. In addition, co-polymers of PPS polymers can be produced as shown in Figure 2D. Thus, additional to the choice of polyol as well as monomer stoichiometry, co-polymerization allowed for tuning mechanical properties of PPS polymers.

The in vivo degradation mechanism PXS elastomers is dominated by surface erosion and is reported elsewhere [12,20]. In vitro degradation rates of PXS however, did clearly not correspond to in vivo degradation rates. In addition, in vitro degradation of PXS elastomers was significantly less than PSS and PMS elastomers, and comparable to the PMtS 1:4 polymer under similar conditions (Figure 3A). This hydrolysis profile was confirmed for hydrolysis in a high pH environment (Figure 3B). Interestingly, during the in vivo biocompatibility study presented here, we observed that the PSS 1:1 polymer appeared to completely degrade within 12 weeks, but had only lost $15.66 \pm 1.75\%$ of its original mass after 105 d in vitro. This in vivo mass loss of PSS 1:1 is similar to the previously observed degradation of PXS 1:1, which had an in vivo half life of 3 – 4 weeks. It is postulated that a higher crosslink density as well as the introduction of more hydrophobic entities (sebacic acid) are responsible for tuning the degradation rate. In vitro hydrolysis under physiological conditions occurred for all PPS polymers and revealed similar differences between the 1:1 and 1:2 stoichiometries, as was observed in vivo for PXS elastomers.

PPS polymers support cellular attachment with the exception of PSS 1:1 and PMS 1:1 elastomers (Figure 4). The in vitro cell attachment and proliferation studies were performed without pre-treating or coating the polymers with adhesion proteins such as fibronectin and collagen. If cellular attachment is not warranted, which can be important in some applications such as contact guidance [8,23], PSS 1:1 and PMS 1:1 can be applied. Alternatively, PSS 1:1 and PMS 1:1 elastomers could be modified with adhesion-promoting proteins and peptides by grafting them onto the exposed hydroxyl groups.

The fibrous capsules during the acute inflammatory response to PPS foreign materials seemed consistent with fibrous capsule thicknesses of previously reported values for soft thermoset elastomers (Figure 5) [2,4,12,17,20,21]. However, the fibrous capsules of the chronic inflammatory response surrounding the higher modulus materials (PMS 1:2 and PMtS 1:4), seemed more pronounced than observed for PSS 1:2 and PMS 1:1, but was still less than reported values for PLA and PLGA polymers, which are frequently reported around 400–600 microns thick (Figure 6) [2,5,24].

PPS polymers are composed of structural units that are endogenous to the mammalian metabolism, but have advantages associated with synthetic polymers. PPS polymers revealed mechanical properties that can be useful for a variety of implantable medical devices. As an

example, human ulnar metacarpophalangeal thumb joint ligament has a UTS and a Young's modulus of 11.4 ± 1.2 MPa and 37.3 ± 5.1 MPa respectively [25], and several human cervical spinal components such as intervertebral discs, as well as their associated ligaments, have mechanical properties [26] that fall within the limits of the PPS polymer platform shown here. This is also true for softer tissues such as nerves [27] and blood vessels [28]. Therefore, PPS polymers potentially offer a material platform for surgical procedures and tissue engineering applications.

5. Conclusions

PPS polymers are synthetic in nature but have the advantage of being composed of structural units endogenous to the human metabolism. We have presented seven polymers in this report. Chemical, physical, and mechanical properties as well as degradation rates of these polymers can be tuned by altering the polyol and stoichiometry of the reacting sebacic acid. Potentially, many co-polymers and composite materials are possible, resulting in a considerable number of polymers accessible through the synthetic scheme presented here. PPS polymers exhibited comparable biocompatibility to materials approved for human use, such as PLGA.

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A **B** **Figure 1.**

(A) A representative ^1H -NMR spectrum of PPS polymers, PMS 1:1 in this case. Signal intensities of the polyols at 3.5 – 5.5 ppm, and of sebacic acid in the polymer were identified at 1.2, 1.5, 2.2 ppm by hydrogens on the carbons labeled 'a' – 'b', and 'c' – 'e' respectively.

