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Cite this: *RSC Adv.*, 2015, 5, 11284

# Manipulation of polycarbonate urethane bulk properties *via* incorporated zwitterionic polynorbornene for tissue engineering applications

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Elastomeric crosslinked materials based on polycarbonate urethane (PCU) and zwitterionic polynorbornene were designed by thiol–ene click-chemistry and crosslinking reaction. The zwitterionic polynorbornene poly(NSulfoZI) with functionalisable double bonds was first treated with L-cysteine *via* thiol–ene click-reaction and subsequently formed a crosslinked structure upon treatment with PCU in the presence of a small amount of hexamethylene-1,6-diisocyanate as a crosslinking agent. The obtained materials possessed improved tensile strength (14–20 MPa) and initial modulus (8–14 MPa). All of these materials showed high breaking strain ( $\epsilon_b$  740–900%) except the material with a high poly(NSulfoZI) content of 28% ( $\epsilon_b$  470 ± 80%). The biodegradability of these materials was enhanced compared to blank PCU, as demonstrated by testing in PBS for five weeks. Moreover, the cytocompatibility was studied by MTT assay. The adhesion and proliferation of endothelial cells (EA.hy926) over a one-week period indicated that cell growth on these designed material surfaces was enhanced. Therefore, these zwitterionic polynorbornene-modified PCU-based materials could be suitable candidates for tissue engineering applications.

Received 15th November 2014  
Accepted 5th January 2015

DOI: 10.1039/c4ra14608e

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## 1. Introduction

Biodegradable polyurethane elastomeric scaffolds are of great interest in soft tissue repair and regeneration.<sup>1</sup> The compliance mismatch between artificial grafts and the elastic nature of arteries could result in intimal hyperplasia (IH) and unwanted inflammatory reactions.<sup>2,3</sup> Therefore, the proper design of these materials for tissue engineering (TE) applications is still challenging. Some biodegradable scaffolds release acidic products during degradation, which lower the pH of the surrounding tissue fluid and hence greatly inhibit the rate of extracellular matrix synthesis.<sup>4</sup> Synthetic materials should be able to promote vascular cell adherence (endothelial cells, vascular smooth muscle cells, *etc.*) without inducing a chronic immune

or inflammatory response after the implantation. In addition, such materials should possess mechanical properties tailored for the specific tissue type and exhibit a wound-healing phenotype at the application site.<sup>5</sup>

The advantage of polycarbonate urethanes (PCUs) for elastic soft TE and other biomedical applications results from their favourable mechanical properties (strength, elastic modulus, *etc.*), chemical properties, durability, tolerance during the healing process and controlled degradation rates that allow the retention of physical properties throughout the remodelling period, even with high porosity.<sup>6–10</sup> However, their exclusive use in TE products is limited because of the unsatisfactory cell adherence and proliferation, hydrophobic nature, biologic inertness and lack of modification sites. To circumvent these obstacles, several strategies have been developed, such as hydrophilic modification of PCUs using poly(ethyleneglycol) (PEG) and, more recently, zwitterionic polymers with high wettability, which could affect the thrombogenic nature and suitability of the surface for cell seeding.<sup>11–13</sup> Zwitterionic polymers, which are highly hygroscopic in nature and have lipid-like biomimetic features, suppress clot formation.<sup>14</sup>

The possibility of precisely tailoring biodegradability and the inherent reproducible nature has shown that a degradable PCU containing anionic dihydroxyl oligomers could be processed to improve cell attachment.<sup>15–17</sup> Recently, a stimuli-sensitive PCU for drug delivery systems was reported with a specific focus on its degradation and biocompatibility both *in vitro* and *in vivo*.<sup>18</sup>

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Poly( $\epsilon$ -caprolactone) and modified PEG have been used in the preparation of PCU-film with a low level of terminal amino groups.<sup>19</sup> The content of the PEG moiety could modulate the mechanical properties such as tensile strength (16–21 MPa), young modulus (2.6–4.8 MPa) and elongation at break (1100–1450%).<sup>20</sup> Wagner *et al.*<sup>21–23</sup> have prepared a family of unique poly(ester-urethane) ureas from poly( $\epsilon$ -caprolactone)diol (PCL), PEG, and 1,4-diisocyanatobutane with either lysine ethyl ester or putrescine as the chain extender. These materials exhibited high tensile strength (8.0–20 MPa) and breaking strain (325–560%). In this case, the PCL block length directly affected the initial modulus and tensile strength, as manifested by the high crystallinity. Moreover, the materials prepared from longer PEG blocks showed faster degradation rates than those prepared from shorter ones. Segmented PCUs synthesised from PCL and 4,4-methylene bis(cyclohexyl isocyanate) using either L-glutamine or ascorbic acid as the chain extender have shown accelerated degradation in acid and alkaline conditions.<sup>24</sup> In another report, the co-spun film of PCL/1,4-diisocyanatobutane/putrescine polymers and collagen showed low tensile strengths (2–13 MPa). The incorporation of collagen decreased the tensile strength and initial modulus while facilitating the adhesion of smooth muscle cells.<sup>25,26</sup> Soft segments, chain extenders and diisocyanates are the main components of elastomeric polyurethane materials and can directly affect the hydrophilicity, water uptake and calcification process.<sup>27</sup> Minor differences in the components of each segment might be amplified because of the repetitive nature of polymers.<sup>28</sup>

To date, PEG has been used to modify PCU materials in order to enhance their biodegradable or cell adhesive properties. However, reports on the incorporation of zwitterionic functionalities on the PCU elastomer backbone are rare. Poly(NSulfoZI) is an important class of zwitterionic materials with clickable functionality that exhibit non-fouling properties and resistance to protein adsorption.<sup>14,29</sup> Furthermore, they resemble natural functional groups such as phosphatidylcholines and are hence analogues to mammalian cell membranes since they both present bilayer-structures with the unique zwitterion functional groups exposed on the surface of the bilayer.<sup>30</sup> In the present work, we fabricated crosslinked materials from PCU elastomer and hydrophilic zwitterionic poly(NSulfoZI) in order to manipulate the biodegradability and mechanical properties as well as to endow them with good cytocompatibility.

