Design, synthesis and properties of polyurethane hydrogels for tissue engineering

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Due to their similarity to natural soft tissues, water-swellable polymeric materials (hydrogels) are, in principle, ideal candidates for scaffolds/matrices in tissue engineering. Polyurethanes (PU), hydrophilic but water-insoluble, can be obtained by the incorporation of hydrophilic soft segments, e.g. poly(ethylene oxide) (PEO). These materials possess the favorable characteristics of the family of PUs as well as the ability to mimic soft tissues.

In this work, new crosslinked PU-hydrogels were prepared in a one-step bulk polymerization process using an aliphatic diisocyanate, PEO, a low molecular weight diol, and a tri-functional crosslinking agent. A porous structure was also obtained by air-incorporation under mechanical stirring at a controlled high speed during the polymerization.

Structural characteristics of the compact (PU-HyC) and the porous (PU-HyP) material were investigated. Molecular weight between cross-links, M_c, and crosslinking density, ρ_x, were typical of a low crosslinking degree. A homogeneous distribution of non-interconnecting pores (ϕ100 μm) was observed in PU-HyP. Both materials showed a high water adsorption. The swelling behavior and weight loss in water was affected by porosity.

For their mechanical behavior in the swollen state, the novel PU hydrogels can be considered for biomedical applications where good mechanical properties are required (i.e. 3D scaffold for tissue engineering).

Introduction

Water-swellable and water-insoluble polymeric materials (hydrogels) are meeting an increasing interest in a wide range of biomedical applications [1, 2]. Hydrogels can be employed for cell (gene therapy and artificial organs) or enzymes microencapsulation and as environmentally responsive or shape-memory materials. Due to their similarity to natural soft tissues, hydrogels are ideal candidates for scaffolds/matrices in tissue engineering.

However, hydrogels in the swollen state exhibit weak mechanical strength and hence they are unsuitable for applications under load. On the other hand, mechanical properties can be, in principle, increased by developing hydrogels having a stronger polymer network, such as the one of polyurethane (PU) block copolymers.

As PUs represent a versatile family of polymers, by the appropriate selection of the base reagents, hydrophilic PUs can be obtained: this usually involves the incorporation of hydrophilic soft segments, e.g. poly(ethylene oxide) (PEO) [3, 4]. PU-hydrogels, hydrophilic but water-insoluble, are generally represented by chemically crosslinked networks. These materials possess the favorable characteristics of the family of PUs as well as the ability to mimic soft tissues.

In this work, new crosslinked, PEO-based, PU hydrogels were prepared both in a compact and in a porous structure by a one-step bulk polymerization using an aliphatic diisocyanate and a tri-functional crosslinking agent. Their structural and chemico-physical properties, swelling degree and mechanical characteristics were studied.

Experimental

Materials

Poly(ethylene glycol) (PEG, Aldrich Chemical Company Ltd.) was vacuum dried at 90–95 °C on a rotary evaporator until residual water was lower than 0.1% w/w (evaluated by Karl Fisher titration, Mettler DL 18 instrument). The molecular weight was accurately determined as 1624 by the ASTM 1638 67T standard method.

Bis(cyclohexyl)methane-4,4′-diisocyanate (HMDI, Desmodur W, Bayer), *tris*(2-hydroxy-ethyl)-amine, 1,4-butanediol, 1,4-diazo(2,2,2)bicyclooctane (DABCO) and dibutyl-tin-dilaurate (DBTDL) (Aldrich Chemical Company Ltd) were used without further purification.

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Synthesis

Hydrogels were prepared by one-step polymerization process both in a compact (PU-HyC) and a porous form (PU-HyP) with a PEO/crosslinking agent molar ratio of 10 : 1. The required amount of catalysts, butanediol and crosslinking agent was added into the molten PEG; the mixture was returned to the rotary evaporator at 90–95 °C until a homogenous solution was obtained. A weighed amount of the solution was poured into a polypropylene beaker, the diisocyanate was added and mechanically stirred at 400 r.p.m. for the compact structure. The porous structure was obtained by air-incorporation at high speed (2000 r.p.m.). The reaction mixture was poured into polypropylene molds and post-cured 20 h at 80 °C.

