A comparative study on poly(xylitol sebacate) and poly(glycerol sebacate): mechanical properties, biodegradation and cytocompatibility

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Abstract
In order to develop degradable elastomers with a satisfactory combination of flexibility and enzyme-mediated degradation rate, the mechanical properties, enzymatic degradation kinetics and biocompatibility of poly(xylitol sebacate) (PXS) has been systematically investigated in comparison with poly(glycerol sebacate) (PGS). Under the same level of crosslinked density, the PXS elastomer networks have approximately twice the stretchability (elongation at break) of their PGS counterparts. This observation is attributable to the relatively longer and more orientable xylitol monomers, compared with glycerol molecules. Although xylitol monomers have two more hydroxyl groups, we, surprisingly, found that the hydrophilic side chains did not accelerate the water attack on the ester bonds of the PXS network, compared with their PGS counterpart. This observation was attributed to a steric hindrance effect, i.e. the large-sized hydroxyl groups can shield ester bonds from the attack of water molecules. In conclusion, the use of polyols of more than three –OH groups is an effective approach enhancing flexibility, whilst maintaining the degradation rate of polyester elastomers. Further development could be seen in the copolymerization of PPS with appropriate thermoplastic polyesters, such as poly(lactic acid) and polyhydroxyalkanoate.

(Some figures may appear in colour only in the online journal)

1. Introduction
The regeneration of mechanically functional soft tissues, such as colon, skin, and muscle, demands very soft biomaterials that can both sustain and recover from various deformations without causing mechanical irritation to the surrounding tissues. The range of Young’s moduli for some living tissues are, for example, 0.01–0.5 MPa for muscle and 0.7–16 MPa for skin [1–3]. Flexible elastomeric polymers (elastomers) that can provide sustainable elasticity and structural integrity to tissues and organs are mechanically more advantageous than thermoplastic (non-elastomers) polymers for soft-tissue engineering. Since the publication of a seminal work on poly(glycerol sebacate) (PGS) in 2002 [4], there have been an increasing number of research studies on the development of biodegradable elastomeric biomaterials for soft-tissue engineering applications [3, 5, 6]. Poly(polyol sebacate) (PPS) is a family of ester-bonded crosslinked elastomers developed for medical applications [6–9]. These polymers are biocompatible, biodegradable, inexpensive [10] and have already shown potential for applications in nerve [11], vascular and myocardial tissue engineering [12–15]. A polyol is an alcohol containing multiple hydroxyl groups (e.g., glycerol,
mannitol, sorbitol and xylitol). Sebacic acid, a dicarboxylic acid with the structure (HOOC(CH₂)₁₆COOH), is a naturally occurring chemical derived from castor oil, which has been proven safe in vivo [16–20].

PGS is the most widely studied PPS member [4, 8, 21–24]. The Young’s modulus of PGS is in the range of 0.05–1.5 MPa [25]. In general, flexible PGS with a low Young’s modulus (<0.5 MPa) has good stretchability (i.e. rupture strains of over 100%), but degrades rapidly (4–6 weeks) [26] and exhibits cytotoxicity in vitro due to the acidic by-products of its degradation [25]. The rapid degradation kinetics of soft PGS has impeded any possible application as a scaffolding material for tissues that have healing rates of several months or years (e.g., muscle) [26, 27]. Although a slow degradation rate, and thus satisfactory cytocompatibility, can be achieved in PGS by increasing the crosslink density of the network, such PGS is brittle with significantly compromised elasticity [28]. Thus it is difficult to achieve a slow degradation rate in pure PGS without compromising the flexibility and stretchability of the network. Indeed, an elastomer, which is as soft as muscle, which degrades slowly and has a satisfactory cytocompatibility, has yet to be developed.

