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ORIGINAL PAPER

Synthesis and characterization of biocompatible poly(ethylene glycol)-functionalized polyurethane using click chemistry

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Abstract Functionalization of azide moiety-containing polyurethane (PU) with alkyne-decorated poly(ethylene glycol) (PEG) was accomplished by Cu(I)-catalyzed Huisgen [3+2] dipolar cycloaddition click chemistry. The azide moiety containing poly(ε-caprolactone)diol was synthesized by the copolymerization of α-chloro-ε-caprolactone with ε-caprolactone using ring-opening polymerization and further used for PU synthesis. The PEG-functionalized PU was characterized using FT-IR, NMR, and GPC. The hydrophilicity of synthesized polymers was measured using contact angle and water content tests. In vitro cytotoxicity results showed that the amphiphilic PEG-functionalized PU exhibits good biocompatible behavior, which supports the importance of functionalized PU for biomedical applications.

Keywords Polyurethane · Click chemistry · Poly(ethylene glycol) · Functionalization · Biocompatibility

Introduction

Due to its blood compatibility and good mechanical properties, polyurethane (PU) has become one of the most representative materials for medical devices [1]. PU block copolymer composed of hard and soft segments has been successfully used as a biomaterial ranging from the heart catheters to orthodontics applications [2–4]. Although PU shows good biocompatibility properties, even then much work has been done for functionalization of PU surface to prevent the blood platelets adhesion [5, 6] and enhancing the application area of PU. For further improvement of PU biocompatibility, researches are focused on functionalization of PU using different functionalities such as fibronectin coating [7], RGD peptides grafting [8],

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peptides [9], heparin [10, 11], plasma treatment [12], and chitosan grafting [13], etc. During the functionalization of polymer, the reactivity of functional groups may be affected by the functionalized moiety as well as the route of functionalization. The careful attention needs to pay for avoiding the secondary reactions and preparing the final application material. Recently click chemistry has emerged as a strategy from polymer synthesis to surface modification [14, 15].

Click reactions are modular, tolerant of wide range of functional groups, simple to perform, and very high yielding. Particularly, the Cu(I)-catalyzed [3+2] Huisgen dipolar cycloaddition between azides and alkynes provides 1,2,3 triazole ring with 1,4 regioselectivity and quantitative transformation under mild conditions. This reaction has been used for small molecule organic synthesis, dendrimers, hyperbranched polymers, and biologically derived macromolecular structures [16–18]. Apart from the synthetic promise, triazole moieties are also interesting conjugation entities as they are proven to be relatively stable to the metabolic degradation and the triazole ring also can participate in the hydrogen bonding [19], which enhance the biological importance of the click coupling.

Recently, Qin et al. [20] prepared the PU-based high speed electroactive devices using click chemistry approach. They synthesized PU using 2,4-toluene diisocyanate (TDI) and diols containing the chromophore moiety. Prez et al. [21] have prepared the linear PU having alkyne groups along the backbone, by applying two different alkyne-functionalized diols with diisocyanate.

In the present attempt, we focused to synthesize the biocompatible poly(ethylene glycol) (PEG)-functionalized PU via click chemistry. The coupling of azide moiety containing PU with acetylene functionalized PEG was achieved successfully using Cu(I)-catalyzed Huisgen [3+2] dipolar cycloaddition reaction. Among the most known biocompatible polymers, polyethylene glycol has received considerable attention of biochemists. Due to its excellent properties such as water solubility, amphiphilicity, and protein adsorption resistance, poly(ethylene glycol) is eminent for its biomedical applications [22].

Synthesis and functionalization of PU were characterized using nuclear magnetic resonance (NMR), attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) and contact angle measurement. The biocompatibility of the samples was measured using in vitro cytotoxicity measurement, confirmed with polarized optical microscopy (POM).

