Antimicrobial Hydantoin-grafted Poly(ε-caprolactone) by Ring-opening Polymerization and Click Chemistry[a]

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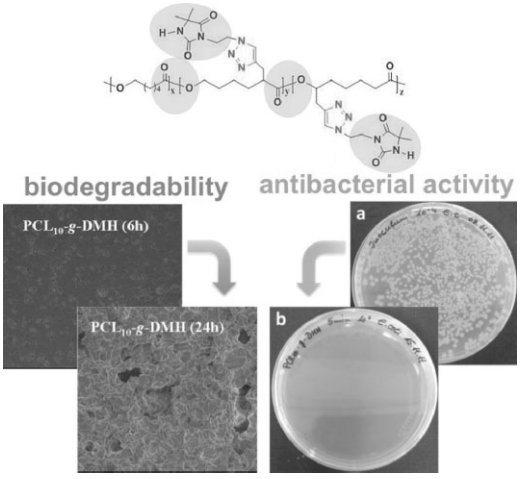
Novel degradable and antibacterial polycaprolactone-based polymers are reported in this work. The polyesters with pendent propargyl groups are successfully prepared by ring-opening polymerization and subsequently used to graft antibacterial hydantoin moieties via click chemistry by a copper(I)-catalyzed azide-alkyne cycloaddition reaction. The well-controlled chemical structures of the grafted copolymers and its precursors are verified by FT-IR spectroscopy, NMR spectroscopy, and GPC characterizations. According to the DSC and XRD results, the polymorphisms of these grafted copolymers are mostly changed from semicrystalline to amorphous depending on the amount of grafted hydantoin. Antibacterial assays are carried out with *Bacillus subtilis* and two strains of *Escherichia coli* and show fast antibacterial action.



1. Introduction

Due to biodegradability, biocompatibility, as well as excellent shaping and molding properties, aliphatic polyesters such as polylactide (PLA), poly(ε-caprolactone) (PCL), and their respective copolymers have been increasingly used in biomedical and engineering fields in recent years, including absorbable implant materials, biodegradable surgical sutures, vascular grafts, bone screws, and carriers in drug delivery.[1–6] Preparation of polyesters containing pendent functional groups has attracted much attention in recent years. Pendent functionalization of aliphatic polyesters can be achieved by polymerization of functionalized lactones via ring-opening polymerization (ROP), post-polymerization modification, or a combination of these two approaches.[7] A series of polymers based on PCL with pendent allyl,[8] cyclopentene,[9] propargyl,[10–12] and hydroxyl,[13,14] have been prepared, which possess functional groups for covalent substitution and grafting of functional units. This is highly desirable for properties modification and/or for additional functionalities in polyesters.

Recently, the "click" reaction of copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition[15,16] between azide and alkyne groups has been developed and represented

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an attractive method for the preparation of clickable and biodegradable polyesters for potential biomedical applications due to the relatively mild reaction conditions and tolerance of other functional groups.[17–22] Such polyesters were generally synthesized by ROP and subsequently modified via a click reaction. For example, Suksiriworapong et al. prepared poly(ε-caprolactone)–poly(ethylene glycol)–poly(ε-caprolactone) with pendent azide groups on the PCL backbone via ROP, and further click modified the polyesters with nicotinic acid and *p*-aminobenzoic acid (PABA) moieties containing alkyne groups.[23]

For many application areas of biodegradable polymers like sutures, implants, wound coatings, etc. on one side and packaging, food storage, etc. on the other side, it is reasonable to have antibacterial functionality besides degradability. For such applications antibacterial functionality could provide self-sterilizing articles and packaging, etc. and complete elimination of bacterial growth just by contact during the use period. For such applications the antimicrobial moiety should be active while attached to the polymer. The concept of having a biodegradable antimicrobial polymer with hydrolysable moiety together with antibacterial units in its inactive form is reported in the literature for the smart release of active ingredients on demand. Such systems are important for targeted antibacterial action. They make use of hydrolysable ester units either as linker between the biocide and polymer backbone or in the backbone attaching biocide units together.[24] Poly(vinyl alcohol-peptide linker-gentamicin) is one such polymer,[25] which has degradable peptide units attaching biocide (gentamicin) to the polymer backbone. The enzyme proteinases from wounds infected with bacteria like *Pseudomonas aeruginosa* cleaved the linker releasing the antibiotic. In these cases the biocides are present in their *inactive* form in the polymers and were released after degradation of either linker or polymer backbone and brought biocides in the active form. There is hardly any literature on dual functional polyesters (hydrolysable and antibacterial) with inherent antimicrobial functionality.

