J Mater Sci: Mater Med (2010) 21:1081–1089
DOI 10.1007/s10856-009-3955-2

Evaluation of in situ curable biodegradable polyurethanes containing zwitterion components

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Received: 25 August 2009 / Accepted: 25 November 2009 / Published online: 10 December 2009

Abstract Porous polyurethane networks containing covalently attached zwitterionic compounds dihydroxypolycaprolactone phosphorylcholine and 1,2-dihydroxy-*N*,*N*-dimethylamino-propane sulfonate have been prepared and characterised. Three polymers were prepared by reacting methyl 2,6-diisocyanato hexanoate functionalised D-glucose as prepolymer A with either polycaprolactone triol alone or with addition of 10 mol% zwitterion as prepolymer B. All polymer compositions were mixed with 10 wt% hydrated gelatin beads. The cured polymers with the gelatin beads showed compression strengths that were still suitable for use in articular cartilage repair. The incorporation of zwitterions yielded more hydrophilic polymers that showed increased water absorption and increased porosity. After four months degradation in phosphate buffered saline, the polymers containing zwitterions had approximately 50% mass loss compared with 30% mass loss for that with polycaprolactone triol alone. All polymers were non-toxic in chondrocyte-based assays. Subcutaneous implantation of these polymers into rats confirmed that the polymers degraded slowly. Only a very mild inflammatory response was observed and the polymers were able to support new, well vascularised tissue formation.

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1 Introduction

Biodegradable polyurethanes offer many advantages for designing polymers for biomedical implants including tissue engineering applications, with systems as injectable liquids, gels or pastes receiving attention because of their potential to be delivered using arthroscopic techniques to defect sites [1]. Ideally, these injectable polymer systems are expected to polymerise in situ to form cross-linked networks without damaging the surrounding tissues. They should also facilitate cell delivery and growth while retaining adequate mechanical strength until new, functional tissue is formed.

Few polymer-based injectable systems with suitable mechanical properties and controlled curing and degradation rates have been reported in the literature. Anseth et al. [2] and Mikos et al. [3] have reported systems that can be polymerised in situ based on polyanhydride and polypropylene fumarate chemistry. These could be cured in situ with low reaction exotherms and were shown to be biocompatible and encourage cell attachment, proliferation, and differentiation of osteoblastic function in vivo [4, 5]. We [6, 7] and others [8, 9] have reported two-part polyester urethane prepolymer systems that can be cured in situ to form materials with a wide range of properties that are useful for a range of biomedical applications. This polyurethane based injectable system has the added advantage that it is easy to tailor the mechanical and degradation properties by changing the composition, structure and molecular weight of the prepolymers.

Polyurethanes containing added zwitterionic species have previously been reported [10, 11] and have been shown to be more hydrophilic and to show improved biocompatibility and increased degradation [8–11]. In this present study we have incorporated two different types of

zwitterion compounds into a two-part polyester urethane prepolymer system to see their effect on the physical and biological properties of the injectable polymer system. In the present study, the prepolymers were prepared from monomers that were selected so that when the polymer network degrades, the degradation products are non toxic, bioresorbed or released from the body due to their low molecular weight and water solubility. Thus, Prepolymer A is a reaction product of methyl 2,6-diisocyanato hexanoate (MLDI) and D-glucose as a polyhydroxy compound. The other, Prepolymer B, was either polycaprolactone triol (PCLT), PCLT with 10 mol% dihydroxypolycaprolactone phosphorylcholine (DPCLPC) or PCLT with 10 mol% 1,2-dihydroxy-*N,N*-dimethylammonio-propane sulfonate (DAPS).

This paper reports on the effect of incorporating these zwitterionic species as part of the polymer network structure on the mechanical properties and in vitro and in vivo degradation of the polymer networks.

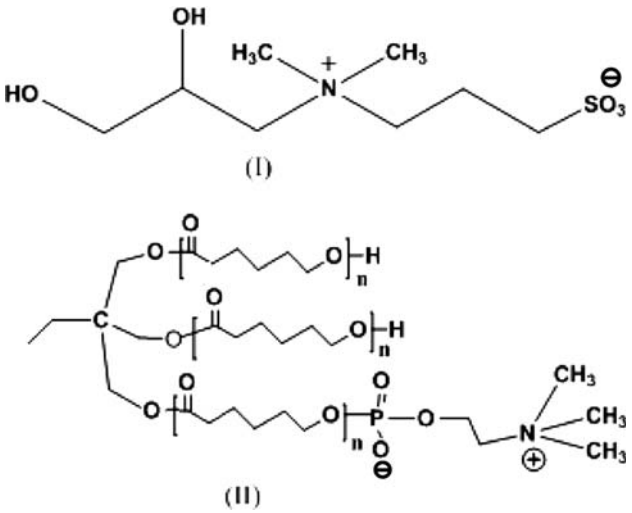

Fig. 1 Structures of the zwitterions included in prepolymer B. (I) 1,2-dihdroxy-*N,N*-dimethylammonio-propane sulfonate (DAPS) and (II) dihydroxypolycaprolactone phosphorylcholine (DPCLPC)