Experimental

Materials

4,4′-Methylenebis(phenylisocynate) (MDI) was purchased from Aldrich and dried in vacuum oven before use. 2-Chlorocyclohexane was purchased from Tokyo Chemical Industry Co. Ltd, Japan. *m*-Chloroperoxybenzoic acid (mCPBA), sodium azide, copper(II) sulfate. $5H_2O$, sodium ascorbate, ε-caprolactone, poly(ethylene glycol) methyl ether (MPEG-OH) of Mn 2,000 g/mol and stannous octate were received from Aldrich. 1,4-Butanediol (BD) was purchased from Duksan Chemical,

Korea and used after drying in vacuo at 60 °C overnight. Poly(ε-caprolactone)diol (PCL) with molecular weight of 3,000 g/mol was received from Solvay Co., UK. *N,N*-Dimethylformamide (DMF) (Junsei Chemical, Japan) was used after freshly purified. All other reagents and solvents were purchased from commercial suppliers and purified by distillation before using.

Synthesis of azide moiety containing PU

A series of azide moiety containing PU was synthesized as represented by Fig. 1. Firstly the α-chloro ε-caprolactone monomer was synthesized by an oxidation of α-chlorocyclohexane in the presence of *m*-chloroperoxybenzoic acid as reported earlier [23]. Ten grams (41 mmol) of *m*-chloroperoxybenzoic acid (mCPBA—70%) was added to a solution of 5 g (38 mmol) of 2-chlorocyclohexanone in 60 mL of dichloromethane at room temperature. After 96 h, the reaction flask was cooled to −20 °C in order to precipitate *m*-chlorobenzoic acid. After filtration and purification, the monomer was characterized by ^{1}H-NMR (Fig. 2). The synthesized monomer was copolymerized with ε-caprolactone using ring-opening polymerization at 110 °C temperature for 24 h in the presence of ethylene glycol as an initiator and $Sn(Oct)_2$ as a catalyst to yield poly(α-chloro-ε-caprolactone-co-ε-caprolactone) (αCl-εCL-co-εCL)diol, containing 35 mol % of α-chloro-ε-caprolactone. The molecular weight of poly(αCl-εCL-*co*-εCL)diol was monitored by gel permeation chromatography (GPC), containing a molecular weight of 3,000 g/mol. This poly(αCl-εCL-*co*-εCL)diol was further treated with sodium azide to get the poly(αN_3-εCL-*co*-εCL)diol and was confirmed by ^{1}H-NMR measurement (Fig. 3). The resultant poly(α-azide-ε-caprolactone)diol was treated with MDI using BD as a chain extender, containing 30–50 wt% hard segment content. A catalyst-free two steps process was applied for preparation of PU. The required amount of diol solution in dry DMF was added dropwise through the dropping funnel into MDI