Recently, we have made a dual functional antimicrobial biodegradable polyester using *N*-halamine as the biocidal unit. For the first time, polyesters of dimethyl succinate (DMS), 1,4-butanediol (BDO), and 3-(*N*,*N*-di-*b*-hydroxyethylaminoethyl)-5,5-dimethylhydantoin (H-diol) in varied molar ratios were made via a two-step melt polycondensation reaction. 3-(*N*,*N*-di-*b*-hydroxyethylaminoethyl)-5,5-dimethylhydantoin (H-diol) was used as antibacterial comonomer. The resulting polyesters were biocompatible as determined by cell viability studies and showed an antibacterial activity against *Escherichia coli*.[26]

N-Halamines are compounds containing one or more covalent nitrogen-halogen bonds, which exhibit long-term stabilities, non-toxicity to humans, biocidal activity against a broad range of microorganisms, and regenerable properties upon exposure to household bleach.[27,28] Both low and high molecular weight *N*-halamine-based antibacterial materials are known in the literature. In one of the approaches, either *N*-halamine containing polymers or low molecular weight *N*-halamine derivatives were added as additives to impregnate the respective polymers in order to obtain antibacterial materials.[29–33] The other recent approaches involve functionalization by chemical reactions to introduce *N*-halamine structures into the desired matrix polymers.[34–38] Worley and coworkers have done extensive work on antibacterial materials based on *N*-halamine. They have provided *N*-halamine based new materials and many novel concepts of attaching *N*-halamine moieties to various polymers either by copolymerization or by polymer analogous reactions and layer-by-layer process.[39]

In this work we show the possibility of introducing antibacterial functionality to polycaprolactone, a highly studied biomaterial by a combination of ROP and click chemistry. A series of novel antibacterial poly(ε-caprolactone)-*graft*-hydantoin copolymers have been successfully prepared by a copper(I)-catalyzed click reaction of azide-functionalized hydantoin with PCL bearing pendent alkyne functionalities and characterized in terms of structure, properties like thermal, mechanical, antibacterial, and degradability.

2. Experimental Section

2.1. Materials

Cyclohexanone was purchased from Aldrich, dried over magnesium sulfate and distilled before use. ε-Caprolactone (ε-CL, Aldrich, 97%) was dried over calcium hydride, distilled under reduced pressure and stored over molecular sieves (0.4 nm) under an argon atmosphere. Stannous(II)octoate (tin bis-2-ethylhexanoate, Aldrich, 95%) was degassed at high vacuum and used as received. Propargyl bromide (80 wt.-% solution in toluene) and 5,5-dimethylhydantoin (DMH) were purchased from Alfa Aesar Co., Ltd. 1-Bromo-2-chloroethane, lithium *N*,*N*-diisopropylamide (LDA) solution (2.0 M in THF/heptanes/ethylbenzene), *m*-chloroperbenzoic acid (70–75%), (+)-sodium-L-ascorbate and copper(II)sulfate were purchased from Sigma–Aldrich and used as received. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (98%), sodium azide (99%) were purchased from Acros and the lipase of *Pseudomonas cepacia* was acquired from Sigma–Aldrich. Tetrahydrofuran (THF) was refluxed over a benzophenone-sodium mixture to remove water and distilled before use. *N*,*N*-Dimethylformamide (DMF) was distilled from calcium hydride under reduced pressure. Dichloromethane (DCM) was dried over calcium chloride and stored in presence of molecular sieves (0.4 nm). All other chemicals and solvents of analytical grade were used as received without further purification.

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2.2. Methods

FT-IR spectra were recorded on a Digilab Excalibur Series system using a Pike Miracle attenuated total reflection (ATR) unit. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Bruker DRX-300 spectrometer using deuterated chloroform ($CDCl_3$) as solvent. ^{1}H–^{13}C heteronuclear multiple quantum correlation (HMQC) experiments were performed on a Bruker DRX-500 spectrometer, with a 5 mm multinuclear gradient probe and using gs-HMQC pulse sequences. Molecular weights of the polymers were determined by GPC using a Knauer system equipped with one PSS-SDV (linear XL, 5 μm, 8.0 mm × 300 mm) column, a differential refractive index detector, $CHCl_3$ as eluent at a flow rate of 0.5 mL · min^{-1}. Thermogravimetric analysis (TGA) was performed on a TGA/SDTA 851e (Mettler Toledo) at a heating rate of 10 °C · min^{-1} under nitrogen from 25–800 °C with a sample size of 8–10 mg. Differential scanning calorimetry (DSC) measurements of polymers were carried out on a DSC 821e unit (Mettler Toledo) under a nitrogen flow at a rate 80 mL · min^{-1}. About 7 mg sample were encapsulated in the DSC aluminum pan, heated quickly to 150 °C and held for 5 min to erase the thermal history. Then, the samples were cooled to −100 °C and subsequently heated to 150 °C at a rate of 5 °C · min^{-1}. The corresponding glass transition temperature (T_g), melting temperature (T_m), crystallization temperature (T_c), melting enthalpy (ΔH_m) and crystallization enthalpy (ΔH_c) were recorded, respectively. X-ray diffraction patterns of the samples were recorded with a Siemens goniometer D5000 using Cu K_α-radiation of $\lambda = 1.54$ Å. The crystal morphology of the polymers was analyzed by a Leica DMRX polarized optical microscope (POM) fitted with a Leica DC200 camera at a magnification of 1:100. Mechanical testing was done using an Instron testing machine (model 4411) using dumbbell-shaped samples (35 mm length, 2 mm width). Films with a thickness of $d = 0.08$–0.1 mm were prepared from chloroform solution.