## 2. Experimental section

### 2.1. Materials

$\beta$ -Mercaptoethanol (98%), L-cysteine (98%), 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) (98%) and  $\alpha,\alpha$ -dimethoxy- $\alpha$ -phenylacetophenone (Irgacure 651, DMPA, 99%) were obtained from Tianjin Heowns Biochemical Technology Co., Ltd. Fluorescein diacetate (FDA) was obtained from Sigma-Aldrich. Di-*n*-butyltin dilaurate (DBTDL), hexamethylene-1,6-diisocyanate (HDI), 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) and all other solvents of analytical grade were obtained from Tianjin Jiangtian Chemical Technology Company Ltd, China. PCU (Chronoflex C,

$M_n = 110$  kDa) was purchased from Cardio International Incorporated, USA.

### 2.2. Polymer synthesis

The zwitterionic polymer poly(NSulfoZI) was synthesised according to our previously established protocol using the same design principle of ring-opening metathesis polymerisation (ROMP); the structural characterisation of the polymer was also reported in the same study.<sup>14</sup>

### 2.3. Preparation of cross-linked PCU film

**2.3.1. Preparation of L-cysteine-functionalised poly(NSulfoZI) by “click-reaction”.** Different amounts of zwitterionic polymer poly(NSulfoZI) (25, 50, 100 and 200 mg) were separately dissolved in 10 mL HFIP-DCM (3 : 1). Subsequently, L-cysteine solution (1 mM, 200  $\mu$ L) and DMPA (2%, 20 mg) were added and stirred until completely dissolved. The mixture was purged with nitrogen for 30 min. The click reaction was carried out under UV exposure using a 365 nm UV-lamp. Finally, the reaction mixture was repeatedly precipitated in anhydrous ether in order to remove the unreacted reagents and then centrifuged at 50 rpm to obtain a solid precipitate. The precipitated product (amine-functionalised) was dried and then re-dissolved in dry THF to produce a homogeneous solution.

**2.3.2. Preparation of PCU-HDI stock solution.** PCU solution (10 wt%) was prepared in DMF in a 100 mL round-bottom flask and kept at 37 °C under continuous stirring for one week. Subsequently, 7 g of PCU solution was mixed with 0.173 mg of HDI crosslinker and stirred until a homogeneous solution was obtained. This solution was used immediately.

### 2.4. Film casting and curing

The as-prepared solutions obtained in Sections 2.3.1 and 2.3.2 were mixed and stirred continuously in the presence of 20  $\mu$ L DBTDL as catalyst at 50 °C for 90 min. This final solution was casted onto a ceramic mould (about  $3.5 \times 3.5$  cm<sup>2</sup>), heated at 70 °C until the solvent was completely evaporated and further cured at 90 °C for 6–7 h. These films containing different weight percent ratios of poly(NSulfoZI) (0%, 3.5%, 7%, 14% and 28%) were symbolically represented as PCU-blank, PCU-3.5, PCU-7, PCU-14 and PCU-28, respectively. Finally, the films were cleaned with ethanol and water, dried at 37 °C in a vacuum dry box until constant weight and stored at –40 °C for further analysis.

### 2.5. Water uptake (%)

Pre-weighed ( $w_0$ ) samples of the different PCU films were incubated in PBS (pH 7.4) at 37 °C for 24 h, taken out, dried with blotting paper and re-weighed ( $w_1$ ). The percent water uptake was calculated as  $(w_1 - w_0)/w_0 \times 100\%$ . At least three samples were tested for each material.

### 2.6. *In vitro* biodegradability

Degradation tests were performed in PBS (pH 7.4) at physiological temperature (37 °C). In brief, weighed samples ( $w_0$ ) of all

films were incubated in 5 mL PBS. The PBS was changed twice per week during the degradation tests. No change in pH was observed during the degradation. The samples were removed from PBS after the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> week of immersion, rinsed three times with deionised water, dried at 37 °C in vacuum and then re-weighed ( $w_2$ ). The mass remaining values were calculated by  $w_2/w_0 \times 100\%$ .

## 2.7. Cell culture

Human endothelial cell hybridoma line EA.hy926 cells were purchased from American Type Culture Collection and cultured in high glucose DMEM supplemented with 10% FBS in 5% CO<sub>2</sub> atmosphere at 37 °C. On the next day, the non-adherent cells were discarded, and the adherent cells were cultured to confluence; the culture medium was changed every three days.

## 2.8. In vitro cytotoxicity

The cytotoxicities of the PCU films were evaluated by MTT assay using a blank as control.<sup>46</sup> Briefly, EA.hy926 cells ( $1 \times 10^4$  cells per well) were seeded in a 96-well plate and cultured for 24 h until 80–90% confluence. The medium was then replaced with serum-free medium. After 12 h, the medium was replaced with fresh growth medium (10% FBS DMEM).

