Polymer 117 (2017) 259–267

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Photopolymers based on ethynyl-functionalized degradable polylactides by thiol-yne 'Click Chemistry'

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ARTICLE INFO

Article history:
Received 23 January 2017
Received in revised form
7 April 2017
Accepted 12 April 2017
Available online 18 April 2017

Keywords:
Thiol-yne Click Chemistry
Microstructuring
Polylactides

ABSTRACT

This work reports the synthesis and characterization of a series of new degradable ethynyl-ended polylactides with different architectures. These ethynyl-functionalized polylactides were blended with a commercial low molar mass tetrathiol to prepare crosslinked polymeric networks by a thiol-yne photoclick reaction that was studied by infrared spectroscopy and photocalorimetry. Cell proliferation assays with human colorectal carcinoma cells showed that the resulting polymeric networks are not cytotoxic. Two-dimensional structures were prepared by patterned curing using direct laser writing of the photopolymerizable formulations. Preliminary cell tests on substrates provided with these polymeric structures demonstrate the possibility to generate cell patterns.

1. Introduction

In recent years, there has been an emerging interest in the development of biomaterials based on the use of biodegradable polymers for applications such as tissue engineering or drug delivery, among others [1–4]. Crosslinked polymeric materials are particularly attractive in this field since the chemical structure and architecture of polymers can be easily controlled and their final properties tailored to fulfill the performance requirements of target applications. Together, crosslinks impart mechanical and temporal stability to the final system. From the different strategies, light-induced crosslinking is particularly attractive as it can be temporally and spatially controlled on exposing the material to the initiating light source [5–7].

For the preparation of crosslinked biomaterials, photopolymerization of multifunctional (meth)acrylate monomers has been mostly used [8–15]. However, despite the demonstrated advantages of the *in situ* formed (meth)acrylate-based networks, the reaction that proceeds through a radical chain-growth polymerization mechanisms generates non-biodegradable high molecular mass acrylic chains [16,17]. These acrylic chains become the major drawback as they are difficult to eliminate from the body.

As an alternative, Anseth and Bowman developed degradable networks formed by a photoinitiated thiol-ene reaction [18–22]. Using a low molecular mass multifunctional thiol with hydrolyzable ester groups and an alkene-functionalized biodegradable macromonomer, a highly crosslinked network was obtained. Degradation of such network resulted in low molecular weight byproducts that are easier to excrete from the body. Additionally, the robust nature of thiol-ene chemistry allows the preparation of well-defined materials for applications in a wide range of disciplines [23,24].

Similarly, the analogous thiol-yne photoreaction can also lead to highly crosslinked polymer networks [25–29]. Nonetheless, in a light induced thiol-yne polymerization, two thiols react with one ethynyl group giving polymer networks with higher crosslinking densities than the equivalent thiol-ene networks [27]. Additionally, the versatility of this chemistry facilitates post-polymerization modification of residual functional groups to produce materials

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http://dx.doi.org/10.1016/j.polymer.2017.04.035
0032-3861/

with improved and tailored properties, as it has be recently pointed by Quick et al. on the preparation and subsequent dual-postfuncionalization of micro-resolved 3D mesostructures prepared by thiol-yne chemistry using low molecular weight monomers as photoresist systems [30]. The radical mechanism of the thiol-yne reaction makes it a very reliable and versatile method that tolerates a variety of functional groups, which is of interest for the synthesis of new functional materials [31–34]. Very recently Blasco et al. reviewed the formation of precision polymeric networks using advanced photoinduced ligation techniques including thiol-yne approach, as emerging and promising alternatives to acrylate or epoxy materials [7]. Regardless the benefits, the preparation of degradable polymeric networks by thiol-yne photocrosslinking of biodegradable polymers has been rarely exploited [35,36]. In a previous work [37], we first demonstrated that the thiol-yne photoreaction was an attractive tool for the preparation of biocompatible crosslinked polymeric materials for cell patterns. The investigated system comprised an ethynyl-functionalized hyperbranched aliphatic polyester and a non-cytotoxic, commercial and multifunctional low molecular mass thiol (as crosslinker of the hyperbranched polyester). Recently, Oesterreicher et al. explored this chemistry for the 3D printing of tailor-made medical devices using low molecular weight monomers and studied the mechanical properties of the obtained networks and their biodegradability [38,39]. Even the thiol-yne chemistry was successfully applied to the covalent post-functionalization of polylactide surfaces to yield antibacterial surfaces [40].