Characterization of the materials

SEM

Specimens, mounted on aluminum stubs, were sputter-coated with gold, and examined with a Leica Cambridge Stereoscan 360 microscope at 3–7 keV acceleration voltage.

M_c and ρ_x

Molecular weight between cross-links, $\overline{M}_c$ [5, 6] was calculated as:

$$\overline{M}_c = \sum_i X_i * M_i/(1.5 * X)$$

where X is the molar ratio of crosslinking agent and X_i and M_i are the molar ratio and the molecular weight of the monomers respectively. Crosslinking density (ρ_x) was obtained from $\overline{M}_c$ [5] and density (measured by weighting samples of known geometry).

FT-IR spectroscopy

ATR FT-IR spectra were performed with a FT-IR Magna 510 Nicolet spectrometer equipped with Omnic 4.1 Software. ATR Spectra-Tech attachment, mod. 300, was used.

One-way ANOVA was used to assess the statistical significance of the compression tests data.

Swelling behavior of PU-HyC and PU-HyP

The swelling degree of PU-HyC and PU-HyP in water at 37 °C (± 0.3 °C) was measured from 30 min up to 96 h.

Cylindrical samples (∅ 13.5 mm) were vacuum dried at room temperature, weighed accurately and then each sample was immersed, individually, in 50 ml of distilled water in a closed bottle. The bottles were placed in a thermostatic bath at 37 °C (± 0.3 °C). At each time interval, the samples were removed, blotted with tissue paper and accurately weighed. The swelling measurements were carried out in triplicate.

The volume of adsorbed solvent, VAS (%), versus time, was calculated as

$$\%\mathrm{VAS} = (W_s - W_d)/\rho_s W_d \times 100$$

where W_s is the weight of the swollen sample and W_d is the weight of the dry sample and ρ_s density of the solvent.

Water stability at 37 °C

The above swollen samples (in triplicate) were dried in a vacuum oven after extracting in water at 37 °C (± 0.3 °C) from 30 min up to 96 h and re-weighed. The percentage weight loss was plotted as a function of time.

Mechanical properties

Mechanical properties of the hydrogels in dry and swollen state were investigated by performing three hysteresis cycles under compressive condition. Tests were performed on cylindrical samples ($n = 3$, $\varnothing = 13.5$ mm, $h = 10.5$ mm) with an Instron model 4200 instrument, at a cross-head rate of 1.3 mm/min (ASTM D695-96). From the experimental curves the following parameters were found: tangent modulus (E), secant moduli at 10, 30, 40% strain (E_{10}, E_{30}, and E_{40}, respectively), and the hysteresis area of the three compressive cycles.

Results and discussion

Polyurethane hydrogels based on PEO were prepared in a one-step bulk polymerization process using an aliphatic diisocyanate, a di-functional chain extender and a tri-functional crosslinking agent. The obtained polymers were insoluble in water and organic solvents.

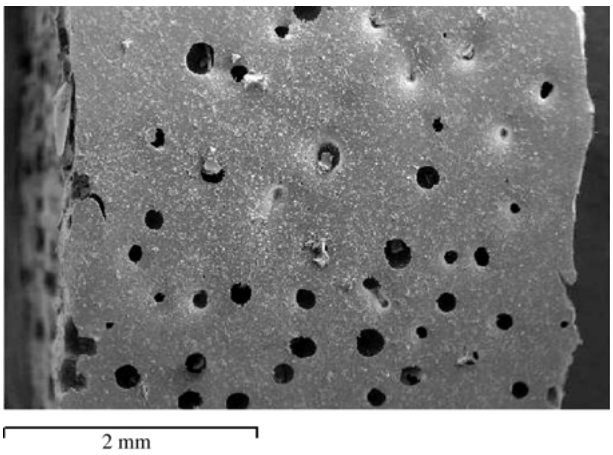

Figure 1 SEM image of a cross section of PU-HyP.