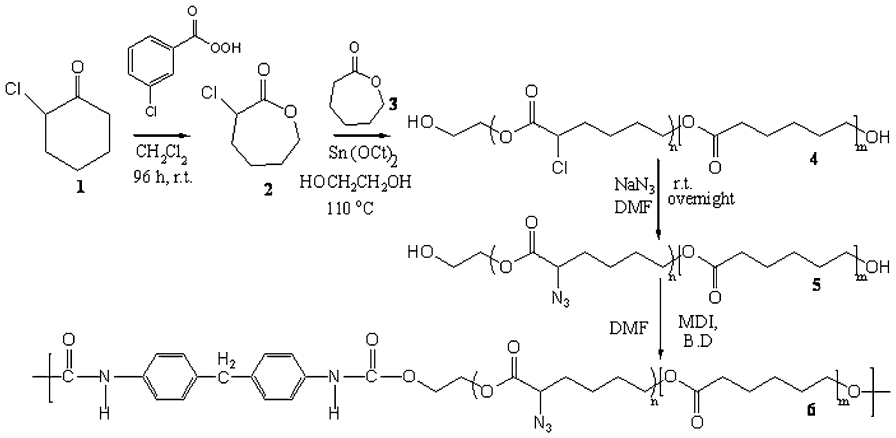

Fig. 1 Synthesis of azide moiety containing polyurethane

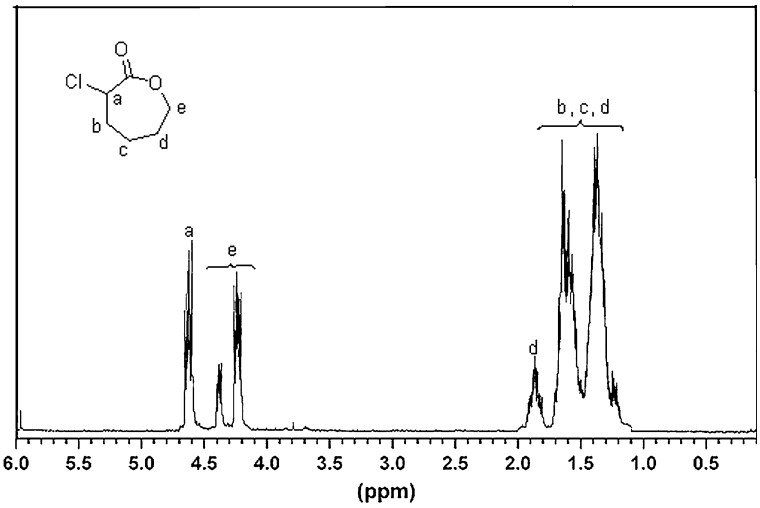

Fig. 2 [1]H-NMR spectra of α-chloro-ε-caprolactone

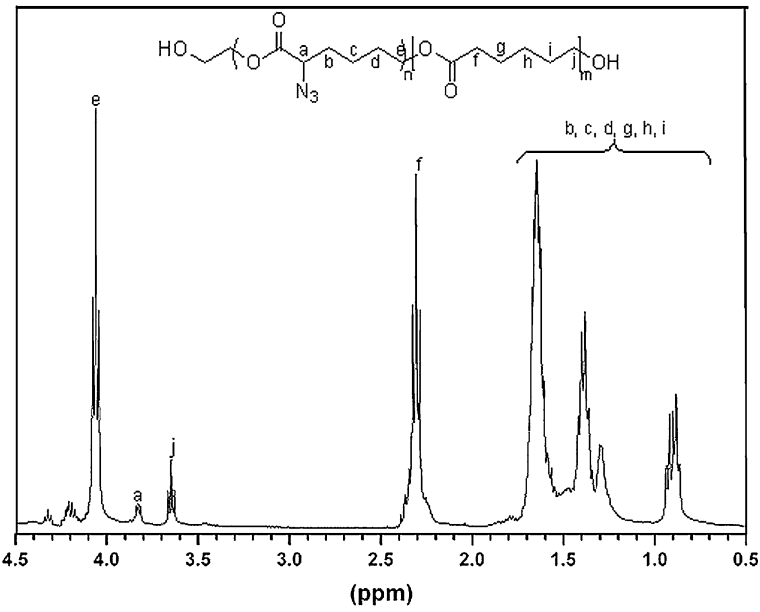

Fig. 3 [1]H-NMR spectra of αN_3-ε*CL-co-*ε*CL-diol

solution in dry DMF in the vessel, and then the temperature was raised and maintained at 65 °C for 3 h. After completion of prepolymer, BD was slowly added to the reaction mixture and stirred at 85 °C overnight. After completion of reaction, the final product was washed with distilled water and dried under vacuum oven.

Synthesis of alkyne moiety-functionalized PEG

The alkynated PEG was synthesized using poly(ethylene glycol) methyl ether (MePEG-OH) as a starting material by following the procedure as reported earlier [24]. First, MePEG-OH (10 g, 5 mmol) was dissolved in dichloromethane (35 mL). A solution of 4-pentynoic acid (0.59 g, 6 mmol) in dichloromethane (6 mL) was added, and the solution was cooled in an ice-water bath. A solution of dicyclohexylcarbodiimide (1.14 g, 6.6 mmol in 10 mL of dichloromethane) was added to the reaction mixture, followed by a solution of 4-(dimethylamino)pyridine (0.25 g in 5 mL of dichloromethane). The reaction mixture was kept in the ice-water bath for 10 min and was stirred overnight at room temperature. The precipitated dicyclohexylcarbamide was filtered off and washed with dichloromethane. The solution was precipitated into a large excess of cold diethyl ether and cooled in a refrigerator at −18 °C for 20 min. The products were then filtered and dried in a vacuum oven overnight at 40 °C. The yield of alkyne moiety-functionalized PEG was 90%. The functionalized PEG was analyzed using ^{1}H-NMR spectroscopy ($CDCl_3$, δ, in ppm): 4.24 (CH_2OOC), 3.62 (OCH_2CH_2O), 1.96 ($C\equiv CH$), 3.30 (CH_3O), and 2.56 ($OOCCH_2$).