In this work, we report the preparation of polymeric networks by thiol-yne photoclick reaction of ethynyl-functionalized polylactides (PLAs) and a low molar mass tetrathiol. Thus, the synthesis of a series of amorphous biodegradable ethynyl-ended polylactides (PLAs) has been approached by ring opening polymerization (ROP) of D, L-lactide and post-polymerization modification of the macromolecule ends. By using different hydroxy-functionalized initiators, PLAs with different architectures have been prepared that include several linear PLAs differing in the number of terminal ethynyl groups (**L_X-YNE** and **LB_X-YNE** with two and four ethynyl groups, respectively), and four arm star PLAs also having four terminal ethynyl groups (**ST_X-YNE**) (Scheme 1). These PLAs were combined with a commercial low molar mass tetrathiol, which has been previously used in the preparation of biomaterials [18,20,37,38], to prepare polymeric networks. The photopolymerization process was investigated by infrared spectroscopy (FTIR) and photocalorimetry (photo-DSC). Thermal properties of PLA precursors and resulting photocrosslinked networks were characterized by DSC. To assess their biocompatibility, cell viability experiments were performed on photocrosslinked thin films of these materials. In addition, the possibility to prepare two-dimensional patterned surfaces of these materials using direct laser writing (DLW) and cell culture on them was also explored.

2. Experimental

2.1. Materials

D, L-Lactide (Sigma-Aldrich) was dried under vacuum at 30 °C for 24 h before use. All other commercially available starting materials were purchased from Sigma-Aldrich and used as received. Pentaerythritol tetrakis(3-mercaptopropionate), which was also purchased from Sigma-Aldrich, was used as a crosslinker and Irgacure 369 (Ciba) was used as photoinitiator.

2.2. Synthesis of pentaerythritol tris(4-pentynoate)

4-(Dimethylamino)pyridinium *p*-toluenesulfonate (DPTS) (6.37 g, 20.39 mmol), pentaerythritol (1.85 g, 13.59 mmol), and 4-pentynoic acid (5.00 g, 50.97 mmol) were dissolved in dry THF (100 mL). The reaction flask was cooled in an ice bath and flushed with argon, then *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (9.77 g, 50.97 mmol) was added. The mixture was stirred at r.t. for 24 h under argon atmosphere. Then, the solvent was evaporated under reduced pressure. The crude product was dissolved in DCM and washed twice with water and once with brine. Finally, the organic layer was dried over anhydrous magnesium sulfate and the solvent was evaporated. The crude product was purified by flash column chromatography on silica gel using hexane/ethyl acetate (2:1) as eluent. Yield: 14%. IR (KBr, ν, cm^{-1}): 3503, 3292, 1735, 1188, 1089. ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 4.19 (s, 6H), 3.56 (d, 2H, $J = 6.9$ Hz), 2.62–2.48 (m, 12H), 2.39 (t, 1H, $J = 6.9$ Hz), 2.00 (t, 3H, $J = 2.6$ Hz).^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 171.8, 82.3, 69.6, 62.5, 60.8, 44.1, 33.4, 14.6. Anal. calcd for $C_{20}H_{24}O_7$: C, 63.82%; H, 6.43%. Found: C, 64.21%; H, 6.31%.

2.3. General polymerization procedure

A solution of the alcohol and 4-(dimethylamino)pyridine ($[alcohol]_0$:$[DMAP]_0 = 0.5$) in dry DCM was dried under argon atmosphere over 4 Å molecular sieves during 12 h. Lactide and this solution were added to a Schlenk flask that was then closed with a rubber septum. The flask was deoxygenated by three freeze-pump-thaw cycles and flushed with argon. The reaction was stirred at 35 °C until disappearance of the lactide signals in ^{1}H NMR spectrum. Then, the reaction mixture was washed twice with 1 N hydrochloric acid and once with brine. Finally, the organic layer was dried over anhydrous magnesium sulfate and the solvent was partially evaporated. The polymer was precipitated by adding the solution dropwise to cold methanol and further centrifugation at 4000 rpm for 30 min. The polymer was washed twice with cold methanol and dried under vacuum at room temperature. Yield: 32–80% (characterization data in Supporting Information).

2.4. General procedure for the esterification of hydroxy-functionalized polylactides with 4-pentynoic acid

4-(Dimethylamino)pyridinium *p*-toluenesulfonate (DPTS) (0.025 M), the hydroxy-functionalized PLA (0.05 M) and 4-pentynoic acid (0.1 M) were dissolved in dry DCM. The reaction flask was cooled in an ice bath and flushed with argon, then *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.1 M) was added. The reaction mixture was stirred at r.t. until the disappearance of the methine signal corresponding to the terminal lactide unit in ^{1}H NMR spectrum. Then, the reaction was washed twice water and once with brine. Finally, the organic layer was dried over anhydrous magnesium sulfate and the solvent was partially evaporated. The polymer was precipitated by adding the solution dropwise to cold methanol and further centrifugation at 4000 rpm for 30 min. The polymer was washed twice with cold methanol and dried under vacuum at room temperature. Yield: 42–80% (characterization data in Supporting Information).

2.5. Characterization techniques

FTIR spectra were obtained on a Bruker Vertex 70 FT-IR spectrophotometer using KBr pellets or polymer films deposited onto KBr pellets by casting, in the 4000-400 cm^{-1} region, with 4 cm^{-1} accuracy. Solution NMR experiments were carried out on Bruker Avance spectrometers operating at 400 MHz for ^{1}H and 100 for ^{13}C, using standard pulse sequences. Chemical shifts are given in ppm relative to TMS and the solvent residual peak was used as internal reference. Elemental analysis was performed using a Perkin-Elmer