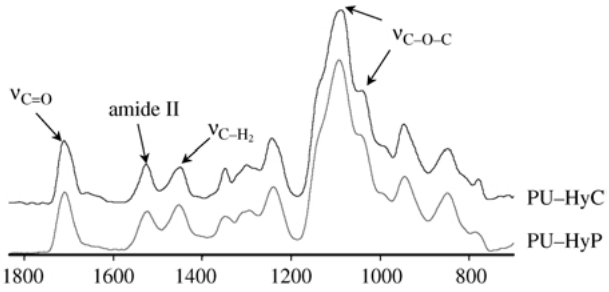

Figure 2 ATR FT-IR spectra of PU-HyC and PU-HyP (1800–700 cm^{-1} zone).

TABLE I Structural parameters and infrared adsorption bands of PU-HyC and PU-HyP

	Density (g/cm^3)	$\overline{M}_c$	ρ_x (mol/cm^3)	ATR FT-IR: urethane bands (cm^{-1})					
				ν_{N-H}	$\nu_{C=O}$	amide II	δ_{C-H2}	amide IV	ν_{C-O-C}
PU-HyC	1.18	1.37×10^4	0.86×10^{-4}	3340	1712	1527	1450	1244	1041
PU-HyP	1.09	1.37×10^4	0.80×10^{-4}	3326	1710	1524	1455	1240	1040

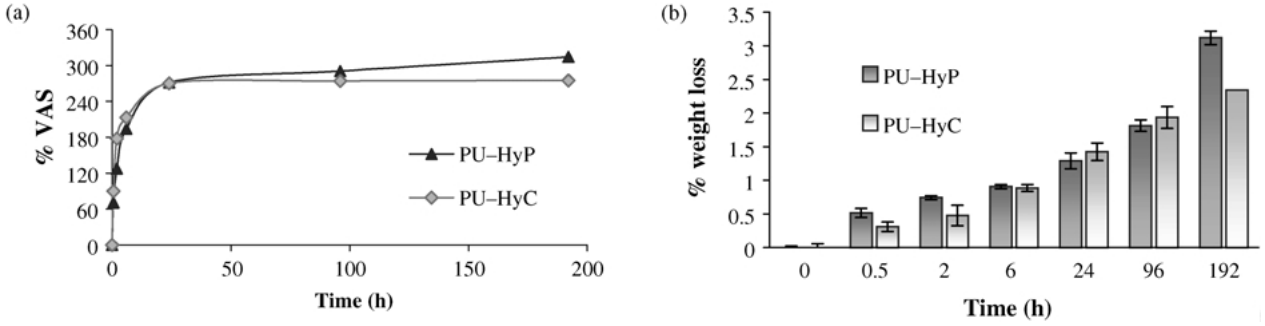

Figure 3 (a) Water swelling and (b) water stability of PU-HyC and PU-HyP.

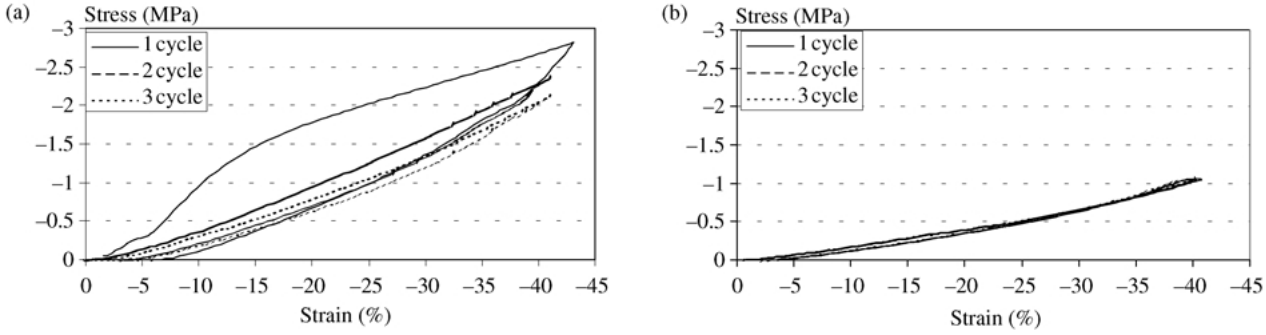

Figure 4 σ/ε curves for the xerogels: (a) PU-HyC and (b) PU-HyP.