PCU films (three for each sample) were added into the medium. After 1, 3 and 7 days of culturing, the supernatant was discarded. After the addition of 20  $\mu$ L of MTT solution (5 mg mL<sup>-1</sup>) to each well, formazan crystals were allowed to form for another 4 h. The medium was then removed carefully, 150  $\mu$ L of dimethylsulfoxide was added to each well and the plate was oscillated at low speed on a volatility instrument for 10 min. Optical density (OD) was measured by an ELISA reader (Titertek multiscan MC) at a wavelength of 490 nm.

The relative cell viability (%) was calculated using the following formula (OD<sub>490</sub> = the absorbance value of experimental wells minus zero wells; avg(OD<sub>490</sub>C') = the average absorbance value of corrected control wells):

$$\text{Relative cell viability (\%)} = \frac{\text{OD}_{490}}{\text{avg}(\text{OD}_{490}C')} \times 100$$

## 2.9. Characterisation

Contact angles were measured by the sessile drop method using a video contact angle instrument (Kruss Easy Drop goniometer, Germany) at room temperature. The contact angle was calculated with the AutoFAST algorithm within the image analysis software.

The mechanical behaviour of the cross-linked PCU-film was investigated with a tensile testing machine (M350-20KN, Testmetric, UK) equipped with a 100 N load cell at a crosshead speed of 10 mm min<sup>-1</sup> in the ambient environment. All the data reported for the tensile modulus, tensile strength and elongation at break represent the average of six tests and were analysed statistically by the *t*-test method.

The chemical compositions of the crosslinked films were determined by PHI-1600 X-ray photoelectron spectroscopy (XPS) with an Mg K $\alpha$  X-ray source at  $2 \times 10^{-8}$  Torr. Low-resolution survey scans were performed at 187.85 eV with a step of

0.8 eV, and high-resolution survey scans were done at pass energy of 29.35 eV with a step of 0.25 eV. Core-level signals were obtained at a photoelectron take-off angle of 45°, and 1s spectra bands were deconvoluted into sub-peaks using the XPSPEAK41 spectrometer software.

<sup>1</sup>H NMR data of the degradation products were typically collected in D<sub>2</sub>O solvent at 25 °C using ECA-500 spectrometers at 400 MHz.

The adherent cells on the different surfaces were stained with FDA and photographed by fluorescence microscopy (Fluorescence OLYMPUS U-RFLT50; microscopy Olympus DP72). Random pictures were taken at six different places of each sample.

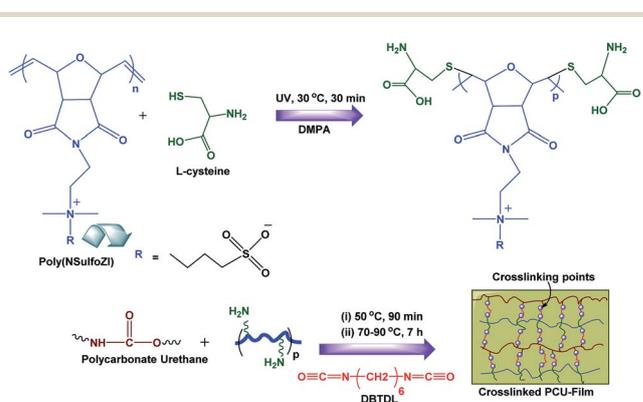
## 2.10. Statistical analysis

All experiments were performed at least three times. Quantitative data are presented as the mean  $\pm$  S.D. Statistical comparisons were made with the Student's *t*-test. *p*-Values <0.05 were considered to be statistically significant.

# 3. Results

## 3.1. Preparation of crosslinked PCU-films

The various crosslinked PCU films with different weight ratios (wt%) of zwitterionic polynorbornene poly(NSulfoZI) were prepared by a two-step method using a combination of click chemistry and crosslinking reaction (curing) at different temperatures and time schedules (Scheme 1). In the first step, the thiol reagent (*L*-cysteine) was reacted with poly(NSulfoZI) with functionalisable double bonds *via* a thiol-ene click-reaction in the presence of DMPA as photo-initiator. Subsequently, the resulting amine-functionalised precursor was treated with PCU solution in the presence of HHDI crosslinker. The stepwise reaction was optimised such that the reaction mixture was stirred at 50 °C for 90 min during the first step, heated at 70 °C until all solvent evaporated and then cross-linked at 90 °C for 7 h. The effects of different poly(NSulfoZI) ratios on the overall properties (mechanical, biodegradable and cell adhesive) of the crosslinked film have been investigated,



Scheme 1 Schematic illustration of the preparation of crosslinked PCU materials using photo-initiated thiol-ene click-reaction and crosslinking.

while the PCU-elastomer and crosslinker (HDI) ratios were kept constant.

### 3.2. XPS of crosslinked PCU film surfaces

The surface chemical compositions of the crosslinked PCU films were analysed by XPS (Table 1). The sulphur (S 2p) peak indicated the successful incorporation of L-cysteine-linked poly(NSulfoZI) into the crosslinked materials. However, due to the positional preference, the low level of zwitterion and the further attenuation of electron signal in the film matrix, the sulphur content of PCU-3.5 approaches the noise level signal. In contrast, the rest of the samples showed a clear S 2p peak.

### 3.3. Hydrophilicity

The surface hydrophilicities of the different PCU films were evaluated by measuring water contact angles (WCA; Table 1). The blank film surface (PCU-blank) exhibited a highly hydrophobic characteristic with a water contact angle of  $106 \pm 6^\circ$ . With increasing hydrophilic zwitterion poly(NSulfoZI) content, the WCA values of the crosslinked films showed a continuous decreasing trend. As the weight ratio of zwitterion increased, the hydrophilicity of the crosslinked films increased significantly. Hence, these results indicated that the thiol-ene click-reaction is an effective approach to prepare materials with suitable hydrophilic features.