Synthesis of PEG-functionalized PU using click chemistry

The click coupling reaction was performed between alkynated polyethylene glycol and azide moiety-functionalized PU (Fig. 4). First the DMF solution of alkynated PEG (2 equivalent of alkyne functions) was added to the stirred DMF solution of azide functionalized PU (1 equivalent of azide functionality) at 60 °C, followed by the addition of $CuSO_4{\cdot}5H_2O$ and sodium ascorbate, respectively (0.1 equivalent each according to the alkyne content), and the reaction stirring was continued for overnight at 60 °C. The excess of PEG was used to ensure the efficient click coupling. The resulting material was precipitated in ether and dried under vacuum.

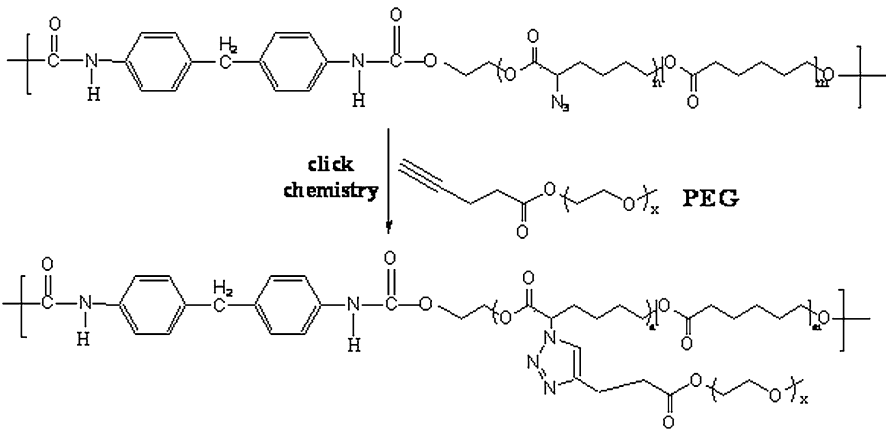

Fig. 4 Functionalization of PU with PEG using click chemistry

The films were prepared by dissolving the PEG-functionalized PU in dimethyl acetamide. The polymer solutions (10% w/v) were poured into clean Petri dishes and the films were prepared by evaporating the solvent at 35 °C for 4 days under vacuum.

Measurements

The FT-IR spectra were recorded using Jasco FT-IR 300E with an attenuated total reflectance (ATR) method. ATR FT-IR spectra were collected using germanium (Ge) crystal as internal reflection element at 45° of incidence angle. The spectra were scanned without any gas flow, at a resolution of 4 cm^{-1}, and 40 scans for each measurement. ^{1}H-NMR spectra were recorded in Bruker 400 MHz NMR spectrometer using TMS as the internal standard and d_6-DMSO as the solvent with 16 transients and 1 s of relaxation time.

Contact angle measurements were performed using advancing contact angle measuring system (RAMHART, Model 100 US). Measurements were carried out using the sessile drop method with 0.5 μL overnight using vacuum oven. The water content of the pristine PU and PEG-functionalized PU films were determined by immersing the films in deionized water. After 24 h, the films were taken out and dried with tissue paper. The water content in % was calculated by Eq. 1

$$\text{Water content}(\%) = [W(\text{wet}) - W(\text{dry})]/W(\text{wet}) \times 100 \quad (1)$$

where W(wet) and W(dry) are weight of the wet and dry samples, respectively [25]. The cytotoxicity of the PEG-functionalized PU was evaluated by minimal essential medium (MEM) testing. Cell attachment was performed using 5-mm polymer films, UV sterilized for 15 min.