2.3. Synthetic Procedures

2.3.1. Synthesis of 2-Prop-2-ynyl-cyclohexanone

A solution of lithium diisopropylamide (LDA, 120 mmol) was added to a 250 mL round-bottom flask purged with argon, cooled in a dry ice/acetone bath, and stirred for 30 min. An argon-purged solution of cyclohexanone (120 mmol, 11.78 g) in 3 mL THF was added dropwise to the LDA solution at −70 °C. Propargyl bromide (120 mmol, 14.28 g) in 10 mL THF was also added dropwise by a syringe and the solution was stirred for additional 60 min. The reaction mixture was then warmed up to room temperature and stirred overnight. Excess aqueous ammonium chloride was added, and the reaction mixture was washed twice with diethyl ether, dried over $MgSO_4$ and concentrated. The following distillation under reduced pressure resulted in a colorless, viscous liquid (61% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm) = 2.56 (ddd, $J = 17.5$ Hz, 4.8 Hz, 2.7 Hz, 1H), 2.48–2.42 (m, 1H), 2.39–2.34 (m, 2H), 2.30–2.21 (m, 1H), 2.14 (ddd, $J = 16.8$ Hz, 8.7 Hz, 2.7 Hz, 1H), 2.07–2.03 (m, 1H), 1.92 (t, $J = 2.7$ Hz, 1H), 1.89–1.86 (m, 1H), 1.70–1.55 (m, 2H), 1.38 (ddd, $J = 25.8$ Hz, 12.3 Hz, 3.6 Hz, 1H). ^{13}C NMR (75 Hz, $CDCl_3$): δ(ppm) = 210.5, 82.6, 69.3, 49.4, 42.1, 33.4, 27.9, 25.2, 18.7.

2.3.2. Synthesis of 3-/7-(Prop-2-ynyl)oxepan-2-one

2-Prop-2-ynyl-cyclohexanone (60 mmol) was added to a solution of *m*-chloroperbenzoic acid (90 mmol, 20.18 g) in 160 mL dichloromethane and then refluxed for 48 h. After cooling to room temperature and filtration, the solution was washed twice with aqueous sodium sulfite and aqueous sodium hydrogen solutions, the solvent was evaporated. Subsequent distillation under oil pump vacuum yielded 64% of the product as an isomeric mixture (3-(70%) and 7-(prop-2-ynyl)oxepan-2-one (30%)), named as alkyne CL. ^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm) = 4.35–4.16 (m, 3H), 2.77–2.72 (m, 1H), 2.66–2.52 (m, 5H), 2.43 (ddd, $J = 16.7$ Hz, 8.1 Hz, 2.7 Hz, 1H), 2.31 (ddd, $J = 17.1$ Hz, 9.2 Hz, 2.6 Hz, 1H), 2.18–2.06 (m, 2H), 2.00–1.88 (m, 5H), 1.72–1.37(m, 6H). ^{13}C NMR (125 Hz, $CDCl_3$): δ(ppm) = 176.0, 174.6, 82.0, 79.5, 78.2, 71.4, 70.8, 68.6, 42.5, 34.8, 33.4, 28.9, 28.8, 28.3, 28.1, 26.1, 22.9, 21.9.

2.3.3. Copolymerization of Alkyne CL and ε-CL via Ring-opening Polymerization

The mixture of 3-/7-(prop-2-ynyl)oxepan-2-one (alkyne CL), ε-CL (ratio 1:9, 1.5:8.5, 2:8) and 0.5 mol-% of $Sn(Oct)_2$ as catalyst were placed in a Schlenk flask purged with argon for 10 min. Then, the polymerization was carried out in an oil bath at 100 °C for 24 h under continuous stirring. After cooling to room temperature, the solution was dissolved in dichloromethane, precipitated in cold methanol and dried in vacuum.

2.3.4. Synthesis of 3-(2′-Chloroethyl)-5,5-dimethylhydantoin (DMH-Cl)

5,5-Dimethylhydantoin (DMH) (96.1 g, 0.75 mol) was dissolved in a solution of 42.1 g (0.75 mol) KOH in 500 mL ethanol. 1-Bromo-2-chloroethane (215.1 g, 1.5 mol) was added in one portion to the solution and then refluxed at 80 °C for 8 h. Evaporation of the reaction mixture resulted in a white solid which was shaken with ethyl acetate and water in a separatory funnel. The organic layer was separated, washed (10% aqueous $NaHCO_3$), dried (Na_2SO_4), and evaporated to isolate the product. Recrystallization from toluene/2-propanol (v/v = 1:9) resulted in white crystals with a yield of 92%. This compound was identified as 3-(2′-chloroethyl)-5,5-dimethyl-hydantoin. ^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm) = 1.46 (6H, CH_3), 3.75 (2H, CH_2), 3.84 (2H, CH_2), 6.61 (1H, N−H).