A homogeneous distribution of non-interconnecting pores (ϕ 100 μm) was observed in PU-HyP (Fig. 1).

Molecular weight between cross-links, $\overline{M}_c$ and cross-linking density, ρ_x, were typical of a low crosslinking degree (Table I) [5].

ATR FT-IR spectra present typical bands of urethane groups, e.g. stretching of NH (ν_{N-H}), stretching of carbonyl ($\nu_{C=O}$), amide II band, bending of C–H$_2$ (δ_{C-H2}), amide IV band, and stretching of –C–O–C–. Very small differences between the spectra of the two materials can be observed, mainly in the 1800–1300 cm^{-1} region, indicating some dissimilar structural characteristics on the surface (Table I and Fig. 2).

Broad bands for bending (about 1450 cm^{-1}) and wagging (about 1350 cm^{-1}) of methylene groups indicate that soft segments, deriving from PEO, are partially in an amorphous conformation in both the PU structures [7].

The water-swelling of the polymers was measured as a function of time. Both polymer samples reach the equilibrium in about 24 h, showing a similar kinetic profile up to 4 days. However, a higher water absorption (8 days) was observed for the porous hydrogel (274% for PU-HyC and 314% for PU-HyP) (Fig. 3).

The weight losses exhibited by the PU-HyC and PU-HyP in water were similar up to 4 days and lower than 2% w/w. As observed for water swelling, after 4 days the behavior of the two hydrogels diverges, showing a higher weight loss for the porous structure. This can be related to the higher swelling which may alter the diffusion properties of the hydrogel thus promoting the release of low molecular weight, water soluble, products. Another hypothesis may be found in a mayor degradation of the porous structure, whose by-products can be released in water.

Compression properties

Whereas the PU-HyP xerogel did not show any different behavior among the three compression cycles (Fig. 4(b)) meaning an elastomeric nature, the PU-HyC did not show a reversible behavior in the three hysteresis cycles (Fig. 4(a)). A mechanical decrease from the first to the third compression cycle was detected, showing a non elastomeric behavior probably due to a different soft segment morphology. The PU-HyC values of E and secant moduli (Table II) are significantly different from those of the PU-HyP xerogel ($p < 0.05$). The first and the second hysteresis cycle breaks gradually the crystalline domains in PU-HyC and change the compression resistance till values, at the third cycle, similar to those of the PU-HyP.

In PU-HyC the large hysteresis area at the first cycle indicates a high energy loss due to the structural modification of the crystalline phase; at the third cycle the hysteresis behavior is very similar to that of the PU-

TABLE II Compression test results for the xerogels and hydrogels

		Xerogel				Hydrogel			
		E (MPa)	E_{10} (MPa)	E_{30} (MPa)	E_{40} (MPa)	E (MPa)	E_{10} (MPa)	E_{30} (MPa)	E_{40} (MPa)
PU-HyC	1st cycle	3.13 ± 0.19	18.62 ± 3.54	8.98 ± 1.60	7.76 ± 1.20	0.20 ± 0.05	0.45 ± 0.10	0.65 ± 0.07	0.79 ± 0.09
	2nd cycle	1.39 ± 0.19	4.80 ± 0.52	5.74 ± 0.64	6.20 ± 0.70	0.19 ± 0.05	0.46 ± 0.10	0.67 ± 0.08	0.80 ± 0.09
	3rd cycle	0.81 ± 0.20	3.07 ± 1.59	4.15 ± 1.56	4.77 ± 1.69	0.20 ± 0.05	0.45 ± 0.12	0.67 ± 0.09	0.80 ± 0.01
PU-HyP	1st cycle	0.84 ± 0.12	1.93 ± 0.34	2.33 ± 0.25	2.63 ± 0.15	0.19 ± 0.01	0.48 ± 0.03	0.66 ± 0.02	0.81 ± 0.01
	2nd cycle	0.76 ± 0.09	1.92 ± 0.30	2.33 ± 0.24	2.70 ± 0.19	0.20 ± 0.03	0.49 ± 0.03	0.68 ± 0.02	0.82 ± 0.00
	3rd cycle	0.85 ± 0.13	1.95 ± 0.27	2.36 ± 0.22	2.74 ± 0.19	0.20 ± 0.06	0.49 ± 0.04	0.68 ± 0.03	0.82 ± 0.01