### 3.4. FT-IR analysis

The FT-IR spectra of the different crosslinked PCU films (Fig. 1) indicated the characteristic peak of thio-ether groups (C-S-C) at around  $688 \text{ cm}^{-1}$ , which was manifested prominently in the crosslinked PCU-28.<sup>31</sup> The absorption peak at  $1103 \text{ cm}^{-1}$  increased with increasing poly(NSulfoZI) content in the films.<sup>32</sup> The peak at  $1242 \text{ cm}^{-1}$  (C-O)<sup>11</sup> also increased, and a new zwitterion peak (-C-O) at  $1191 \text{ cm}^{-1}$  was observed.<sup>33</sup>

The ester carbonyl (-C=O) peak at  $1738 \text{ cm}^{-1}$  was shifted slightly and dominated by a carboxylic acid (-COOH) peak at about  $1696 \text{ cm}^{-1}$ , which confirmed the successful introduction of the poly(NSulfoZI) segment into the crosslinked PCU film.<sup>34</sup> A new characteristic vibration peak appeared at  $1045 \text{ cm}^{-1}$ , which was attributed to the sulfonate groups of zwitterions in these films.<sup>33</sup>

Table 1 XPS chemical composition and water contact angle of the crosslinked PCU films

Sample ID	C 1s (%)	O 1s (%)	N 1s (%)	S 2p (%)	Water contact angle ( $^\circ$ )
PCU-blank	69.7	19.6	10.7	0.0	$106 \pm 5$
PCU-3.5 <sup>a</sup>	84.4	14.8	0.7	0.1	$89 \pm 3$
PCU-7	83.9	14.6	1.2	0.3	$84 \pm 4$
PCU-14	83.4	15.0	1.2	0.4	$77 \pm 3$
PCU-28	86.3	10.7	2.4	0.6	$73 \pm 4$

<sup>a</sup> The number (3.5, 7, 14 or 28) in each sample ID represents the weight percentage ratio of zwitterion [poly(NSulfoZI)] in the crosslinked materials, while for PCU-blank it is 0.

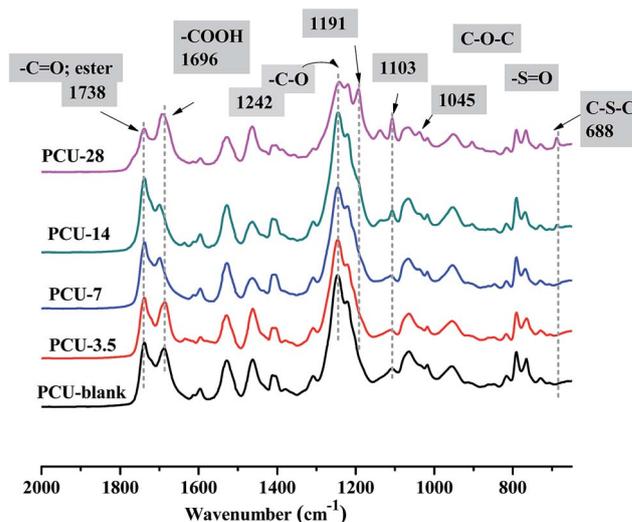


Fig. 1 FT-IR spectra of PCU-blank and crosslinked PCU films.

### 3.5. Mechanical properties

The mechanical characteristics of these crosslinked PCU films (Table 2) indicated that the material elastic properties were improved by increasing the weight ratio of the zwitterionic poly(NSulfoZI). The blank film (PCU-blank) showed a tensile strength of  $12 \pm 4 \text{ MPa}$ , which gradually increased with the incorporation of zwitterionic polymer; among all samples, the maximum tensile strength was observed for PCU-14 ( $20 \pm 3 \text{ MPa}$ ; Fig. 2).

Upon doubling the percent ratio of zwitterion (PCU-28), the tensile strength suddenly dropped to  $10 \pm 2 \text{ MPa}$ . The breaking strain exhibited the same; the breaking strain of PCU-blank ( $740 \pm 60\%$ ) was enhanced by increasing the zwitterion ratio, reaching a maximum for PCU-14 ( $900 \pm 40\%$ ), and then dropped to  $470 \pm 80\%$  for PCU-28. This may be explained by the crosslinking reaction at different temperatures, as facilitated by HDI between PCU and the amine-terminated product.

### 3.6. Water uptake (%)

The water uptake of the different crosslinked PCU film samples incubated for 24 h (Fig. 3) showed an increasing trend with zwitterion ratio in the films. The water uptake value of PCU-28 was the highest ( $9.3 \pm 1.7\%$ ), while that of the blank film (PCU-blank) was only  $2.1 \pm 0.5\%$ . The reason for this is obviously related to the significant amount of hydrophilic zwitterions incorporated into the crosslinked films, allowing the entrapment of more water, which is also in accordance with the WCA values (Table 1).