2 Materials and methods

2.1 Materials

Anhydrous D-glucose, phosphorus trichloride, ethylene glycol, stannous 2-ethyl hexanoate, polycaprolactone triol (PCLT, MW 300) were purchased from Aldrich (Sydney, Australia). PCLT was degassed at 70°C for few hours prior to use to remove absorbed moisture. Dichloromethane was dried over molecular sieves (3 Å) and distilled under vacuum. Gelatin beads (70–140 μm when dry) crosslinked with 0.1% glutaraldehyde were synthesised as previously described [12]. Methyl 2,6-diisocyanato hexanoate (MLDI) was purchased from Kyowa Hakko Kogyo Co Ltd, Japan and was distilled before use. Number average molecular weights were calculated based on hydroxyl numbers of the polyol (glucose) in accordance with the American Society for Testing and Materials (ASTM) method E 1899-02. 1,2-dihydroxy-*N,N*-dimethylammonio-propane sulfonate (DAPS) and dihydroxypolycaprolactone phosphorylcholine (DPCLPC) (Fig. 1) were synthesised following published procedures [13–15].

2.2 Synthesis of prepolymer A

Anhydrous D-glucose (5.0 g) was placed in a dry three-neck flask equipped with a magnetic stirrer and under nitrogen and MLDI (29.8 g) was added to the flask, followed by 0.1 wt% stannous 2-ethyl hexanoate as catalyst. The reaction mixture was stirred and heated to 50°C for 71 h under a nitrogen atmosphere. The homogenous prepolymer mixture was then degassed under vacuum (0.1 torr) at 50°C for 1 h before it was transferred to a vial under nitrogen atmosphere and stored sealed at 4°C until required.

Prepolymer A was evaluated after dissolving in tetrahydrofuran (THF) by warming the solution at 50°C for 1 h and then filtering through 0.45 micron filter prior to GPC analysis. GPC was performed on a Waters Associates Liquid Chromatograph system (Waters 717) equipped with a differential refractometer and four μ-Styragel columns (10^5, 10^4, 10^3 and 100 Å). The mobile phase was THF at a flow rate of 1 ml/min. The system was calibrated with narrow disperse polystyrene standards and molecular weights are reported as polystyrene equivalents. The viscosity of the prepolymer was measured at 23°C using a Bohlin Rheometer CSR 10.

2.3 Synthesis of prepolymer B

Three separate prepolymer B mixtures were prepared. These comprised PCLT alone, PCLT mixed with 10 mol% DPCLPC and PCLT mixed with 10 mol% DAPS.

2.4 Polymer synthesis

All polymers were prepared by reacting prepolymer A with one of the three prepolymer B mixtures, such that the reaction had equimolar amounts of isocyanate and hydroxyl functional groups according to the procedure described below. The three polymers that were prepared by varying the Prepolymer B composition were designated as PU-1 (where Prepolymer B is PCLT), PU-2 (where Prepolymer B is PCLT and 10 mol% DPCLPC) and PU-3 (where Prepolymer B is PCLT and 10 mol% DAPS). All formulations, PU-1, PU-2 and PU-3, contained 10 wt% of hydrated gelatin beads.

Degassed Prepolymer A (typically 2.50 g) was weighed into a cavity (20 × 20 × 10 mm) in a polytetrafluoroethylene (PTFE) block. Degassed and dried prepolymer B components (as above) were weighed and added to the prepolymer A, to give a 1:1 molar ratio to prepolymer A. The mixture was stirred using a spatula for ~5 s and then stannous 2-ethyl hexanoate catalyst (0.1 wt% of prepolymer A) was added and stirred for several minutes. Hydrated gelatin beads, 0.3 ml, were added to this mixture and stirred for about 1 min. The viscous mixture was then taken into a 2.5 ml syringe and 0.24 g of the mixture was placed into cylindrical cavities (6 mm D × 12 mm H) in a multi-cavity PTFE mould, which was then firmly sealed with a PTFE cover. This was left to cure overnight at 37°C to give porous cylindrical polymer test specimens. Completion of the reaction was monitored by FTIR to verify the disappearance of the NCO peak from the cured polymer.

2.5 Polymer properties

The mechanical properties of polymer samples were determined using an Instron Universal Testing System, Model 5568 (Instron Corporation; series IX Automated materials testing System) equipped with Merlin 2002 software. Five cylindrical test specimens (6 mm × 12 mm) were tested at a cross head speed of 1 mm/min (ASTM F451-95) and average values for compression modulus and ultimate compressive strength were determined. Specimens were equilibrated for 24 h at 23°C and 45–50% humidity before testing. Porosity was calculated from Scanning Electron Microscopy (SEM) images from the cross-sectional area occupied by polymer.