2.3.5. Synthesis of 3-(2′-Azido-ethyl)-5,5-dimethylhydantoin (DMH-N_3)

3-(2′-Chloroethyl)-5,5-dimethylhydantoin (1.90 g, 10 mmol) was initially dissolved in 6 mL DMF before sodium azide (0.91 g, 14 mmol) in 3 mL distilled water was added dropwise with a syringe. The reaction mixture was stirred under argon atmosphere at 80 °C for 48 h. After removal of DMF under reduced pressure, the residue was dissolved in diethyl ether and washed with distilled water three times before drying over anhydrous $MgSO_4$. The product was concentrated by rotary evaporation and obtained in a yield of 93%.

2.3.6. Synthesis of Poly(ε-caprolactone)-*graft*-Hydantoin Polymers by Click Chemistry

DMH-N_3 (1.3 equiv. compared to the alkyne group of alkyne PCL) was added to a solution of 400 mg alkyne PCL in DMF (4 mL) in a

flask with a magnetic stirring bar, and the solution was bubbled with argon for 30 min. 5 mol-% $CuSO_4 \cdot 5H_2O$ and 10 mol-% sodium ascorbate (NaAsc) was individually dissolved in 3 mL of water, followed by bubbling with argon for 10 min. After dropping aqueous $CuSO_4$ and NaAsc solutions separately in sequence into the Schlenk flask with a syringe under vigorous stirring at room temperature, 10 mol-% DBU was added. The reactive mixture was degassed by three freeze–pump–thaw cycles, stirred at 35 °C for 24 h, and then exposed to air. After that, the solution was passed through a neutral aluminum oxide column to remove the copper catalyst and evaporated. The final product was dried in a vacuum oven at room temperature.

2.4. Enzymatic Degradation

To investigate the enzymatic degradation, polymer films (10 mm × 10 mm × 0.08 mm), cast from chloroform solutions, were placed in vials containing 10 mL of phosphate buffer solution (Na_2HPO_4/KH_2PO_4, pH 7.0 at 25 °C) with 0.20 mg · mL^{-1} *Pseudomonas cepacia* lipase at a constant temperature of 37 °C. At predetermined degradation time intervals, the specimens were removed from the medium, rinsed with distilled water, dried under vacuum at room temperature for 48 h and weighed. Weight loss percentages of the copolyesters were obtained according to the relationship:

$$\text{Weight loss}\,(\%) = (W_0 - W_r)/W_0 \times 100\% \quad (1)$$

where W_0 is the initial weight, and W_r is the dry weight of the specimens after degradation.