(B) FTIR analysis of PPS polymers.

Figure 2.

(A) Typical tensile stress versus strain curves of low Young's modulus PPS elastomers (PXS 1:1 and PSS 1:1). (B) Typical tensile stress versus strain curves of PPS elastomers with higher Young's moduli. (C) First and last 10 cyclic compression cycles of a 1000 times to 50 N on PXS 1:1. (D) Co-polymers composed of low- and high modulus PPS polymers, PXS 1:1 and PMtS 1:4 respectively, with 25/75, 50/50 and 75/25 PXS/PMtS w/w ratios.

A **B** **Figure 3.**

(**A**) Degradation of PPS polymers in PBS at 37 °C for 105 d. (**B**) Degradation of PPS polymer in 0.1 N NaOH.

A

Figure 4.

(A) Attachment and proliferation of HFFs on PPS polymers. The (*) indicates significant difference ($p < 0.05$) to the other PPS polymers for that time point. Representative phase contrast micrographic images of HFFs on PSS 1:1 (B), PSS 1:2 (C), PMS 1:1 (D), PMS 1:2 (E), PMtS 1:4 (F), and PLGA (G). Images are 10x magnification. Bars represent 150 μm .

Figure 5.

Representative images of H&E stained sections demonstrating the acute inflammatory response to subcutaneous implanted PPS polymers. After 10 d, (A) PSS 1:1, (B) PSS 1:2, (C) PMS 1:1, (D) PMS 1:2, (E) PMtS 1:4 revealed mild inflammatory responses as compared to (F) PLGA. Images are 10x and bars represent 200 μ m. C = fibrous capsule, S = skin and M = muscle.

Figure 6.

Representative images of H&E stained sections demonstrating the chronic inflammatory response to subcutaneous implanted PPS polymers, 12 weeks after implantation. The PSS 1:1 elastomer had completely degraded at this time. (A) PSS 1:2, (B) PMS 1:1, (C) PMS 1:2, (D) PMtS 1:4 and (E) PLGA. Images A – D are 20x and bars represent 100 µm. Image E is 10x and bar represents 200 µm. C = fibrous capsule and M = muscle.

Figure 7.

Initial in vitro analysis of PPS polymers for musculoskeletal tissues. **(A)** Attachment and subsequent proliferation of an OS cell-line derived from human bone on PMtS 1:4, compared to PLGA **(i)**. OS cell morphology was quantified by cell area (A in μm^2) **(ii)** and circularity **(C)** **(iii)**. In addition, representative phase-contrast images of OS cells on PLGA **(iv)** and on PMtS 1:4 **(v)** are shown. **(B)** Attachment and proliferation of RMS cells derived from human muscle on PSS 1:2 and compared to PLGA **(i)**, as well A **(ii)** and C **(iii)** (* $p < 0.05$). Representative images of RMS on PLGA **(iv)** and PSS 1:2 **(v)** also revealed a difference in morphology. **(C)** Seeding of primary BACs on PMS 1:2 and PLGA revealed a similar attachment but different cell numbers after 4 and 6 d **(i)** (* $p < 0.05$). A **(ii)**, but not C **(iv)** showed a difference between the polymers (* $p < 0.05$). Representative images of BACs on PLGA **(iv)** and PMS 1:2 **(v)** revealed cell number difference at 6 d. **(D)** HUVECs attached and proliferated on PXS 1:1 elastomers similar to PLGA **(i)**, and revealed a comparable cell shape **(ii)** **(iii)**, also demonstrated by phase contrast images of HUVECs on PLGA **(iv)** and PXS 1:1 **(v)**. All phase contrast images are 10x, bars represent 50 μm .

Scheme 1.