2.6 In vitro degradation

An in vitro degradation study was carried out according to ASTM method F 1635-04. The medium was 0.1 M phosphate buffered saline (PBS) pH 7.4, and was maintained at this pH. Polymer samples were each placed in an individual vial immersed in excess PBS (Sample:PBS, 1:300) and incubated at 37°C. Samples at different time intervals (30, 60 and 120 days) were removed from the media, rinsed with deionized water to remove any salts within the pores of the polymer and dried on the surface with tissue before measuring the wet mass and dimensions of the samples. The samples were then further dried under vacuum for 5 days at 40°C to determine dry mass. Results were reported as an average of six replicates.

2.7 In vitro cytotoxicity

Human chondrocytes (Edward Keller, Australia) were used for in vitro cytotoxicity assays and were maintained in complete chondrocyte medium (F12:DMEM, 1:1, containing 10% FCS). Prepolymer A and all the monomers used for Prepolymer B mixtures (PCLT, DAPS, DPCLPC) were tested for their cytotoxicity based on the ISO 10993-5 (Biological evaluation of medical devices). The assay used in this study was the CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (MTS) performed according to the manufacturers instructions (Promega). Each monomer at various concentrations was added to microtitre wells containing 80% confluent cells and incubated for 24 h. The culture medium was removed and MTS reagents added, incubated for a further 4 h and then absorbance read at 490 nm. Cytotoxicity was calculated from absorbance values by comparing the relative percentage of cells remaining attached to the microtitre wells in the presence of the different monomers compared with untreated control wells. Results were expressed as percentage cell attachment, where >75% attachment indicated no cytotoxicity.

2.8 Subcutaneous rat implantation study

In vivo implantation of polymers was undertaken according to ISO 10993 International Standard Biological evaluation of medical devices—Part 6: Tests for local effects after implantation 2007. Eight week old, Specific Pathogen Free, female, Sprague–Dawley (Crj:CD(SD)IGS) rats were obtained from the Animal Resource Centre (Canning Vale, WA, Australia). This study was approved by the CSIRO Animal Care and Ethics Committee (Ethics number AEC 02-03).

Prior to surgery animals were randomly divided into groups of eight animals for implantation of PU-1, PU-2 and PU-3 samples. Each group of rats was implanted with a different preformed polymer, with two implants (6 mm × 12 mm) per rat as described previously [16]. A control animal group underwent sham surgery where no polymer was inserted.

A total of four samples per polymer per time point were used for in vivo analysis. Thus, two rats from each polymer group and the sham control group were sacrificed at 2, 4, 8, and 14 months post implantation using an intraperitoneal overdose of sodium pentobarbitone (Nembutal® 60 mg/ml) at a dose rate of >120 mg/kg body weight. Polymer samples were excised to include at least 10 mm of surrounding tissue. One sample was immediately fixed in cold formalin for 6 h. The other sample was cut in half and the macroscopic appearance of the implant and surrounding tissue recorded prior to fixation in formalin. The heart, lung, liver, kidney and spleen were also removed, weighed, and the ratio of each organ weight to total body weight was recorded along with macroscopic appearance prior to fixation in cold formalin. After 6 h of fixation, formalin was replaced by cold 70% ethanol and samples stored at 4°C prior to embedding in paraffin. Samples and organs were

cut along different planes (vertically and horizontally) and 5 μm sections prepared and stained with H&E.

2.9 Histological evaluation

Polymer sections were evaluated histologically according to ISO 1993-6 to assess capsule formation, inflammatory cell infiltration, necrosis, fatty infiltration, fibroblast in-growth into polymer, neovascularisation, and degradation of the polymer. The surrounding capsule thickness was recorded as thin (<5 cell thick) moderate (6–10 cells thick) and pronounced (≥11 cells thick). Inflammatory cell infiltration refers to neutrophils, mononuclear cells (MNC), macrophages and giant cells. Neovascularisation was recorded as the number of capillaries observed in the new tissue. Inflammation and neovacularisation was graded using a 5 point scale. A grade of 0 = no cells or capillaries, 1 = 1–5 clusters of cells or capillaries, 2 = 5–9 clusters of cells or capillaries, 3 = 10–15 clusters of cells or capillaries and 4 ≥ 15 clusters of cells or capillaries per 100 × field. Fibroblast infiltration was recorded as the percentage of the cross section of the cylindrical polymer containing fibroblasts (average of four samples per time point per polymer). Polymer degradation was the percentage of polymer loss either from coalescing pores or fibroblast infiltration.