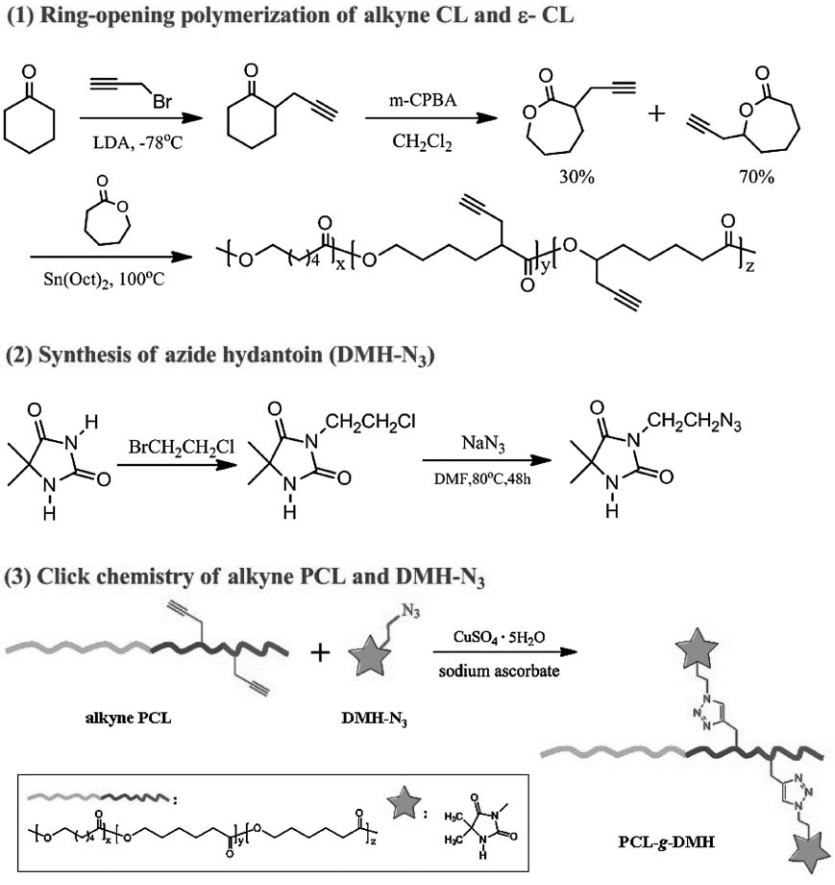
2.5. Antibacterial Test

To obtain inocula for the antibacterial tests, a single colony of the respective test organism, preserved on tryptic soy agar at 4 °C, was transferred to the corresponding liquid nutrient with an inoculation loop. The inoculated broth was incubated with shaking at 37 °C until the optical density at 578 nm had increased by 0.125, indicating a density of viable cells of 10^7–10^8 cfu · mL^{-1}, and diluted to an approximate concentration of 10^6–10^7 cfu · mL^{-1} to result in the final inoculum; the exact number of colony forming units per mL was determined subsequently by the spread plate method. *E. coli* (DSM No. 1077, K12 strain 343/113, DSZM Braunschweig), *E. coli* (DSM No. 1058, ML3 strain, ATCC 15223, DSMZ), and *Bacillus subtilis* (*B. subtilis*, DSM No. 1088, IFO 13169, DSZM) were employed as test organisms; tryptic soy broth (TSB, Sigma–Aldrich; $c = 30$ g · L^{-1} in distilled water) was used as nutrient for *E. coli* strains and peptone/meat extract (5 g · L^{-1} peptone and 3 g · L^{-1} meat extract in distilled water) for *B. subtilis*. To evaluate the antibacterial properties of the grafted polymers, films exhibiting a size of approximately 5 mm × 5 mm were inoculated with 10 μL inoculum and covered with a second film each. After given time intervals (5, 15, and 30 min), these "sandwiches" were vigorously washed by immersion in 10 mL of a 0.03% sodium thiosulfate solution in sterile water. A 100 μL aliquot of each solution was then serially diluted with sterile potassium buffer solution (pH 7.4), 100 μL of each dilution were spread on nutrient agar plates. By counting of the formed colonies after incubation at 37 °C for 24 h, the number of viable cells in the solutions was determined and the reduction was calculated respective to the inoculum in the appropriate dilution stage.

3. Results and Discussion

3.1. Synthesis of Poly(ε-caprolactone)-*graft*-Hydantoin Polymers by Ring-opening Polymerization and Click Chemistry

By following a procedure of the known approach by Ritter and coworkers,[6] the functionalized lactone with propargyl groups was prepared in two steps. Firstly, 2-prop-



Scheme 1. Preparation of side-chain hydantoin-containing PCL by ROP and click chemistry.

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2-ynylcyclohexanone was prepared from commercially available cyclohexanone by reaction with LDA in THF at −78 °C, followed by quenching with a toluene solution of propargyl bromide. The distilled product was further reacted with *m*-chloroperbenzoic acid by the Baeyer–Villiger oxidation. Subsequent distillation of the crude product gave an isomeric mixture 3-/7-(prop-2-ynyl)oxepan-2-one (alkyne CL) (70:30 molar ratio) in 64% yield.

Azide functionalized hydantion (DMH-N_3) was synthesized by a reaction of DMH with 1-bromo-2-chloroethane, followed by transformation of chlorine end groups to azide groups via a substitution reaction between chlorine-substituted hydantion (DMH-Cl) and excess sodium azide in DMF at 80 °C (Scheme 1). The presence of the terminal azide group was confirmed by a stretching frequency at 2120 cm^{-1} in the FT-IR spectra (Figure 1) and a methylene resonance at $\delta = 3.72$ ppm ($-CH_2CH_2N_3$) in the ^1H NMR spectra (Figure 2).

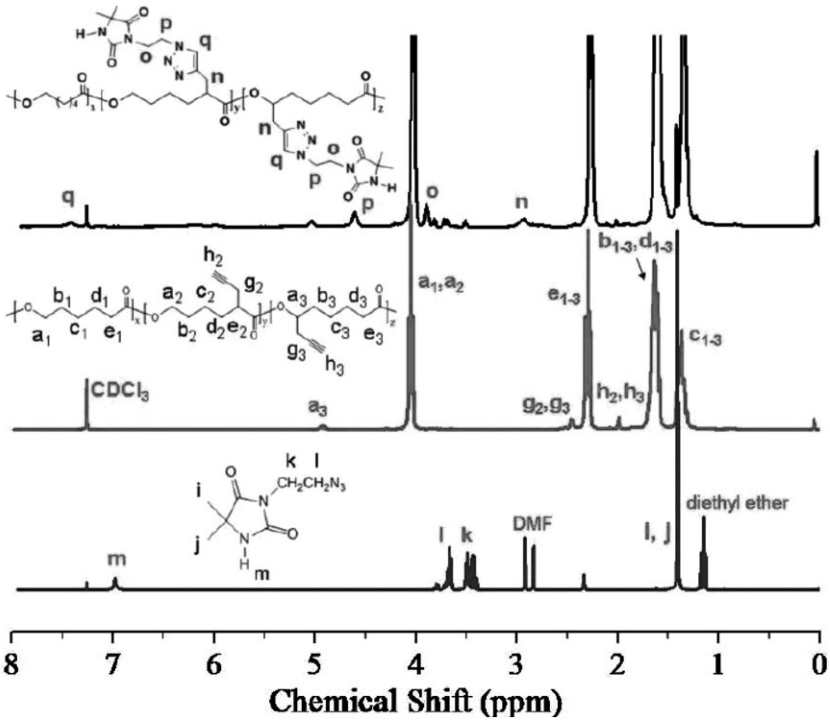

Figure 2. ^1H NMR spectra of DMH-N_3, alkyne PCL_{10}, and PCL_{10}-*g*-DMH.