A potential approach to enhance the stretchability of PPS-based elastomers is the use of relatively longer polyol monomers (such as xylitol), which can space out crosslinks and thus enhance the flexibility of the polymer network, compared with their PGS counterparts. However, long polyol monomers have more than three hydroxyl groups, and so the effect of the extra –OH groups on the hydrolysis of the PPS network is an open question. On the one hand, the hydroxyl groups are hydrophilic and thus may enhance water absorption of the network. On the other, the large sized –OH groups can have a steric hindrance effect on the hydrolysis of the ester bonds of the network [29]. Hence, it is necessary to carry out an investigation on the effect of long polyol monomers on the elastic mechanical properties and hydrolysis of PPS networks. The aim of this work, therefore, was to investigate whether the mechanical properties, enzymatic degradation rates and cytotoxicity) of PPS-based elastomers. To this end, enhance the flexibility and reduce the hydrolytic rate (as extra –OH groups of polyol monomers could simultaneously

2.2. Synthesis

According to previously published methods [7, 8, 30], PGS and poly(xylitol sebacate) (PXS) can be synthesized in two steps. A pre-polymer was first synthesized by polycondensation of an 1:1 molar ratio of either glycerol or xylitol (purity 99%) mixed with sebacic acid (purity 99%). The monomers reacted at 130 °C for 24 h under a nitrogen gas purged at a flow rate of ∼130 cm³ min⁻¹. The produced pre-polymer was then dissolved in tetrahydrofuran (THF) at a concentration of 50 vol.%. The PGS/THF or PXS/THF solution was then cast onto glass slides, and the THF was evaporated in ambient conditions. The above cast sheets were then cured at 130 °C under vacuum for 1 to 12 days, followed by cooling to room temperature. Finally, PGS or PXS sheets of ∼0.5 mm in thickness were peeled off the glass slides.

2.3. Determination of the esterification degree in PGS and PXS

In this work, the degree of reaction was defined to be the percentage of the esterified carboxylic groups in the PPS material system. The degree of esterification was determined by two methods: the measurement of water (an esterification by-product) and an acid group titration of the PPS polymers. In the first method, the reactant chemicals were weighed before and after the reaction, and the weight loss during the polycondensation synthesis was assumed to be the amount of water by-product. The percentage of carboxylic acid converted to sebacate ester was then given by

\[
\text{Reaction} \% = \frac{m_{H_2O}}{m_{\text{initial}}} \times 100
\]

where \(m_{H_2O}\) is the total weight of the monomer mixture (1:1) before synthesis, \(m_{\text{initial}}\) is the weight of water evolved, \(M_{\text{sebacate}}\) is the molecular weight of sebacic acid, \(M_{\text{polyol}}\) is the molecular weight of polyol (either glycerol or xylitol) and \(M_{H_2O}\) is the molecular weight of water.

In the titration method [31], 1 g specimens were dissolved (prepolymer) or swelled (powder of crosslinked polymer) in an ethanol (25 wt.%)/toluene (75 wt.%) mixture, in a flask sealed with Parafilm™ film. The crosslinked polymers were ground into a powder and soaked in the solvent for 24 h to achieve a high degree of swelling. The carboxylic groups of these dissolved prepolymers or highly swollen elastomers were then titrated with a standard 0.1 M solution of potassium hydroxide in ethanol. The bromothymol blue solution (ten drops) was used as a pH indicator and the endpoint of the titration was indicated by the change of colour in the solution from yellow to bluish-green. For the crosslinked specimens, the endpoint of titration was seen to be reached when the indicator colour remained bluish-green for 1 h. The reaction percentage was given by

\[
\text{Reaction} \% = \left(1 - \frac{N_{\text{unreacted-COOH}}}{N_{\text{reacted-COOH}}} \right) \times 100
\]

\[= \frac{m_{H_2O} \times C/m_{H_2O}}{2/(M_{\text{sebacate}} + M_{\text{polyol}})} \times 100\]

2.4. Materials

The raw materials of PGS and PXS, i.e. glycerol (purity 99%), xylitol (purity 99%) and sebacic acid (purity 99%), and all solvents were purchased from Sigma-Aldrich® (Castle Hill, Australia). Purasorb® PDL 20 is a poly (D,L-lactic acid) (PDLLA, PuraBiochem, Gorinchem, the Netherlands) purchased with an inherent viscosity of 2.0 dl g⁻¹. Purasorb® PDLLA was completely amorphous and was used without further purification. The esterase of porcine liver was purchased from Fluka, Sigma-Aldrich® (Castle Hill, Australia).
where \( N_{\text{unreacted-COOH}} \) is the number of unreacted –COOH groups, \( N_{\text{eqal-COO}} \) is the total number of carboxylic groups, \( V_1 \) is the volume in litres of 0.1 M KOH used in the titration process, \( V_0 \) is the volume for the blank test (without a specimen), \( C \) is the concentration of KOH (0.1 M) and \( m_0 \) is the mass of the tested specimen.