General synthetic scheme of polyol-based polymers. Xylitol (**1**), sorbitol (**2**), mannitol (**3**) and maltitol (**4**) were polymerized with sebacic acid (**5**) in different stoichiometries, yielding PXS (**6**), PSS (**7**), PMS (**8**) and PMtS (**9**). Simplified representations of the polymers are shown.

Table 1

Polycondensation conditions, composition, melting temperatures and molecular weight distributions of PPS pre-polymers. T_m was the observed temperature below which a transition between opaque waxy polymers to viscous clear liquid polymers was observed. M_n ; number average molecular weight. M_w ; weight average molecular weight. PDI ; polydispersity index (M_w/M_n).

Pre-polymer	Composition by ¹ H-NMR	T_m^a (°C)	M_w (g/mol)	M_n (g/mol)	PDI
PXS 1:1	1.10 : 1.00	~80	2443	1268	1.9
PXS 1:2	1.08 : 2.00	~100	6202	2255	2.7
PSS 1:1	0.91 : 1.00	~80	6093	3987	1.5
PSS 1:2	0.89 : 2.00	~100	23013	8990	2.6
PMS 1:1	0.99 : 1.00	~100	3182	2038	1.6
PMS 1:2	1.06 : 2.00	~120	10097	4379	2.3
PMS 1:4	1.18 : 4.00	~130	13265	2992	4.4

^a T_m s are temperatures where the polymer revealed a transition from a white, opaque wax to a clear flowing liquid.

Table 2

Curing conditions, physical and mechanical properties of PPS polymers. M_c : the molecular weight between crosslinks, and n : crosslink density, both calculated with Eqn. 1 (Materials and Methods).

Polymer (curing condition)	Young's Modulus (MPa)	Ultimate Tensile Stress (MPa)	Ultimate Elongation (%)	Contact Angle (°)	T_g (°C)	Hydration by Mass (%)	ρ (g/cm ³)	n (mol/m ³)	M_c (g/mol)
PXS 1:1 (120 °C, 2 Pa, 4 d)	0.82 ± 0.15	0.61 ± 0.19	205.16 ± 55.76	26.5 ± 3.6	7.3	5.10 ± 0.12	1.18 ± 0.02	112.2 ± 30.5	10517.4 ± 102.1
PXS 1:2 (120 °C, 2 Pa, 4 d)	5.33 ± 0.40	1.43 ± 0.15	33.12 ± 4.85	52.7 ± 5.7	22.9	1.74 ± 0.1	1.16 ± 0.02	729.32 ± 57.3	1585.1 ± 43.7
PSS 1:1 (120 °C, 2 Pa, 5 d)	0.37 ± 0.08	0.57 ± 0.15	192.24 ± 60.12	9.6 ± 3.0	18.1	6.28 ± 0.27	1.13 ± 0.04	50.6 ± 10.3	22320.1 ± 85.3
PSS 1:2 (120 °C, 2 Pa, 4 d)	2.67 ± 0.12	1.16 ± 0.33	65.94 ± 24.87	36.6 ± 3.1	26.9	1.80 ± 0.09	1.16 ± 0.02	365.3 ± 21.5	3175.2 ± 132.1
PMS 1:1 (140 °C, 2 Pa, 5 d)	2.21 ± 0.21	0.79 ± 0.10	50.54 ± 9.01	32.2 ± 9.0	16.5	4.45 ± 0.14	1.18 ± 0.02	302.4 ± 60.2	3902.2 ± 109.1
PMS 1:2 (140 °C, 2 Pa, 5 d)	12.82 ± 2.90	3.32 ± 0.76	44.99 ± 11.81	40.4 ± 9.3	32.2	1.82 ± 0.15	1.16 ± 0.03	1754.2 ± 242.4	661.3 ± 219.2
PMS 1:4 (150 °C, 2 Pa, 5 d)	378.0 ± 33.0	17.64 ± 1.30	10.90 ± 1.37	26.3 ± 8.4	45.6	1.40 ± 0.03	1.18 ± 0.01	n/a	n/a