Sn(Oct)$_2$-mediated ring-opening copolymerization of alkyne CL and ε-CL with different feed ratios of monomers was performed at 100 °C for 24 h, yielding copolymers of alkyne functionalized poly(ε-caprolactone) (alkyne PCL) (Scheme 1). The corresponding copolymers were designated as alkyne PCL_X, whereas X shows the amount of alkyne CL in the feed. As depicted in Figure 1, the FT-IR spectrum of alkyne PCL showed the characteristic peak of the alkyne C−H stretching band at 3 272 cm^{-1}. The molar content of alkyne CL incorporated into the copolymer was calculated by integration of the ^1H NMR spectral signals (Figure 2) at $\delta = 1.98$ ppm (−C≡C−*H* from alkyne PCL) compared to the signals at $\delta = 4.92$ ppm (−OC*H*CH_2− of the polymer backbone from alkyne PCL) and 4.03 ppm (−O$CH_2$$CH_2$− of the polymer backbone from alkyne PCL and PCL). As shown in Table 1, the incorporation ratio differed just a little from the feed ratio, suggesting a nearly complete consumption of alkyne functionalized lactone during the polymerization. The GPC traces (in Figure 3) of the copolymers displayed unimodal peaks with number-averaged molecular weights ($\overline{M}_n$) ranging from 20 300 to 49 900 g · mol^{-1}, and a polydispersity index (PDI) in the range of 1.75–1.66.

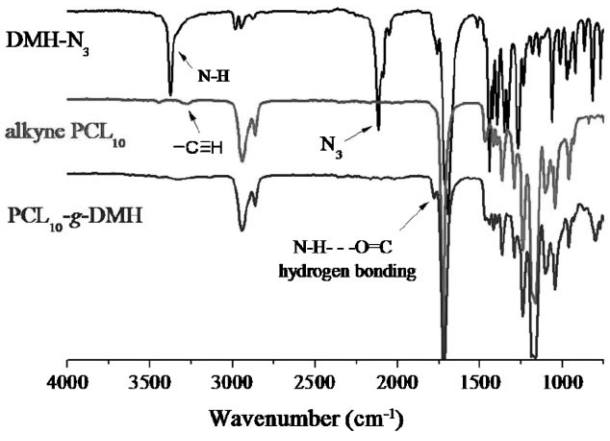

Figure 1. FT-IR spectra of DMH-N_3, alkyne PCL_{10}, and PCL_{10}-*g*-DMH.

The hydantoin moiety was attached onto the backbone through copper(I)-catalyzed Huisgen's 1,3-dipolar cycloaddition reaction using Cu(I) and DBU as catalysts at 35 °C for 24 h (Scheme 1). The success of the click reaction to yield poly(ε-caprolactone)-*graft*-hydantoin (PCL-*g*-DMH) was confirmed by the disappearance of the azide and alkyne signals at 2 120 and 3 272 cm^{-1} in the FT-IR spectra, respectively, and the appearance of a new signal for the methane proton of the triazole ring at 7.45 ppm in the ^1H NMR spectrum. Furthermore, peaks at $\delta = 123.4$ and 144.6 ppm were found in the ^{13}C NMR spectrum (Figure 4), corresponding to the carbons of the triazole ring. The peak assignment of the triazole ring was confirmed by the ^1H–^{13}C HMQC NMR spectrum of PCL_{10}-*g*-DMH (Figure 5), which clearly showed one separate cross-resonance due to the correlation of proton a at 7.45 ppm with the

Table 1. Chemical compositions and molecular weights of the polymers.

Sample	Feed molar ratio (alkyne CL/ε-CL)	Incorporation ratio[a)] (alkyne CL/ε-CL)	Content of grafted DMH[b)]	$\overline{M}_n$[c)] $\times 10^{-4}$ [g · mol^{-1}]	PDI
Alkyne PCL_{10}	10/90	6.3/93.7		4.99	1.66
Alkyne PCL_{15}	15/85	10.0/90.0		2.60	1.75
Alkyne PCL_{20}	20/80	14.7/85.3		2.03	1.71
PCL_{10}-*g*-DMH			6.7/93.3	7.01	1.50
PCL_{15}-*g*-DMH			10.6/89.4	3.68	1.54
PCL_{20}-*g*-DMH			15.5/84.5	2.41	1.56

[a)]Determined from ^{1}H NMR spectra; [b)]determined by ^{1}H NMR spectra from the signal at 4.59 ppm (DMH−$CH_2C\underline{H}_2$−) and the signals at 4.92 and 4.03 ppm (−OC$\underline{H}$$CH_2$− and −OC$\underline{H}_2$$CH_2$− of the polymer backbone); [c)]determined by GPC in $CHCl_3$.

carbon signal b at 123.4 ppm. As shown in Table 1, the content of grafted hydantoin was estimated by integration of the ^{1}H NMR signal at $\delta = 4.59$ ppm (DMH−CH_2CH_2−) against the signals at 4.92 and 4.03 ppm (−OC*H*CH_2− and −OC$H_2$$CH_2$− of the polymer backbone). It was clear that the degree of the hydantoin grafting density could be tailored by the amount of pendent alkyne in the polymer starting material. From GPC traces of PCL-*g*-DMH copolymers in Figure 3, a clear shift toward higher molecular weight in comparison with the starting alkyne PCL polymers was observed. Whereas, the PDI of PCL-*g*-DMH after click reaction did not show a dramatic change (Table 1), indicating no degradation of the polymer chain.