### 3.7. Degradability study

The degradation of the different crosslinked PCU films was studied in PBS (pH 7.4; Fig. 4) for five weeks; a decrease in percent mass remaining with time was observed. This trend was regular and linear with the zwitterion weight ratios in the crosslinked film samples. The percent mass remaining for

Table 2 Mechanical properties of the crosslinked PCU films

Sample ID	Tensile strength (MPa)	Breaking strain (%)	Initial modulus (MPa)	Tensile fracture strength (MPa)
PCU-blank	12 ± 4	740 ± 60	3 ± 1	13 ± 5
PCU-3.5	14 ± 3	770 ± 55	8 ± 3	14 ± 4
PCU-7	16 ± 2	830 ± 90	14 ± 2	15 ± 3
PCU-14	20 ± 3	900 ± 40	11 ± 4	20 ± 4
PCU-28	10 ± 2	470 ± 80	13 ± 2	9 ± 3

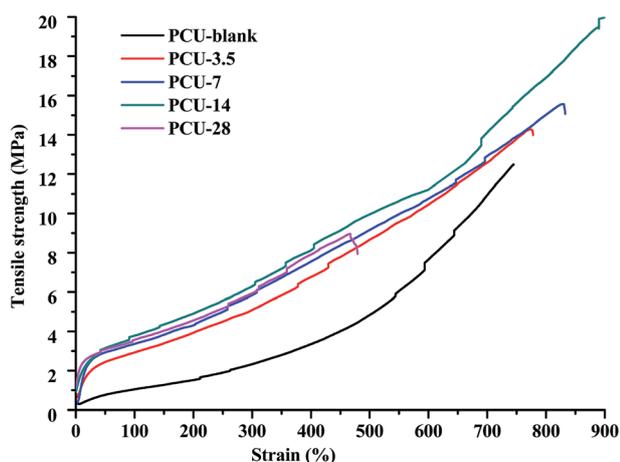
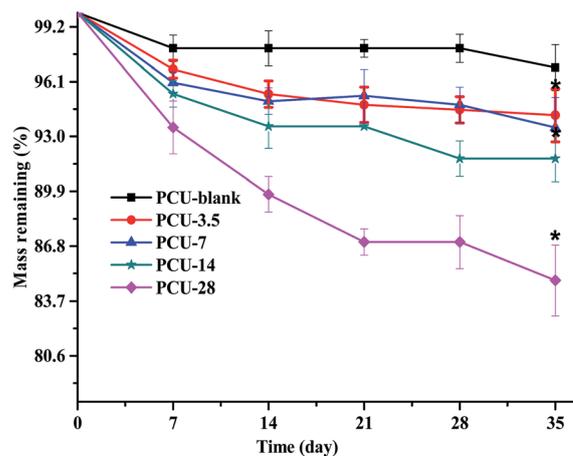
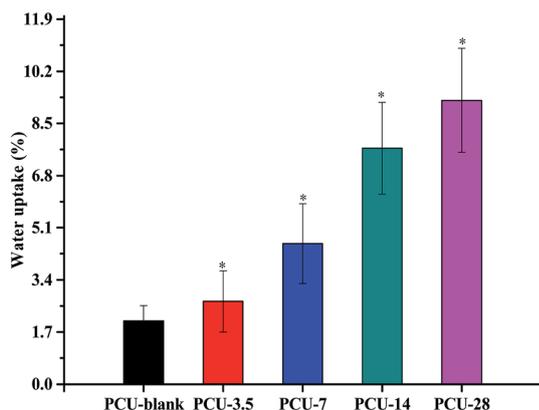


Fig. 2 Typical stress–strain curves of the crosslinked PCU films.

Fig. 4 Graphical illustration of percent mass remaining after incubation of different crosslinked PCU-film samples in PBS (pH ~ 7.4) at 37 °C for 5 weeks ( $\bar{x}$  = SD,  $n$  = 3, \* $p$  < 0.05 vs. PCU-blank).Fig. 3 Water uptake (%) of the different crosslinked PCU-films incubated for 24 hours in water at 37 °C ( $\bar{x}$  = SD,  $n$  = 3, \* $p$  < 0.05 vs. PCU-blank).

blank PCU after five weeks was 97%, while that of the film with the highest zwitterion content (PCU-28) was 85%. This further indicated that the degradation profile of the elastomeric PCU could be manipulated by varying the amount of hydrophilic zwitterions in the hard segment.

**3.7.1. Degradation analysis by  $^1\text{H}$  NMR.** The degradation products of the blank-PCU film (PCU-blank) and crosslinked films (PCU-14 and PCU-28) were collected after 28 days and analysed by  $^1\text{H}$  NMR (Fig. 5) in order to further investigate the

possible degraded or cleaved segments of the polymer backbone. The blank-PCU film (PCU-blank) showed two peaks at 3.4–3.7 ppm and 1.2–1.4 ppm, which could be assigned to the terminal and intermediate methylene protons of the diol, respectively.<sup>35–37</sup> This water soluble product resulted from the hydrolysis of the polycarbonate segment of PCU. From the peaks at 1.3–1.4 ppm, 1.1 ppm and 0.8 ppm, which could be ascribed to  $\text{CH}$ ,  $\text{CH}_2$  and  $\text{CH}_3$  protons of the diisocyanate residue, respectively, it could be hypothesised that the PCU-blank contained diisocyanate in the hard segment.<sup>38,39</sup>

In addition to the above peaks, the degradation products of PCU-14 and PCU-28 showed some of the following characteristic peaks: 3.1 ppm and 1.8–2.6 ppm were assigned to the intermediate and side chains of zwitterionic methylene protons, respectively, which were the characteristic peaks of poly(NSulfoZI).<sup>40</sup> Furthermore, in the PCU-14 spectrum, the characteristic peaks clearly observed at 4.21 ppm and at 3.10–3.32 ppm corresponded to  $\alpha$ -carboxyl and the residual methylene protons of cysteine, respectively.<sup>41</sup> Therefore, it can be envisaged that the degraded constituents were all biocompatible, non-toxic and water soluble.