2.4. Mechanical properties

Dog-bone-shaped specimens of dimensions \( 12.5 \times 3.25 \times t \) mm (length \( \times \) width \( \times \) thickness, \( t = \sim0.5 \) mm) were punched out of the PP sheet materials. The tensile testing was performed with an Instron 5860 mechanical tester, the testing conditions being room temperature, a cross-head speed of 10 mm min\(^{-1}\), and a 100-N load cell [32]. For soft tissues (e.g. cardiac muscle), the maximum strain \( \varepsilon \) in vivo is typically \( \sim15\% \) [33]. Hence, the mechanical properties of specimens at strains lower than 15% are relevant to the clinical applications. Because the samples of PPS are in the rubbery state, their stress–strain behaviour can be described by [34]

\[
\sigma = \nu RT \left( \lambda - \frac{1}{\lambda^2} \right) \approx 3\nu RT \varepsilon, \tag{3}
\]

where \( \sigma \) is the engineering stress, \( \nu \) is the strand density, \( R \) is the universal gas constant, \( T \) is the absolute temperature, \( \lambda = 1 + \varepsilon \) is the extension ratio and \( \varepsilon \) is the tensile strain. At low strains of \( \lesssim10\% \), linearized equation (3) only causes an error of \(<8\%\). Therefore, the Young’s modulus of each specimen in this work was determined by the \( \sigma-\varepsilon \) curves up to a strain of 10%.

2.5. Water swelling of PGS and PXS

The aim of this experiment was to compare the diffusion rates of water molecules in the two PPS networks, which have the same degree of crosslink density. The water-vapour-swollen specimens were prepared by placing them in a sealed container above distilled water at 37 °C, in which a 100% relative humidity was established. The swollen specimens were removed from the above sealed container at various intervals and surface water was removed with filter paper. After the mass of the swollen samples \( (m_{\text{eq}}) \) was measured in a sealed vial, the specimens were dried in a vacuum oven at room temperature for one week and weighed again to determine their mass in the dry condition \( (m_0) \). The weight swelling percentage was calculated as

\[
\frac{m_{\text{eq}} - m_0}{m_0} \times 100. \tag{4}
\]

2.6. Enzymatic degradation

The experimental procedures were published previously [27]. Briefly, the experiment was conducted using 48-well tissue culture plates. Discs (diameter of 11 mm) of the PPS polymers were weighed (giving a mass \( m_{\text{eqal}} \), sterilized in 70% alcohol, and dried in a tissue culture hood. Each specimen was then soaked in 0.5 ml culture medium in a well, with the addition of esterase at a concentration of \( \sim1.5 \) units of esterase per mm\(^2\). These culture plates were placed in an incubator at 37 °C, with the esterase being added every day. At intervals of 1, 2, 3 and 4 weeks, the specimens were removed and weighed (giving a mass \( m_{\text{eq}} \) after which they were washed with water and dried under vacuum at room temperature. The weight change percentage was calculated by using

\[
\text{Weight loss} (\%) = \frac{m_{\text{eq}} - m_{\text{eqal}}}{m_{\text{eqal}}} \times 100. \tag{5}
\]

where \( m_{\text{eqal}} \) is the initial dried mass, and \( m_{\text{eq}} \) is the vacuum dried weight, measured after incubation.