3 Results and discussion

3.1 Prepolymer and polymer properties

All prepolymers were liquids at ambient temperature. GPC results showed prepolymer A to be a mixture of products (Fig. 2) showing M_n 1104, M_w 1916 and a polydispersity of 1.73. The instantaneous viscosity of Prepolymer A was ~220,00 cP at 23°C and after a fall on initial increase of the shear rate, then became essentially independent of the shear rate at 160,000 cP (Fig. 3). When reacted with each prepolymer B and gelatin beads, the mixture remained injectable through an 18 gauge needle for about 20 min, until the viscosity of the mixture had increased due to onset of cross linking. The set time was dependent on the temperature and catalyst concentration and so could potentially be optimized to meet specific needs. Incorporation of a predetermined quantity of water into the prepolymer reaction mixture allowed the formation of a porous structure due to release of carbon dioxide during curing from reaction of the water with isocyanate. The water addition was achieved through incorporation of the hydrated gelatin beads, which also act as biodegradable porogens and as a vehicle for delivery of cells. Table 1 summarizes the mechanical properties of the three polymer materials after curing overnight at 37°C. The incorporation of gelatin beads

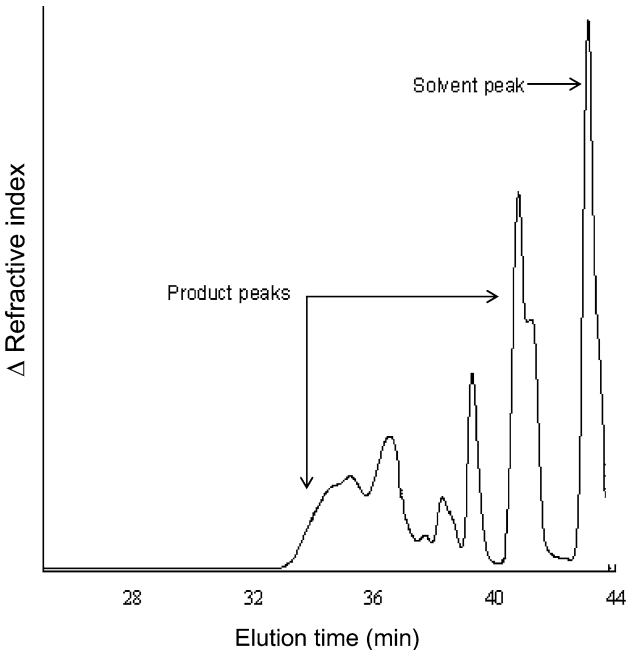

Fig. 2 GPC molecular weight distributions of Prepolymer A: D-glucose end capped with methyl 2,6-diisocyanato hexanoate

decreased both the modulus and compressive strength significantly in all polymers compared to that for a material equivalent to PU-1, but without the gelatin beads, which had a compressive strength of 30 ± 4 MPa and a compressive modulus of 521 ± 200 MPa. This was due to the much more porous structure created by the reaction of isocyanate with the water in the hydrated gelatin beads. The compressive strength of all three polymers was found

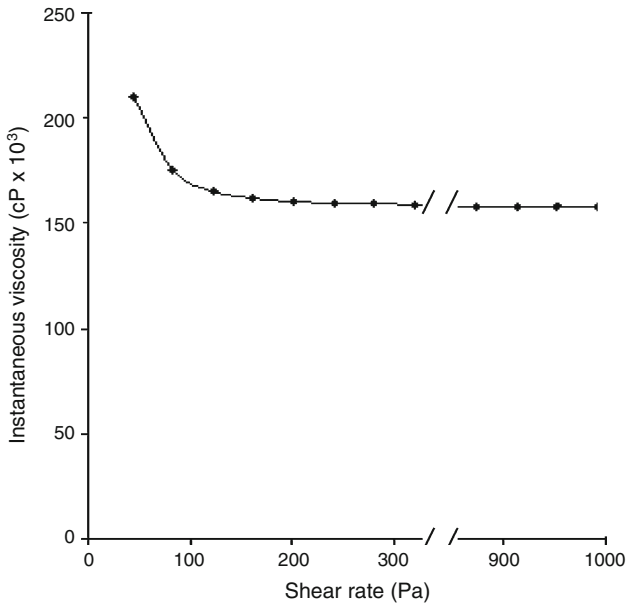

Fig. 3 Viscosity of prepolymer A: D-glucose end capped with methyl 2,6-diisocyanato hexanoate at ambient temperature 23°C

Table 1 Formulation and mechanical properties of polymer/gelatin bead constructs, each construct containing 10 wt% hydrated gelatin beads