3.2. Thermal Properties of Alkyne Functionalized Poly(ε-caprolactone) Copolymers and Grafted Copolymers

Thermal properties of the alkyne PCL and PCL-*g*-DMH graft copolymers were evaluated by TGA and DSC measurements

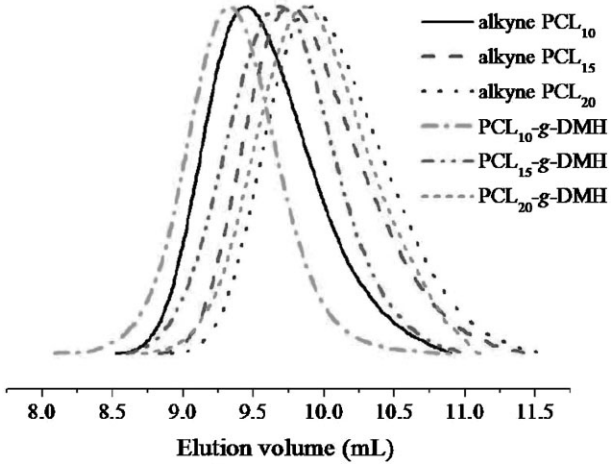

Figure 3. GPC profiles of alkyne PCL and PCL-*g*-DMH polymers.

(the curves are available in the Supporting Information). Table 2 provides a summary of the DSC results from the first cooling and second heating scan, revealing the glass transition temperature (T_g), melting temperature (T_m), crystallization temperature (T_c), melting enthalpy (ΔH_m), and the crystallization enthalpy (ΔH_c), as well as the values of the corresponding decomposition temperatures (T_d) from TGA analysis. As expected, all copolymers showed a high thermal stability with T_d values above 250 °C. The T_d of the PCL-*g*-DMH graft copolymers after click reaction increased apparently compared to the original alkyne PCL copolymers, revealing that the grafting of the hydantoin moiety improved the thermal stability of the graft copolymers. Furthermore, the graft copolymers showed a significant increase of the T_g with increasing content of grafted hydantoin. This was due to the rigidity of the hydantoin moiety, which caused a restricted segmental mobility and thereby an increase of the glass transition temperature. However, the T_m and T_c of the alkyne PCL copolymers decreased with increasing incorporation of alkyne functionalized lactone, because these disrupted the formation of crystalline domains, which are characteristic for PCL and other aliphatic polyesters.[40,41] In addition, the PCL-*g*-DMH graft copolymers with high amounts of the grafting moiety were also amorphous; only the polymer with 10 mol-% of grafted hydantoin comprised crystallinity.

3.3. Crystal Structure and Morphology of Alkyne Functionalized Poly(ε-caprolactone) Copolymers and Grafted Copolymers

The aforementioned results indicated a significant influence of the grafted hydantoin moiety on the crystallization tendency of PCL. Therefore, it was also important to determine the crystal structure of the copolymers in order to illustrate the relationship between chemical composition and crystallization behavior of the copolymers. In

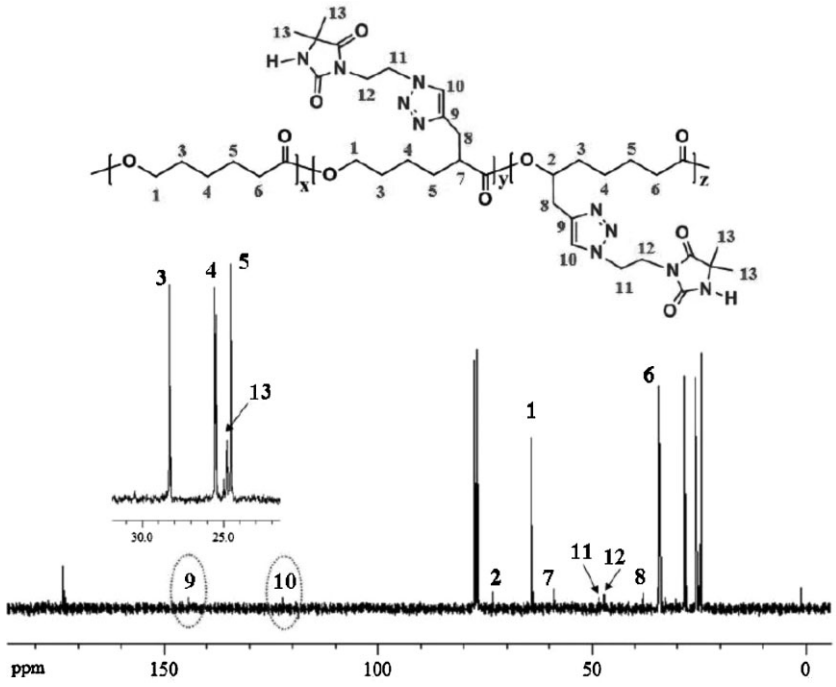

Figure 4. ^{13}C NMR spectrum of PCL_{10}-*g*-DMH polymer.