2.7. Cytocompatibility in vitro (ISO 10993)

A standard cytotoxicity assessment method set by the International Organization of Standardization (ISO 10993) was used. Similar procedures have been published elsewhere [35, 36]. Briefly, extractant media are prepared by soaking the test or control materials in standard cell culture media (10% foetal calf serum, 1% L-glutamine and 0.5% penicillin/streptomycin) at a concentration of 0.2 g of material per ml of culture medium for 24 h at 37 °C and 5% CO\(_2\) conditions in an incubator. PDLLA was used as the control material in this work. The material-free culture medium was the negative control. Mouse fibroblasts, SNL (STO-Neo-LIF, purchased from the University of California, Davis, USA) were seeded in standard media at a density of \( \sim2000\) cells/well in 96-well plates. When 70% confluence of the cells was established (at day 3), the medium was entirely replaced with the extractant medium. The above cultures were then placed into an incubator for an additional two days. At the end of the incubation period, the quantification of the cytotoxicity was performed using a commercial kit, Tox-7 (Sigma-Aldrich). Spent culture media were collected, and the lactate dehydrogenase (LDH) levels in the media were determined (DEAD LDH). Finally, living cells of each well were treated with a lysis solution of Tox-7, and the LDH levels per well were determined (LIVE LDH). The cytotoxicity can be expressed as

\[
\text{Percentage of dead cells} (\%) = \frac{\text{DEAD LDH}}{\text{DEAD LDH} + \text{LIVE LDH}} \times 100. \tag{6}
\]

2.8. Cell proliferation

Cell proliferation was assessed using a commercial AlamarBlue™ assay kit (Life Technologies). Culture media wells were seeded with SNL fibroblasts (2500 cells per ml) in each well of a 48-well plate and cultured in the presence of sterilized PGS or PXS (test material) or sterilized PDLLA discs (material control). After culture for 48 h, 0.1 ml of the AlamarBlue™ indicator was added to each well (except for the background controls) and incubated for a further 5 h. The medium was then transferred to a new plate and measured by the UV of the plate reader at wavelengths of 570 and 600 nm. This procedure was repeated every 48 h until confluence was reached. Cell proliferation was quantified by the percentage
The mechanical properties of a polyester network are directly related to the degree of esterification. Table 1 provides the degrees of esterification have been estimated by the mass loss of the water by-product and the titration of acid groups. PPS materials were pre-polymerized at 130 °C for one day and cured at 130 °C for up to 12 days.

| Polymers and Pre-polymerization conditions | Curing time at 130 °C (day) | Percentage of esterification (%) | Molar fraction of evaporated polyol (%) |
|------------------------------------------|-----------------------------|----------------------------------|---------------------------------------|
|                                         |                             | By mass loss (P₁)                | By titration for –COOH (P₂)            |                                      |
| PGS 130 °C/1 day                         | 0                           | 77.8 ± 0.2                       | 74.3 ± 0.9                            | 1.4                                   |
|                                         | 2                           | 143 ± 12                         | 90.7 ± 0.2                            | 20                                    |
|                                         | 3                           | 154 ± 8                          | 94.3 ± 0.1                            | 22                                    |
|                                         | 4                           | 166 ± 6                          | 95.5 ± 0.1                            | 23                                    |
|                                         | 7                           | 172 ± 4                          | 95.5 ± 0.1                            | 28                                    |
| PXS 130 °C/1 day                         | 0                           | 65.2 ± 8                         | 64.5 ± 10.8                           | 0                                     |
|                                         | 2                           | 100 ± 9                          | 86.8 ± 0.2                            | 3                                     |
|                                         | 3                           | 127 ± 12                         | 92.4 ± 0.1                            | 8                                     |
|                                         | 4                           | 146 ± 14                         | 93.0 ± 0.1                            | 13                                    |
|                                         | 7                           | 162 ± 7                          | 97.0 ± 0.1                            | 15                                    |
|                                         | 9                           | 165 ± 6                          | 98.0 ± 0.1                            | 16                                    |
|                                         | 12                          | 165 ± 6                          | 98.0 ± 0.1                            | 16                                    |

Reduction of AlamarBlue™ using the following equation [37]:

\[
\text{\% Reduced} = \left(\frac{\varepsilon_{\text{OX}}(\lambda_2)A(\lambda_1) - \varepsilon_{\text{OX}}(\lambda_1)A(\lambda_2)}{\varepsilon_{\text{RED}}(\lambda_1)A(\lambda_2) - \varepsilon_{\text{RED}}(\lambda_2)A(\lambda_1)}\right) \times 100,
\]