Polymer code	Prepolymer B	Molar ratios of prepolymer A & prepolymer B components	Compressive strength (MPa)	Compressive modulus (MPa)
PU-1	PCLT	1.0:1.0	1.0 ± 0.1	22.2 ± 12
PU-2	PCLT & DAPS	1.0:0.9:0.1	1.6 ± 0.4	51.6 ± 20
PU-3	PCLT & DPCLPC	1.0:0:9:0.1	0.3 ± 0.1	2.9 ± 0.9

to be comparable to cartilage (~1–3 MPa) [17]. Polymer PU-3, containing DPCLPC, showed the lowest compressive strength (0.27 ± 0.09 MPa) and compressive modulus (2.9 ± 0.9 MPa) compared to the other two polymers. Interestingly, PU-2, containing DAPS, showed a higher compressive strength and compressive modulus compared to PU-1 (Table 1). Both PU-2 and PU-3, which contain the zwitterion components, showed higher initial water absorption, ~30% water in the first week compared to ~5% for PU-1. DAPS and DPCLPC both exist as zwitterions at neural pH and may have contributed to the increased water absorption over and above what is observed due to the presence of gelatin alone.

The high porosity of the polymers, with their gelatin beads, was shown by SEM (Fig. 4), with an average porosity in the range of 51–58%, with PU-1 having the lowest average porosity (51%). The SEM images showed that PU-1, PU-2 and PU-3 had average pore sizes of 200, 350 and 440 μm, respectively. The pore morphology in PU-1, with no added zwitterion, was found to be spherical and small compared to those of PU-2 and PU-3 which showed relatively large, interconnected pores.

The major chemical functional groups linking various segments in the polymer systems reported in this study are ester and urethanes, which are susceptible to hydrolytic degradation. The major degradation products upon complete degradation of these linkages include caproic acid, lysine, glucose, trimethylol propane and methanol as well as minor amounts of zwitterionic species incorporated into the system. All these species are water soluble and expected to be released from the body. During polymer degradation it is likely that some water soluble fractions may be released from the implant site before complete degradation to low molecular weight species. While it is difficult to test each of these species, we have conducted in vivo studies to assess any adverse tissue reactions to degradation products. We have previously reported [7] the biodegradation of similar cross linked polymer networks in an animal study (sheep). Based on histology evaluations, no adverse tissue reactions were evident resulting from polymer degradation in these systems.

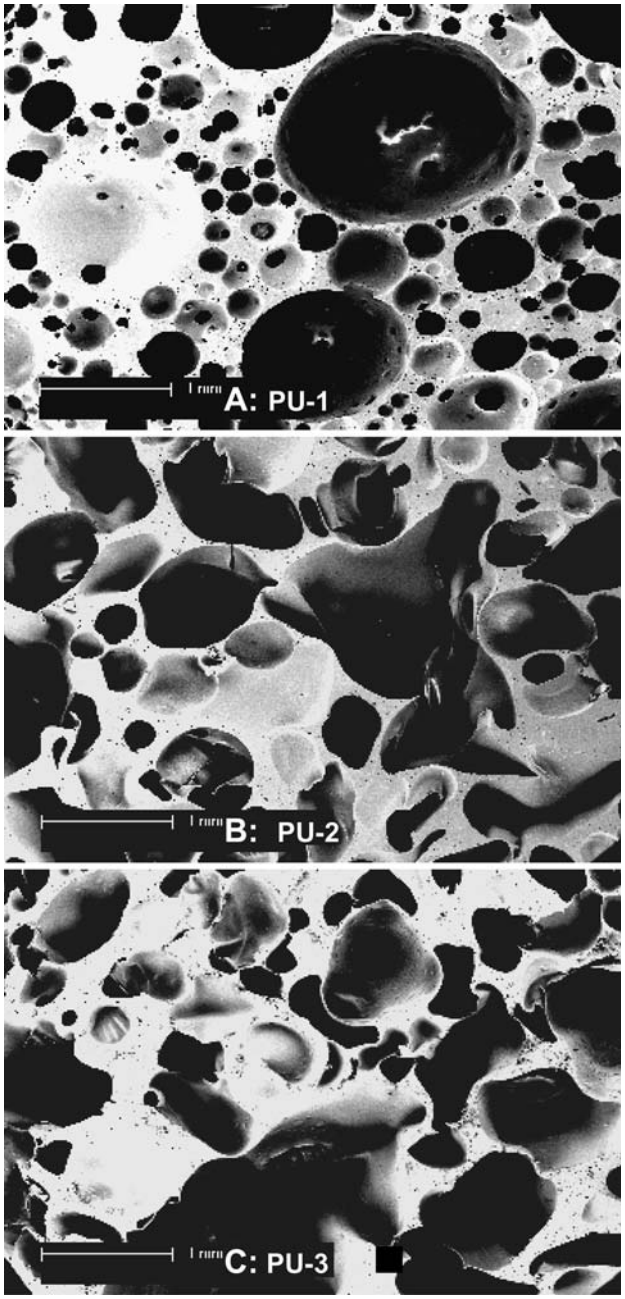

Fig. 4 SEM images of porous cylindrical plugs of **a** PU-1, **b** PU-2 and **c** PU-3. Bars = 1 mm