Results and discussion

Azide moiety containing PUs were coupled with alkyne-functionalized PEG through 1,2,3 triazole ring using Cu(I)-catalyzed Huisgen [3+2] cycloaddition click chemistry between azide and alkyne. Initially, α-chloro-ε-caprolactone was synthesized successfully by the oxidation of α-chlorocyclohexane in the presence of *m*-chloroperoxybenzoic acid and further copolymerized with ε-caprolactone in the presence of ethylene glycol as an initiator and Sn(Oct)2 as a catalyst, to get poly(αCl-εCL-*co*-εCL)diol with controlled molecular weight 3,000 g/mole. The molecular weight was controlled to get the good shape memory properties in PU [26], which is responsible for reversible phase transformation of shape memory polymers as the smart materials for biomedical applications [2]. The synthesized poly(αCl-εCL-*co*-εCL)diol was further treated with sodium azide to get the poly(αN3-εCL-*co*-εCL)diol. The resulted poly(α-azide-ε-caprolactone)diol was treated with MDI using 1,4 butanediol as a chain extender, containing 30–50 wt% hard segment content (Table 1). For studying the effect of PEG-functionalization on PU, the 40 wt% hard segment PU was synthesized using the commercial PCL-diol (PU-40) of the same molecular weight. ^{1}H-NMR spectra confirm the synthesis of PU as shown in Fig. 5.

Table 1 Compounding formulations of PU-based materials

Sample code	Composition			Hard segment content (%)	Molecular weight (g/mol)
	MDI (mol %)	PCL-diol (3,000 Mw) (mol %)	BD (mol %)		
PU-40	6	1	5	40	60,183
PUG-30[a]	4	1	3	30	83,000
PUG-40[a]	6	1	5	40	63,000
PUG-50[a]	9	1	8	50	56,000

[a] Samples containing the poly(α N_3-εCL-*co*-εCL)diol

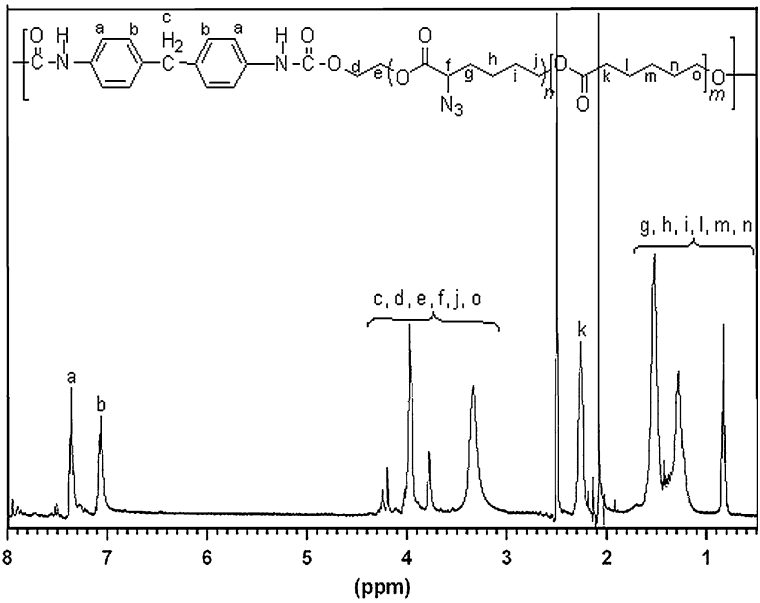

Fig. 5 ^{1}H-NMR spectra of polyurethane

The ^{1}H-NMR analysis indicates the peaks at 3.87 (C*H*–N_3), 1.55 (–OCH$_2$*CH*$_2$), 3.35 (–OC*H*$_2$CH$_2$), 3.87 (Ph–*CH*$_2$–Ph), 4.15 (C(= O)O*CH*$_2$), 7.10 (phenyl), and 7.35 (phenyl).

Figure 6 presents the FT-IR spectra of the PU synthesized in this study. It can be seen from this figure that the reaction between –O–H group of diol and –N=C=O group of diisocyanate was completed successfully. The appearance of a narrow absorption peak at 3,340 cm^{-1} corresponds to the –N–H stretching vibration, as well as disappeared the IR absorption for –O–H stretching and –N=C=O stretching vibration near 3,400 and 2,274 cm^{-1}, respectively. The IR peaks at 2,930 and 2,860 cm^{-1} correspond to aliphatic –C–H stretching. The absorption peaks at 1,725 and 1,650 cm^{-1} correspond to –C=O in ester and C=C stretching, respectively, and the IR peak at 1,500–1,575 cm^{-1} is due to –N–H bending [27]. The peak around