(7)

where \( A(\lambda_1) \) and \( A(\lambda_2) \) are the absorbance values of test wells measured at wavelengths \( \lambda_1 \) = 570 nm and \( \lambda_2 \) = 600 nm, respectively, and \( A'(\lambda_1) \) and \( A'(\lambda_2) \) are the values of absorbance at the same wavelengths for negative control wells containing only culture medium and AlamarBlue™. The other parameters are as follows: \( \varepsilon_{\text{OX}}(\lambda_1) = 80.586 \), \( \varepsilon_{\text{OX}}(\lambda_2) = 117.216 \), \( \varepsilon_{\text{RED}}(\lambda_1) = 155.677 \) and \( \varepsilon_{\text{RED}}(\lambda_2) = 14.652 \).

2.9. Statistical analysis

All experiments were performed with five specimens per experimental group, and the data were shown as the mean ± standard error (SE). One-way analysis of variance with Tukey’s post-hoc test was performed to analyse any significant differences and any p-value less than 0.05 was considered to be significant.

3. Results and discussion

3.1. Degree of esterification

The mechanical properties of a polyester network are directly determined by the levels of polymerization and crosslinking, i.e. the degree of esterification. Table 1 provides the degrees of reaction in the PGS and PXS pre-polymers and the crosslinked PGS and PXS, determined by two methods: measurement of mass loss and titration of carboxylic acid groups. It was found that the percentages of the esterified carboxylic group in the PPS prepolymers (i.e. cured for zero days) determined using the two methods were consistent for both PGS and PXS, being ∼75% and ∼65%, respectively (table 1). However, for the crosslinked PPS polymer, the percentages of reaction determined using the two methods were significantly different. The nominal reaction percentage of over 100% measured by the ‘water’ loss indicated the evaporation of volatile polyol. During the crosslinking treatment of PGS and PXS, the melted polymer sheets exposed a large surface area under vacuum, which increased the evaporation of xylitol, a volatile component. Thus, the mass loss measured actually contained both the water by-product and evaporated glycerol or xylitol monomers, and resulted in an over-estimated percentage from equation (1), which was formulated under the assumption that the weight loss during the crosslinking treatment was the amount of water by-product.

The loss of polyl during polymerization can be calculated from the nominal percentage of reaction (\( P₁ \)) determined from mass loss and ‘actual’ percentage of reaction (\( P₂ \)) determined by the titration technique. The difference between \( P₁ \) and \( P₂ \) is given by

\[
P₁ - P₂ \% = \left[ \frac{(m_{\text{H}_2\text{O}} + m_{\text{xylitol}})/M_{\text{H}_2\text{O}}}{2 \times m/(M_{\text{sebacic}} + M_{\text{polyol}})} - \frac{m_{\text{H}_2\text{O}}/M_{\text{H}_2\text{O}}}{2 \times m/(M_{\text{sebacic}} + M_{\text{polyol}})} \right] \times 100
\]

\[
= \left[ \frac{m_{\text{xylitol}}/M_{\text{H}_2\text{O}}}{2 \times m/(M_{\text{sebacic}} + M_{\text{polyol}})} \right] \times 100.
\]

Then the molar percentage of lost xylitol is given by

\[
\text{Loss of polyl}\% = \frac{m_{\text{polyl}}/M_{\text{polyl}}}{m/(M_{\text{sebacic}} + M_{\text{polyol}})} \times 100
\]

\[
= \frac{2 \times M_{\text{H}_2\text{O}}(P₁ - P₂)}{M_{\text{polyl}}}. \quad (9)
\]

The results (table 1) revealed that the loss of polyl during the synthesis of pre-polymer could be disregarded. However, the evaporation of the polyl was severe during the crosslinking treatment of the cast thin sheets, and the loss of glycerol (small molecules) was higher than xylitol (relatively large molecule), with the maximum loss percentage being 28 and 16%, respectively. The above results were attributable to the processing conditions, described as follows. During the pre-polymerization process, a relatively large amount (~30 cm²) surface area to the vacuum environment in the reaction container, whereas a thin sheet (~1 g) exposed a very limited surface area (~20 cm²) during the curing process. This issue remains to be addressed.