### 3.8. *In vitro* biocompatibility

**3.8.1. MTT assay.** The cytocompatibility of a synthetic material is an important parameter if it is intended for

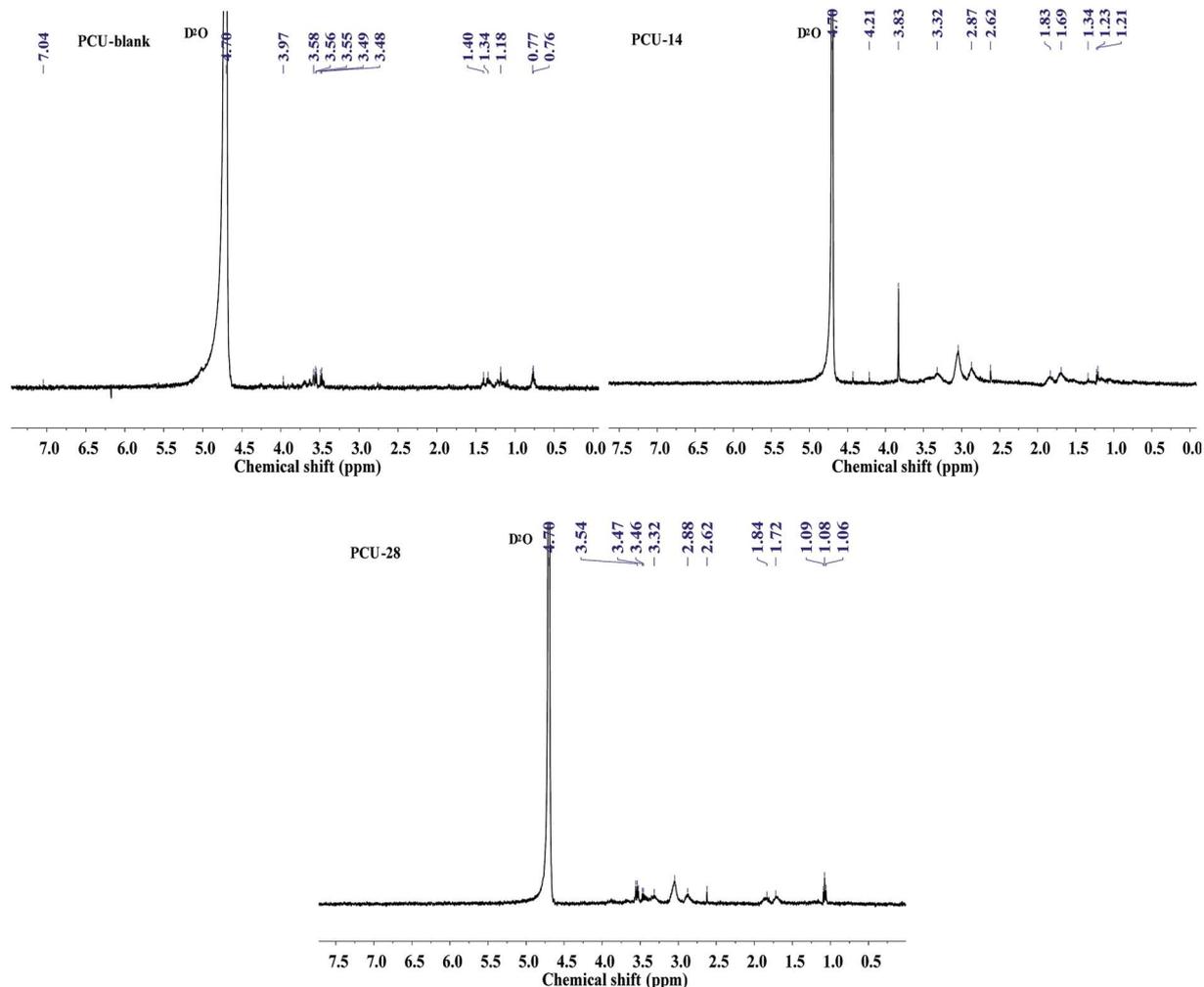


Fig. 5 Representative  $^1\text{H}$  NMR spectra ( $\text{D}_2\text{O}$  solvent) of the blank (PCU-blank) and crosslinked PCU films after being incubated in PBS (pH  $\sim$  7.4) at  $37^\circ\text{C}$ . The degraded residues were collected after 28 days and freeze-dried before analysis.

biomedical applications. In this regard, the cell viabilities of these crosslinked films were evaluated by MTT assay for 1-, 3- and 7-day culture periods (Fig. 6). The results showed that the

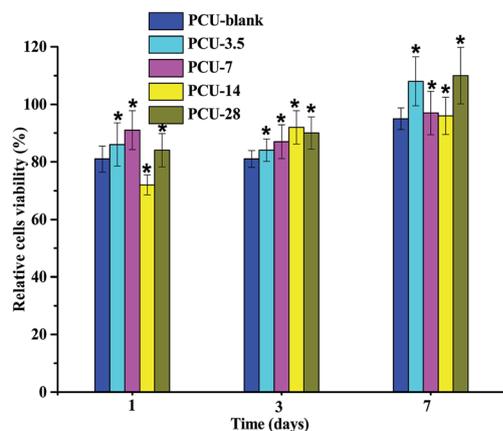


Fig. 6 MTT assay of different crosslinked PCU samples after 1, 3 and 7 day(s) ( $\bar{x}$  = SD,  $n$  = 3,  $*p < 0.05$  vs. PCU-blank).

designed materials were non-toxic and exhibited good cytocompatibility towards ECs (EA.hy926). Moreover, all of these designed crosslinked materials exhibited improved relative cell viability ( $>90\%$ ) after the 7<sup>th</sup> day of culturing. This further indicated that the biocompatibilities of these designed materials were very good, in addition to their improved mechanical and biodegradable properties.