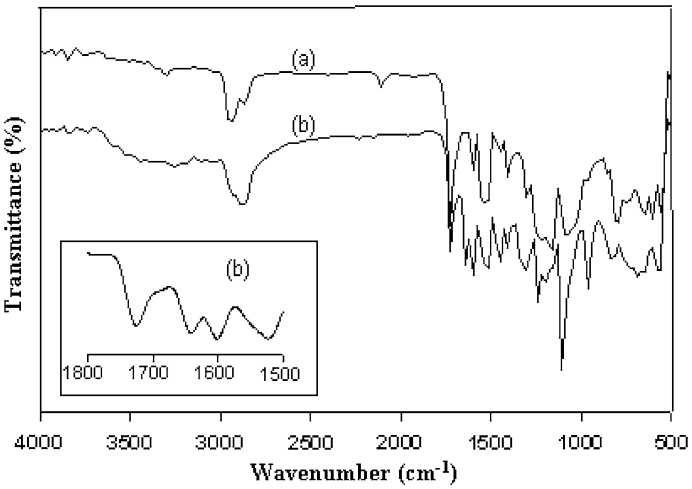

Fig. 6 FT-IR spectra of azide moiety containing PU (*a*) and PEG-functionalized PU (*b*)

2,100 cm^{-1} confirms the azide moiety containing PU. FT-IR results also confirm the successful functionalization of PU with PEG, where the functionalization of PU is confirmed by the appearance of new characteristic peak around 1,640 cm^{-1} due to the triazole ring formation and the disappearance of peak at 2,100 cm^{-1} [28]. The broad peak at 3,400 cm^{-1} may be due to the physically adsorbed water molecules. The functionalization of PU is also confirmed by ^{1}H-NMR, where the appearance of NMR peak at 8.2 ppm corresponds to the proton in triazole ring and disappearance of peak at 3.87 ppm (azide) [21], further supports the PEG-functionalized PU (Fig. 7).

The PEG-functionalized PU was also characterized by contact angle measurement (Table 2). The contact angle of functionalized PU decreased in comparison of pristine PU. The functionalized PU samples show more hydrophilicity than the pristine PU. The contact angle also increases with an increase of the hard segment content. As shown in Table 2, the water content increased from 11% for non-functionalized PU to 25% for PEG-functionalized PU. It indicates that the water content increases due to an increase of the hydrophillicity with the content of PEG functionalities on PU. The water content further decreased to 21% as the hard segment content increased in PU. The water content with the hard segment content may be dependent on the phase separation between hard and soft segments of PU; however, the main reason may be due to a relative amount of PCL as soft segments against hard segments [29]. That is, as the PEG moiety is attached with PCL in PU component, the PEG functionality decreases with the increase of hard segment content. As a result, the hydrophillicity decreases with the increase of hard segment content of PU.

To evaluate the cytotoxicity of non-functionalized and functionalized PUs, the fibroblasts growth on polymer surface was tested out. The fibroblasts (L929 mouse fibroblasts obtained from American Type Cell Culture) with 10% horse serum (pH 7.0) with final concentration of 5 mg/mL were seeded on the polymeric samples.