The titration method produced reasonable results (table 1). The results showed that the percentage of esterified carboxylic groups determined from mass loss and titration of carboxylic acid groups were consistent for both PGS and PXS, being ∼75% and ∼65%, respectively (table 1).
groups in PGS increased steadily during the first three days of crosslink treatment and reached a saturation plateau of 95% by the end of four-days of curing, after which there was no considerable increment in the reaction percentage. With the PXS system, a saturation plateau of 97% was reached by the end of seven-days of curing, after which there was no considerable increment in the reaction percentage.

3.2. Mechanical properties of PGS and PXS

3.2.1. Effects of curing time on mechanical properties. As a typical elastomer, no stress whitening or plastic deformation artefacts were visually observed during the tensile testing. The shifting profiles of the stress–strain curves of PGS and PXS with curing time are demonstrated in figure 1. Figure 1 indicates that the PXS network collectively produced a wider range of mechanical properties than the PGS, including Young’s modulus, ultimate tensile strength (UTS) and elongation at break. The maximal UTS and elongation at rupture, for example, were ~2.5 MPa and >750% in the PXS group, whereas these properties were ~1.5 MPa and ~500% in the PGS group.

As expected, the average values of UTS and Young’s moduli ($E$) of the PGS and PXS polymers increased with curing time ($p > 0.05$), whereas the strain at break ($\varepsilon_{\text{max}}$) decreased, as shown in figure 2. The values of Young’s modulus and UTS of PGS and PXS plateaued after curing for four and seven days, respectively, which is consistent with the results of reaction degrees (table 1). The Young’s moduli of the PXS polymers (0.05–2.0 MPa) had a wider range than that of the PGS polymers (0.05–1.5 MPa) (figure 2(a)). However, the PXS polymers collectively exhibited significantly larger strains at break than the PGS polymers (figure 2(b)). After treatment for crosslinking at 130 °C for four (PGS) or seven (PXS) days, for instance, the PGS and PXS polymers had a similar Young’s modulus (1.2–1.5 MPa). However, the PXS polymer showed a rupture strain of ~200%, whereas the breaking strain value of the PGS counterpart was ~100%. The enhanced stretchability of PXS is primarily attributed to the relatively longer and more orientable xylitol monomers, compared with glycerol molecules.

The UTS and $\varepsilon_{\text{max}}$ of PGS and PXS samples synthesized under different reaction conditions are plotted in figure 3 against the strand density calculated from the rubbery modulus.
using equation (3). The tensile strength linearly rises as the strand density increases (figure 3(a)), while the elongation to break decreases monotonically following a power law (figure 3(b)). Taylor and Darin [38] have derived a theory for the dependence of the extension ratio at break ($\lambda_{\text{max}} = 1 + \varepsilon_{\text{max}}$) and UTS on the strand density:

$$\lambda_{\text{max}} = k_1 v^{-0.5},$$  \hspace{1cm} (10)

$$\text{UTS} = k_2 v \left\{ 1 - \frac{1}{(1 + k_3 \lambda_{\text{max}})^{0.5}} \right\}$$

$$\approx k_2 v \quad \text{when} \ \lambda_{\text{max}} \text{ is high (i.e. UTS is low),}$$  \hspace{1cm} (11)