**3.8.2. Endothelial cell adhesion and proliferation.** The adhesion and proliferation of ECs were investigated by FDA staining of the cultured cells after different time periods (1, 3 and 7 days) and photographed by immunofluorescence microscopy. The quantitative results obtained from these fluorescence micrographs (using image-J software) are shown in Fig. 7. The microscopic images (Fig. 8) clearly indicated the cell morphologies and shapes of the adhered EA.hy926 at different time intervals.

After the first day cell culturing, there was no difference in EC growth on the different surfaces. After three days of culturing, all the surfaces including the blank PCU-film exhibited significant adhesion and proliferation of cells. The

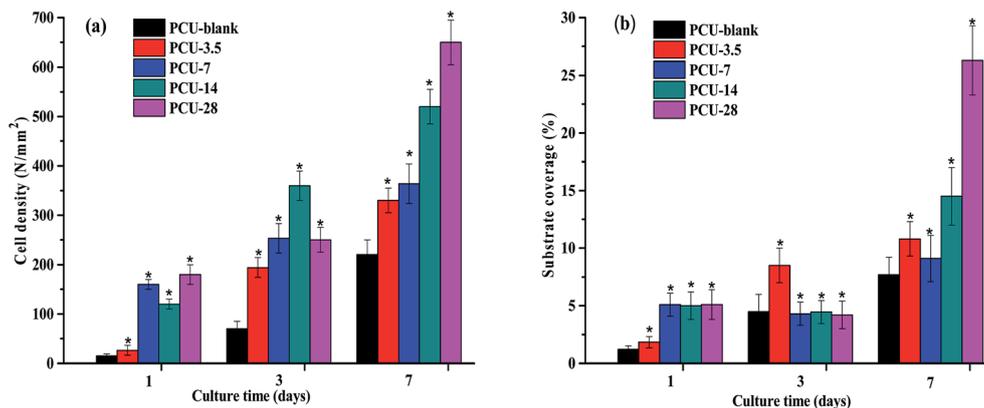


Fig. 7 Quantitative representations of ECs cultured on the crosslinked PCU film surfaces: (a) EC (EA.hy926) density ( $N\ mm^{-2}$ ) as a function of culture time (days); and (b) surface coverage (%) ( $\bar{x} = SD$ ,  $n = 3$ ,  $*p < 0.05$  vs. PCU-blank).

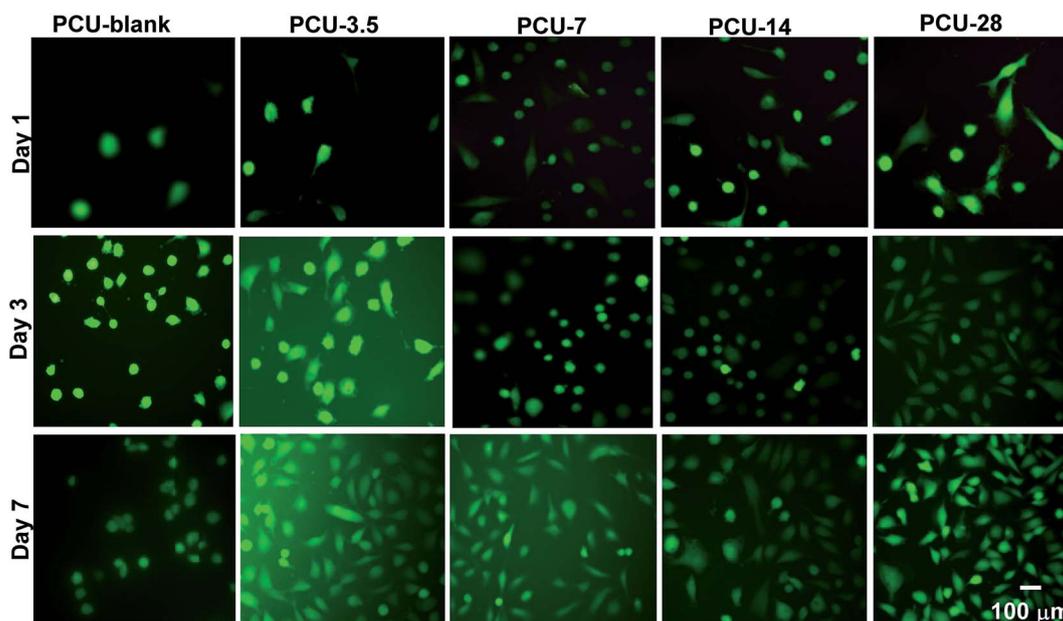


Fig. 8 Representative immunofluorescence micrographs showing EC (EA.hy926) adhesion and proliferation on different crosslinked PCU surfaces for a culture time of one week.