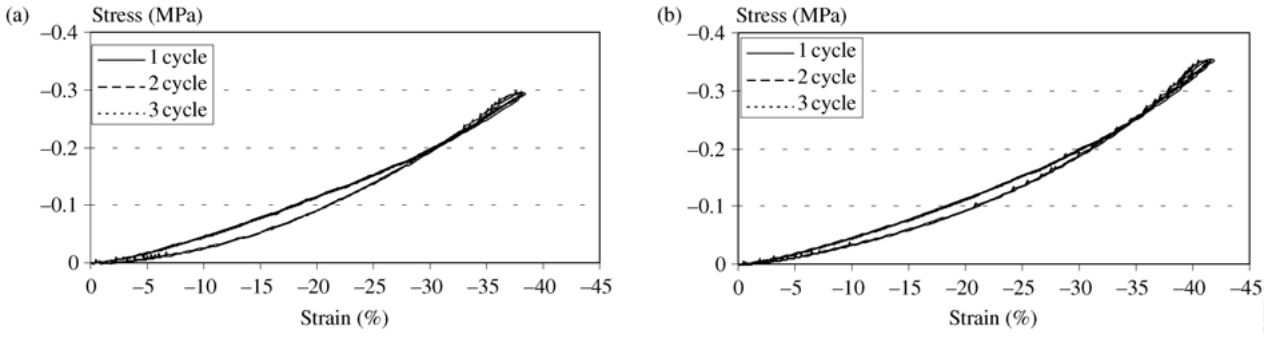

Figure 5 σ/ε curves for the hydrogels: (a) PU-HyC and (b) PU-HyP.

HyP. The hysteresis area at each compression cycle for PU-HyP is not relevant, as the loaded and unloaded curves are almost identical, meaning a very small energy loss during the deformation recovery.

The values of the tangent and secant moduli decrease for both the hydrogels compared to those of the xerogels (Table II). The swollen state lead to a comparable mechanical behavior for PU-HyC and PU-HyP, as tangent and secant moduli values are not significantly different between them $(p > 0.05)$. Both hydrogels showed an elastomeric behavior at each compression cycle and the hysteresis area is almost zero, indicating the absence of non-reversible structural modifications (Fig. 5).

The swollen state in PU-HyC is able to give to the hydrogel a deformation reversibility, probably due to the water molecules in the crystallites that transform the semi-crystalline polymer (dry state) to an ''elastomeric'' hydrogel (swollen state). On the other hand, the PU-HyP maintains a mechanical behavior indicating an elastomeric nature also at the swollen state. The porosity present in PU-HyP hydrogel has not caused a decrease in compressive properties as the moduli values are comparable to the PU-HyC ones.

Conclusions

The PU formulation introducing a low crosslinking degree allowed to obtain high swelling, not water soluble, hydrogels. These materials also present an elastomeric behavior in the swollen state.

Porosity was introduced in the polymeric structure and it affected diffusion and swelling kinetic profile of the hydrogel. However, new technologies should be further investigated in order to obtain interconnecting pores, fundamental in allowing cell colonisation.

For their mechanical behavior in the swollen state, the novel PU hydrogels can be considered for biomedical applications where good mechanical properties are requested (i.e. 3D scaffold for tissue engineering).

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