where $v$ is the strand density (calculated by equation (3)), and the exponent $n$ has a theoretical value of 0.5. $k_1$, $k_2$ and $k_3$ are constants determined by the polymer network structure, i.e. molecular weight of the uncrosslinked polymer and molecular weight of the vulcanizate per crosslinked unit [38]. In general, $k_1$ indicates stretchability (elongation) and $k_2$ reflects the resistance of the network against deformation (strength). The PXS network has higher values in both $k_1$ and $k_2$ than PGS, indicating that PXS gains a better stretchability without compromising its deformation strength. This theory is based on the concept that the UTS is determined by the extent of strand orientation during deformation, and that $\lambda_{\text{max}}$ is determined from the average Gaussian end-to-end distance of the network strands (proportional to the square root of the number of chain bonds) and the extended strand length (proportional to the number of chain bonds). In figure 3(a) the UTS data are found to be approximately linear with strand density in agreement with the prediction of Taylor and Darin [38]. The values of $\varepsilon_{\text{max}}$ for the PGS and PXS samples versus the strand density in figure 3(b) fit to a power law with exponents of 0.541 and 0.515 respectively, with a 95% confidence interval being (0.48, 0.52) and (0.49, 0.51), respectively.

Figure 3. Plots of (a) UTS and (b) elongation at break versus strand density (calculated from $E$) and linear fit analysis using Origin software. The PGS and PXS materials were pre-polymerized at 130 °C for one day, and treated for crosslinking at 130 °C for up to seven and 12 days, respectively.

More importantly, figure 3(b) consistently shows that with the same crosslink density PXS is more stretchable than its PGS counterparts; with the rupture strain value of PXS being double that of PGS, especially at the high end of strand density. The higher UTS (figure 3(a)) and greater rupture elongation (figure 3(b)) of PXS indicated that PXS can offer a more reliable elastic performance than the PGS counterpart with similar Young’s modulus. Moreover, to achieve the same stretchability (e.g. 200% elongation at break), the PXS network can have a much higher crosslink density (i.e. 250 mole cm$^{-3}$), whereas the strand density of the PGS network has to remain lower than 50 mole cm$^{-3}$ (figure 3(b)). Slow degradation rates of elastomers are directly determined by the high crosslink density of the network. Hence, it is possible with PXS to achieve significantly reduced degradation kinetics without compromising elastic stretchability, compared with PGS, as described in section 3.3.

Figure 4. Swelling ratios of PGS and PXS versus swelling time in (a) THF and (b) water vapours. The PGS and PXS materials were pre-polymerized at 130 °C for one day, and treated for crosslinking at 130 °C for up to 1.5 and 2 days, respectively. The strand densities in the two polymers were ~25 mole m$^{-3}$. 

(a) Swelling in THF vapor

| Time (h) | PGS | PXS |
|---------|-----|-----|
| 0       | 10  | 10  |
| 5       | 20  | 22  |
| 10      | 30  | 35  |
| 15      | 40  | 45  |
| 20      | 50  | 55  |

(b) Swelling in H2O vapor

| Time (h) | PGS | PXS |
|---------|-----|-----|
| 0       | 10  | 10  |
| 5       | 20  | 22  |
| 10      | 30  | 35  |
| 15      | 40  | 45  |
| 20      | 50  | 55  |
Figure 5. The weight loss of the PGS and PXS polymers after incubation at 37 °C in the tissue culture medium with the addition of esterase (0.3 units of enzyme per mg of biomaterial) for up to 35 days. The PGS and PXS materials were pre-polymerized at 130 °C for one day and treated for crosslinking at 130 °C for seven and nine days, respectively.

3.3. Diffusion rates of water and THF molecules in PGS and PXS

The level of water and THF absorption in highly crosslinked PPS is very low, to the extent that the swelling measurement cannot detect any considerable difference in water and THF diffusion in the PGS and PXS networks. Hence, the PGS and PXS samples of low crosslink density were selected for the studies described in this section. At the same level of crosslink density (∼25 mole m⁻³ in the present study), the PGS network became swollen slower than that of PXS in THF (figure 4(a)). The above observation is attributable to the hydrophobic nature of THF molecules and the hydrophilic properties of the PGS and PGS networks, i.e. the PXS networks are more hydrophilic than those of the PGS due to the increased number of –OH groups in the former. It is thus not surprising to observe that the level of water absorption was higher in the PXS than in the PGS networks (figure 4(b)). Apparently, the above observation was attributed to the two extra –OH groups on each xylitol monomer.

