Design, synthesis and properties of polyurethane hydrogels for tissue engineering

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Due to their similarity to natural soft tissues, water-swallowable 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, $\rho_c$, were typical of a low crosslinking degree. A homogeneous distribution of non-interconnecting pores ($\phi100\mu m$) 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).

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Introduction
Water-swallowable 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-diazor(2,2,2)bicyclooctane (DABCO) and dibutyl-tin-dilaurate (DBTDL) (Aldrich Chemical Company Ltd) were used without further purification.
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.

\[ M_c \text{ and } \rho_s \]
Molecular weight between cross-links, \( M_c \) [5, 6] was calculated as:

\[ M_c = \sum_i X_i \times M_i / (1.5 \times X) \]

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 (\( \rho_s \)) was obtained from \( M_c \) [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 (\( \varnothing = 13.5 \text{ mm} \)) 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

\[ \% \text{VAS} = \left( W_s - W_d \right) / \rho_s W_d \times 100 \]

where \( W_s \) is the weight of the swollen sample and \( W_d \) is the weight of the dry sample and \( \rho_s \) 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 (\( n = 3, \ \varnothing = 13.5 \text{ mm}, \ h = 10.5 \text{ mm} \)) 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 (\( E_{10}, E_{30}, \text{ and } E_{40} \), 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 trifunctional crosslinking agent. The obtained polymers were insoluble in water and organic solvents.