3.2 In vitro degradation

As expected, incorporation of hydrophilic DPCLPC and DAPS compounds increased water absorption and the resulting polymers were more susceptible to hydrolysis. The mass loss gradually increased in all three polymers with time and PU-1 showed the lowest mass loss compared to

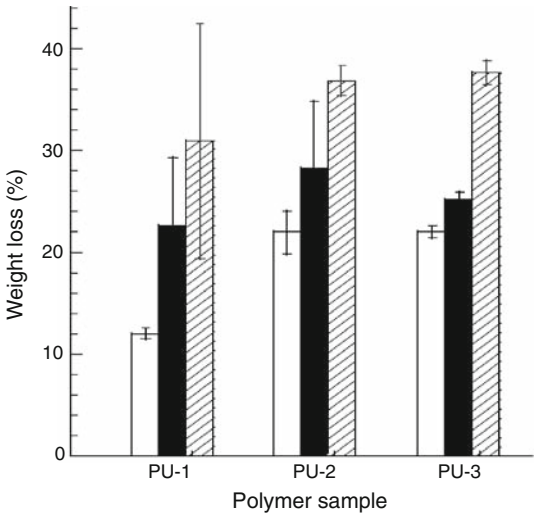

Fig. 5 Weight loss (%) of PU-1, PU-2 and PU-3 during the in vitro degradation test at 1 ■, 2 □ and ▨ 4 month time intervals

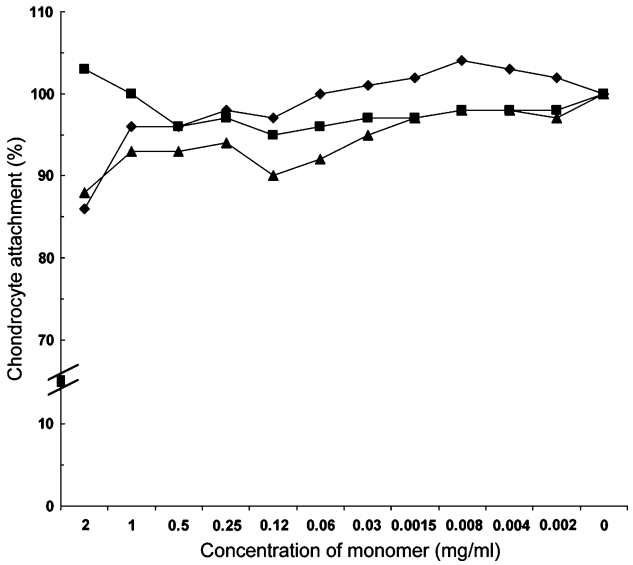

Fig. 6 In vitro cytotoxicity test showing human chondrocyte attachment after treatment with different concentrations of monomers: PCLT ◆; DPCLPC ■; DAPS ▲

PU-2 and PU-3 at all three time intervals (Fig. 5). This could be explained due to the hydrophobic nature of PU-1 which made the polymer relatively less susceptible to hydrolytic attack. Initially between one to two months, PU-2 showed a greater mass loss than PU-3 but the mass loss was comparable at the end of 4 months in both polymers. At the final 4 months time point, PU-1 showed 30 ± 11% mass loss compared to 36 ± 1.4% mass loss in PU-2 and 37 ± 1.2% in PU-3. Even though the PU-2 and PU-3 showed significant swelling due to hydration, both polymers retained their original cylindrical shape throughout the degradation period. This could be attributed to a network structure that allowed overall shape retention until a substantial proportion of the functional groups were degraded.

In a separate study, increasing the amount of either DPCLPC or DAPS from 10 mol% up to 30 mol% led to increased degradation with a mass loss of almost 60% in 2 months (data not shown).

3.3 In vitro cytotoxicity of monomers

In vitro cytotoxicity of Prepolymer A and all Prepolymer B components used in making the polymers were tested against human articular chondrocytes at different concentrations of monomers ranging from 0.002 to 2 mg/ml (Fig. 6). For isocyanate compounds the reaction products arising from reaction with water were examined. All compounds showed no effect on cell attachment indicating that all were non-toxic to chondrocytes up to a concentration of 2 mg/ml.

3.4 In vivo rat subcutaneous implantation studies

The test polymers, PU-1, PU-2 and PU-3, had been designed for potential use for delivery of chondrocytes for cell therapy in order to form new cartilage. The in vivo study was to investigate the biocompatibility and biodegradation of these polymer constructs. Throughout the study, all animals implanted with polymers gained weight and appeared healthy, mirroring the sham control rats. Also, all major organs were histologically normal and the ratio of wet weight organs to body weight were similar to the sham surgical control animals throughout.