3.4. Enzymatic degradation of PGS and PXS

The aim of this study was to test whether extra –OH groups of xylitol have a steric hindrance effect on the hydrolysis of ester bonds of PXS networks. To this end, it is essential to select PGS and PXS polymers of very similar crosslink density, if exactly the same is impossible. Figure 2(a) indicates that the Young’s moduli of PGS crosslinked for seven days and PXS crosslinked for nine days are both approximately 1.7 MPa, and the crosslink densities in these two elastomer networks are approximately 240 mole m⁻³. Moreover, these two polymers are of interest because of their good cytocompatibility (section 3.5). Hence, these two elastomers were selected for the enzymatic study of this work.

The weight loss of the PGS samples crosslinked at 130 °C for seven days was on average 13% after incubation for 35 days, whereas the PXS samples crosslinked at 130 °C for nine days lost approximately 8% of their original weight (figure 5). The enzyme-mediated degradation of polyesters is a hydrolysis process. In the PXS network, the extra –OH groups could influence hydrolysis in two ways. First, the –OH groups attract water molecules in the polymer network, and thus could potentially enhance the availability of water molecules around each ester bond (figure 6). However, the availability of water...
molecules does not necessarily increase their chance to attack ester bonds. Indeed, the large sized –OH groups could shield an ester bond from water attack [29].

It should be mentioned here that the copolymerization of PPS with other polyesters, such as poly lactide (PLA) or polyhydroxyalkanoates (P3HB, P3HV, etc) is another approach to further enhance stretchability, while tuning down hydrolysis of elastomeric polyesters. These non-crosslinkable thermoplastic polyester chains can greatly space out crosslinks. At the same time, the side chains, –(CH$_2$)$_n$–CH$_3$, can sterically hinder ester-bond hydrolysis [39] and may effectively slow down the mediation of enzyme molecules.

3.5. Biocompatibility of PGS and PXS

SNL mouse fibroblasts were used to conduct the in vitro cytotoxicity assessment of the PGS and PXS polymers. PGS and PXS polymers cured at 130 °C for short (2-day) and long (4- or 7-day, respectively) periods were assessed. A visual examination showed that the cell morphology remained normal after two days’ culture in the negative control (i.e. extractant-free medium), positive control (i.e. containing the PDLLA extractant) and the testing medium of PGS and PXS cured for four or seven days (figures 7(a), (b), (d) and (f)). However, cytotoxicity was apparent in the extract media of the PGS and PXS cured at 130 °C for two days (figures 7(c) and (e)). Quantitative LDH assessment confirmed that the
in the extractant media of PGS and PXS cured at 130 ◦C for two days, was most likely caused by either unreacted carboxylic acid groups and/or the carboxylic acids produced by the aqueous hydrolysis of PPS ester groups, which resulted in severe acidity in the extracts. However, PPS cured for a sufficiently long period showed good compatibility, which is comparable with both the negative and positive (PDLLA) controls. Improved compatibility as a result of a long curing time can be attributed to the high crosslink density of the materials, in which most carboxylic groups are converted into ester groups (>94%) and the hydrolytic kinetics of the ester groups is slow due to the hindered diffusion rate of water in a highly crosslinked polymer network. The low cytotoxicity of PXS has been achieved while maintaining good elasticity (εmax is ∼200%, figure 2(b)).

4. Summary

In order to develop flexible and slowly degradable polyester elastomers, PXS and PGS, two members of the PPS family with three or five hydroxyl groups respectively, have been comparatively investigated in terms of mechanical properties, enzymatic degradation and biocompatibility. Under the condition of the same crosslinked density, the PXS elastomer networks have approximately double the stretchability (elongation at break) of their PGS counterparts. This observation is attributable to the relatively longer and more orientable xylitol monomers, compared with glycerol molecules. Although xylitol monomers have two more hydroxyl groups, these hydrophilic side chains do not necessarily accelerate water attack on the ester bonds of the network. Rather, they have a hindrance effect on the hydrolysis of ester bonds. Hence, the PXS network degrades considerably slower than their PGS counterpart. In conclusion, the use of polyols of more than three –OH groups in the fabrication of polyester elastomers can enhance flexibility while tuning down the degradation rate of polyester elastomers.

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