EC morphology indicated that after the third day, the cells adhered on the blank PCU-film showed round morphologies, which is an indication of restricted cell growth and proliferation. On the other hand, all the crosslinked films exhibited good proliferation of ECs, as the cells adapted spindle-shaped structures and cell spreading was observed on the surfaces. Furthermore, cell growth and proliferation were more pronounced on these material surfaces after seven days of culturing. The cultured cells on the different surfaces were expressed quantitatively in terms of cell density ( $N\ mm^{-2}$ ) as a function of culture time (Fig. 7a), which showed a higher cell density after the seventh day of culturing. Moreover, the cell spreading was expressed in terms of surface coverage (covered area in %; Fig. 7b); as the cells found a more appropriate growth environment, they proliferated on the surface and covered a

greater area, which also supported the expanded morphologies seen in Fig. 8.

## 4. Discussion

Scaffolds designed from polymeric biomaterials have recently attracted attention because the inherent biocompatible and biomimetic natures of certain polymers are suitable for clinical applications. However, there is still an urgent need to develop a proper fabrication technique in order to avoid further complication during cell-material interactions. The thiol-ene click-reaction is an excellent approach for the functionalisation of materials because it requires mild experimental conditions, small concentrations of benign catalyst and no cleanup, produces high yields, has a rapid reaction rate and is insensitive

to ambient oxygen and moisture.<sup>42–44</sup> The primary characterisation techniques indicated that the material hydrophilicity was significantly improved, which is a pre-requisite for living cell growth. An ideal TE material should be resorbable in nature and should not eventually undergo mechanical failure, and its biodegradation should not produce cytotoxic components.<sup>45</sup>

In our previous work, we tried to create tissue-mimicking materials with enhanced mechanical and biomedical properties.<sup>6–10,46</sup> The main issue with these materials is the compliance mismatch;<sup>1,2</sup> once implanted into target tissues, they may cause severe inflammatory and chronic reactions, which in turn results in implant failure. On the other hand, controlling the biodegradability rate of the final material is critical for certain therapeutic applications. Hence, on one hand, these materials need substantial elasticity because most of the biodegradable materials available for use in TE indicate that rigid substances could be best suited for hard-tissue applications.<sup>20</sup> The biodegradable and biocompatible PCU networks with labile moieties susceptible to hydrolysis could provide an effective platform for this kind of material design.<sup>47</sup>

Upon increasing the weight ratio of the zwitterionic poly(NSulfoZI) while keeping all other parameters constant, the biodegradability of the material was enhanced. The mass remaining for the PCU-28 crosslinked film (PCU-28) was 85% after a five-week incubation in PBS (pH = 7.4) at 37 °C, whereas that of the blank film (PCU-blank) was ~97%. The degradation of these materials mainly occurred *via* the hydrolysis of urethane/ester linkages in the main crosslinked chains. We note here that the hydrolytic degradation may not be solely responsible for the total mass loss of these films; some minor side reaction may have also occurred. The mass loss could partly be attributed to the dissolution or bond cleavage due to more water-uptake and the high affinity for aqueous solution of the incorporated constituents in the crosslinked PCU films.<sup>48</sup>

Due to the presence of highly hydrophilic zwitterion poly(NSulfoZI) in the crosslinked film, our results were consistent with the previous analysis. Therefore, the degradation of the poly(NSulfoZI)-PCU crosslinked materials could be tuned by varying the crosslinked density in the matrix.<sup>49,50</sup> The blank PCU-film exhibited the minimal degradation (<5% mass loss) and lower water uptake; the degradation was accelerated (>15% mass loss) after the incorporation of 28% hydrophilic zwitterion poly(NSulfoZI). The pH was continuously monitored and showed no detectable change, which could be attributed to the counterion balance by the zwitterionic functionality in the degraded residue.

The high reactivity of the isocyanate groups of HHDI with the amine groups in zwitterions and PCU resulted in extensive crosslinking and enhanced mechanical properties of the crosslinked films. The mechanical properties were gradually improved with increasing zwitterionic ratio, and among all the films, the highest strength was exhibited by the crosslinked film with a zwitterion content of 14% (PCU-14). However, the mechanical properties, particularly the elongation at break, declined upon doubling the percent ratio of the zwitterion (Table 1, PCU-28 with 28% zwitterion). The zwitterion probably formed a continuous rigid-network structure, which is rigid and

stiff.<sup>2</sup> The brittle behaviour of homopolynorbornene has also been previously reported,<sup>51</sup> which resulted in poor resilience of the material under relatively large deformation (>70%).

The cytocompatibility was tested by MTT assay for a one-week period. These materials were non-cytotoxic and exhibited improved cell viability. Moreover, the prepared crosslinked film exhibited enhanced EC (EA.hy926) adhesion and growth, and the cells showed good proliferation on the surfaces after days 3 and 7. Hence, it could be envisaged that these materials would provide a suitable platform for TE and implantable material applications.

## 5. Conclusion

Crosslinked materials based on elastomeric polycarbonate urethane and zwitterionic poly(NSulfoZI) were prepared by a convenient click-chemistry and crosslinking reaction route. The obtained materials combined the unique characteristics of both parent materials, such as excellent mechanical properties and biocompatible properties. In particular, the biodegradability of these materials after five weeks of testing was enhanced with increasing zwitterion content. Moreover, the cytocompatibility against model endothelial cells (EA.hy926) for a one-week period showed enhanced cell growth and proliferation in comparison to blank PCU. Therefore, these materials with easily tailored structures could serve as suitable candidates for tissue engineering applications.

## Acknowledgements

This work has been financially supported by the International Cooperation from Ministry of Science and Technology of China (Grant no. 2008DFA51170 and 2013DFG52040), the National Natural Science Foundation of China (Grant no. 31370969), Ph.D. Programs Foundation of Ministry of Education of China (no. 20120032110073), and the Program of Introducing Talents of Discipline to Universities of China (no. B06006).

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