A thin capsule was produced around each polymer which was apparent throughout the study. The cell thickness varied slightly between each type of polymer and over time, but the capsule at each time point was less than five cells thick (Table 2; Fig. 7). At the 14 month time point the capsule surrounding the polymer samples was less apparent, in particular with PU-3, due to fibroblast infiltration (60%, Table 2).

At 2 months, all the explanted polymers were spongy and slightly flexible, but had maintained their initial shape. Histologically, only a mild degradation (10–15%) of the polymers was observed. Discrete pores were evident in all polymers, with PU-1 having the smallest pores. Fibroblast infiltration was mostly confined to pores along the interface between the polymer and the tissue, with less than five pores in the centre harbouring fibroblasts. PU-3 had the least fibroblast infiltration, at 5–10%, whereas PU-1 and PU-2 had approximately 15–20% fibroblast infiltration (Table 2; Fig. 7). All fibroblast infiltration was associated with neovascularisation. All polymers had a mild influx of macrophages and plasma cells. Occasionally giant cells

Table 2 Histological analysis of subcutaneous implanted polymers in rats over time

Polymer	Time after implantation (months)	Inflammation (grade)				Fibroblast infiltration (%)	Neo-vascular-isation (grade)	Capsule (cells)	Polymer degradation (%)
		Neutrophils	MNC	Macrophages	Giant cells				
PU-1	2	0	1	1	0	15	2	Thin	10
	4	0	1	1	0	15	2	Thin	15
	8	0	1	1	0	20	2	Thin	35
	14	0	0	1	1	35	2	Thin	40
PU-2	2[a]	0	1	1	1	15	2	Thin	10
	4	0	1	1	1	15	1	Thin	20
	8[a]	2	1	1	0	20	2	Thin	30
	14	2	2	1	0	30	2	Thin	40
PU-3	2[a]	0	1	1	1	5	1	Thin	15
	4	0	1	1	1	5	1	Thin	20
	8[a]	1	1	1	1	20	2	Thin	25
	14	2	1	1	1	60	2	Thin	60

[a] indicates that one of the four samples was not included in the assessment because of prior animal welfare concerns. MNC—mononuclear cells refers to lymphocytes and plasma cells. Scores were determined as described in the Materials and Methods

were observed in pores at the tissue interface of PU-2 and PU-3 (Table 2).

At 4 months, explanted polymers were similar to the 2 month samples (Table 2). All polymers were moderately spongy and flexible and the shape of the polymers had not altered. Polymer degradation had increased slightly and ranged from 10 to 20% for all polymers. Discrete smaller sized pores were still evident, although, some had coalesced giving the appearance of larger pores. Fibroblast infiltration was still more notable in pores that were in contact with the tissue, but with some pores in the centre of the polymer also containing cells. Neovascularisation was more advanced and new collagen was deposited within the implant where degradation had occurred.

By 8 months, all polymers were well integrated with the tissue. All were still spongy and flexible and had maintained their cylindrical shape. Polymer degradation was more advanced than previous time points ranging from 30 to 40% (Table 2). The polymers still contained discrete pores but these were larger due to more pores coalescing, allowing more extensive fibroblast infiltration (ranging from 15 to 25%). Fibroblasts had extensively infiltrated many of the pores along the polymer-tissue interface. Well vascularised tissue was evident throughout more regions of all polymer samples. Occasional macrophages and giant cells were present in the polymer pores of all polymers except for PU-3 where only macrophages were observed.

At 14 months, polymer degradation had increased to 30–35% degradation for PU-1 and PU-2, with a fibroblast infiltration of 30–35% (Table 2; Fig. 7). In contrast, PU-3 had degraded by 60% with approximately 60% of the implant containing fibroblast infiltration (Table 2; Fig. 7). At the longer time point (14 months), foreign body or solid state carcinogenesis [18, 19] was observed. This is a well known phenomenon in rats after long term implantation, mainly when the material is large, relatively smooth and degrades slowly or not at all [20]. In the present study, this was very limited and was only observed in association with one of the four polymers implanted from each of the groups. Since this was not uniformly observed in all samples, it is unlikely that the chemical nature of the polymers were responsible for such sarcomas as they occurred in association with only one of the implanted polymers in each group at the longest time point; the other implanted polymers had no associated carcinogenesis.