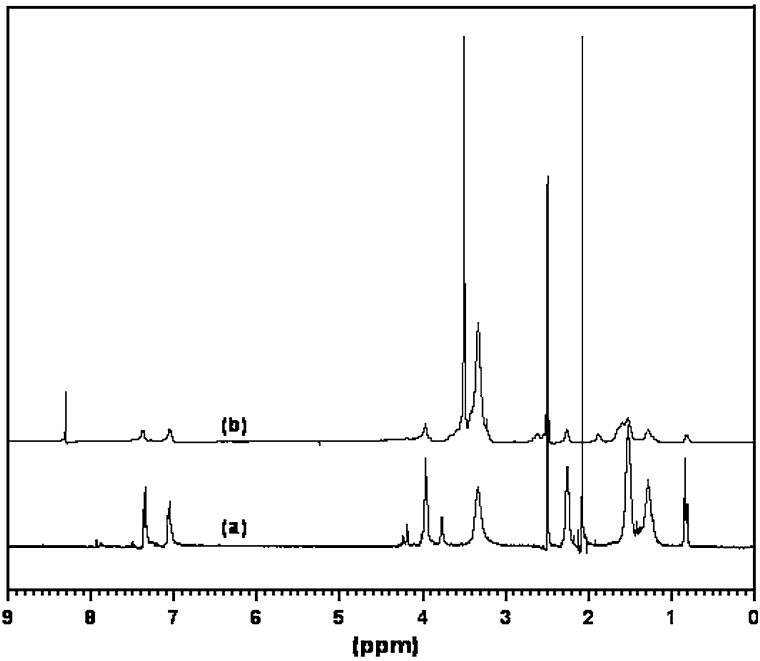

Fig. 7 ^{1}H-NMR spectra of azide moiety containing PU (*a*) and PEG-functionalized PU (*b*)

Table 2 Surface properties of pristine and functionalized PUs

Sample code	Water content (%)	Water contact angle (°)
PU-40	11.3	77 ± 3
PUG-30[a]	25.3	48 ± 3
PUG-40[a]	23.7	55 ± 3
PUG-50[a]	21.6	61 ± 3

[a] Samples containing the poly(α N_3-εCL-*co*-εCL)diol

After 24 h incubation, the monolayer was observed by POM. The optical images of Fig. 8 show clearly that all the samples are compatible for cell attachment. The pristine PU has less cell attachment than the functionalized PU. The cell attachment decreases with increasing the hard segment content, which further supports the effect of PEG concentration on hydrophillicity and biocompatibility of materials.

Conclusions

The controlled functionalization of PU with poly(ethylene glycol) was achieved successfully via click chemistry, which followed the reaction of azide moiety containing PU and alkyne moiety containing poly(ethylene glycol). The click chemistry for functionalization of polymer surface is a simple route, which allows

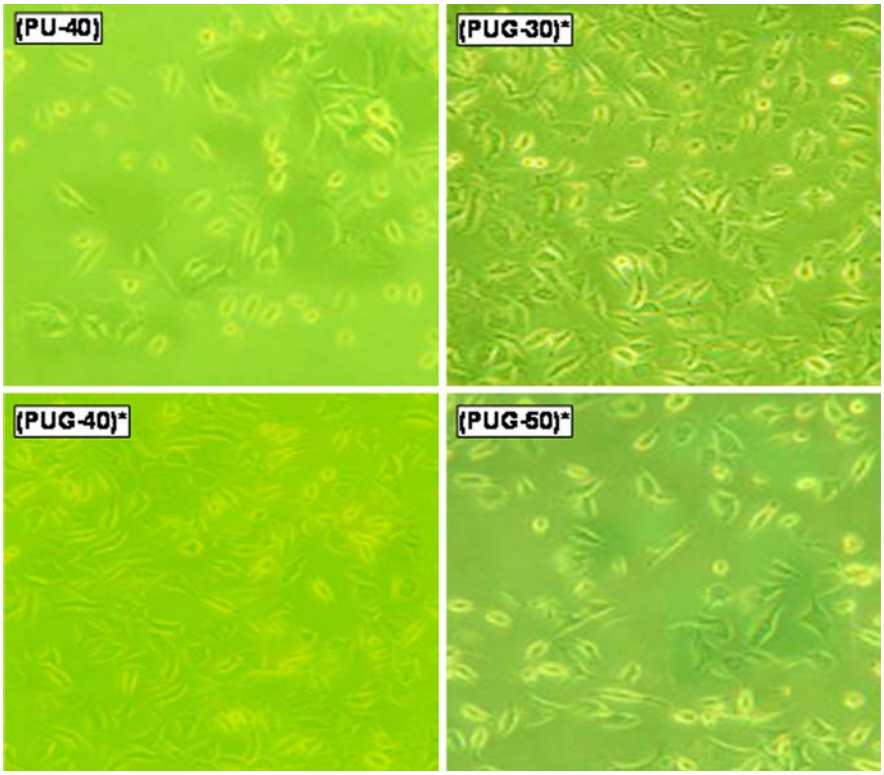

Fig. 8 The POM images obtained from minimal essential medium cell culture experiments using fibroblasts

the mild reaction condition, rapid, and versatile synthesis of poly(ethylene glycol)-functionalized PU. The functionalized PU shows good both biocompatibility and hydrophillicity, which represents the potential biological applications of functionalized PU.

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