4 Conclusion

This study has demonstrated that incorporation of 10 mol% of both DPCLPC and DAPS zwitterions and 10 wt% gelatin beads has produced polymers that are more hydrophilic, with increased water uptake and increased polymer degradation compared with the base polymer formulation. The low reaction exotherm, in situ curing and mechanical properties of these polymers make them suitable as an injectable polymer for cartilage like applications. These modified polymers incorporating the gelatin beads showed lower compressive modulus and compressive strength, but for DPCLPC containing polymer would still be suitable for cartilage cell therapy applications, although the DAPS containing polymer may be too weak. The in vitro cell assays clearly demonstrated that the monomers used in each polymer formulation were

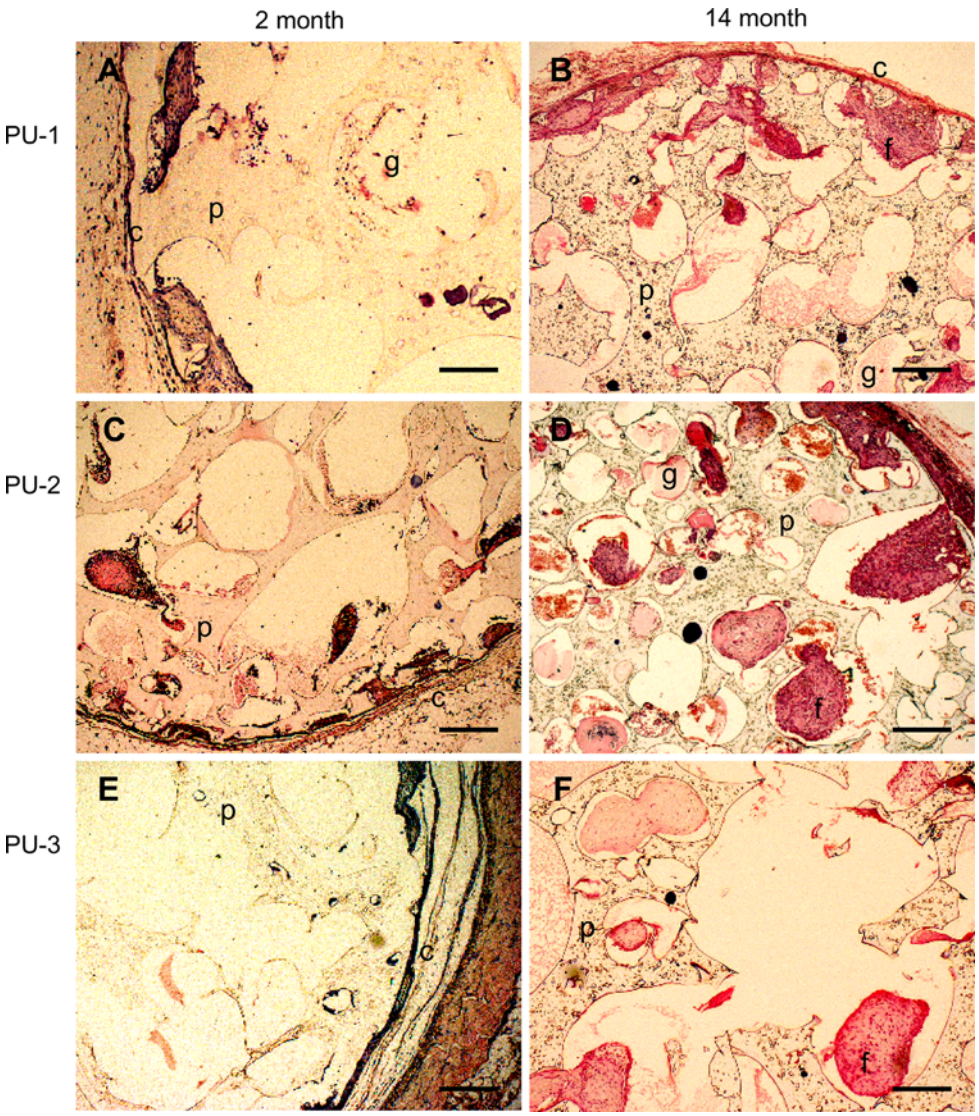

Fig. 7 Micrographs of rat subcutaneous implants of PU-1 (row 1; **a**, **b**), PU-2 (row 2; **c**, **d**) and PU-3 (row 3; **e**, **f**) at 2 months (column 1; **a**, **c**, **e**) and 14 months (column 2; **b**, **d**, **f**) post implantation. Features that are labelled are: capsule (c), polymer (p), invading fibroblasts (f) and gelatin beads (g). Bars = 300 μm)

not cytotoxic using human chondrocytes. Subcutaneous rat implants have indicated that all the polymers were well tolerated by the host. Only a thin capsules were induced and very few inflammatory cells were observed in the vicinity of the polymers. The pores in the polymers along with the gelatin beads create and environment that provides for cell migration and good nutrient flow. This allowed new, well vascularised tissue to develop with fibroblast infiltration and new collagen deposition. Hence, the polymer compositions containing the added zwitterion component, especially the one with DPCLPC, could be useful in delivering cells and growth factors for cartilage repair and for other cell therapy or tissue engineering applications.

Acknowledgment The authors would like to thank Professor Wei-Bor Tsai (Department of Chemical Engineering, National Taiwan University, Taiwan) for providing the gelatin beads.

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