this study, the crystalline structure and morphology of alkyne PCL and the PCL-*g*-DMH graft copolymers were studied by X-ray and POM, respectively. Figure 6 shows the X-ray patterns of alkyne PCL and the graft copolymers. It could be noticed that PCL_{10}-*g*-DMH and PCL_{20}-*g*-DMH graft copolymers exhibited two prominent diffraction reflexes at $2\theta = 21.4^{\circ}$ and 23.8°, just as alkyne PCL, which proved the similarity regarding the crystal structure among copolymers with grafted hydantoin moiety and alkyne PCL. The grafting of low amounts, i.e. up to 10 mol-%, did not disturb the PCL crystal structure to a large extent. In addition, the crystalline morphologies of alkyne PCL and the PCL-*g*-DMH graft copolymers were investigated by POM, as shown in Figure 6. For alkyne PCL, the size of spherulites decreased and the Maltese cross patterns were not very pronounced with the incorporation of a higher amount of alkyne group. PCL-*g*-DMH graft copolymers showed irregular and disordered spherulites with a smaller diameter, which can be attributed to the reduced crystallization ability after grafting of the hydantoin moiety.

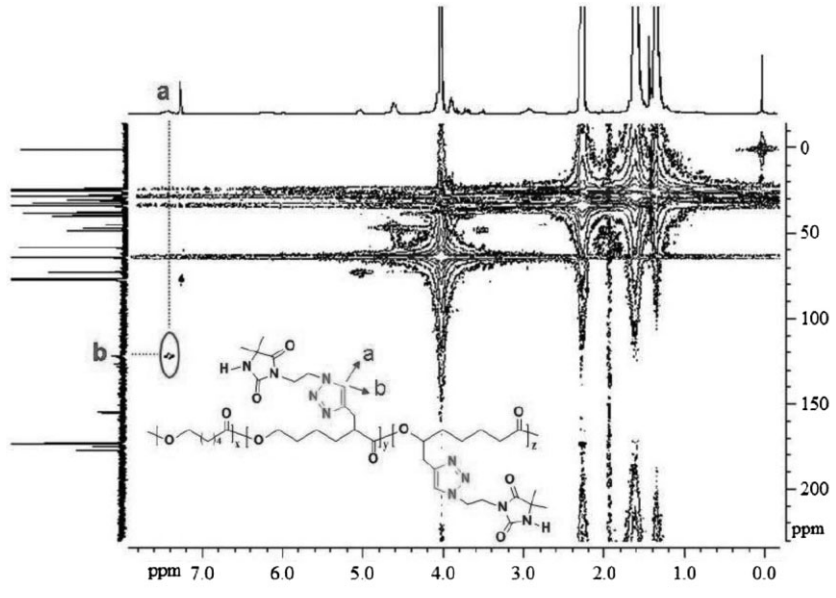

Figure 5. ^{1}H–^{13}C HMQC NMR spectrum of PCL_{10}-*g*-DMH polymer.

3.4. Degradability of Alkyne Functionalized Poly(ε-caprolactone) Copolymers and Grafted Copolymers

The degradation behaviors of alkyne PCL and PCL-*g*-DMH graft copolymers were investigated by immersing them in phosphate buffer solution (pH = 7.0) with *Pseudomonas Cepacia* lipase at 37 °C. The biodegradation behaviors were monitored by weight loss measurements and SEM. As shown in Figure 7, alkyne PCL degraded very fast. After 6 h, more than 74% of the initial weight was lost. After 24 h, the alkyne PCL polymer films were degraded completely. PCL-*g*-DMH graft copolymers degraded initially more slowly than the alkyne PCL polymer. At later stages, the rate of weight loss increased and reached 87% after 24 h. Surface morphology changes during degradation were followed by SEM. After 6 h of degradation by lipase, the surface of alkyne PCL was uniformly eroded with the formation of a network-like structure. In the case of PCL_{10}-*g*-DMH, the surface exhibited initially a rugged pattern. After 6 h, exterior erosion appeared clearly. As degradation proceeded up to 24 h, the surface was strongly eroded to leave sponge-like structures.

3.5. Mechanical Properties and Antibacterial Activities of Polymers

The mechanical properties of alkyne PCL_{10} and PCL_{10}-*g*-DMH polymers were studied and the corresponding data

Table 2. Thermal properties of alkyne PCL and PCL-*g*-DMH copolymers.

Sample	T_g [a)] [°C]	T_m [a)] [°C]	ΔH_m [a)] [J · g^{-1}]	T_c [a)] [°C]	ΔH_c [a)] [J · g^{-1}]	T_d [b)] [°C]
Alkyne PCL_{10}	−56	49	46.1	18	52.4	289
PCL_{10}-*g*-DMH	−48	47	38.5	5.2	40.1	364
Alkyne PCL_{15}	−52	43	42.6	1	46.8	291
PCL_{15}-*g*-DMH	−42	–	–	–	–	350
Alkyne PCL_{20}	−50	38	39.8	−7	41.2	297
PCL_{20}-*g*-DMH	−37	–	–	–	–	312

[a)]Glass transition temperature (T_g), melting temperature (T_m), crystallization temperature (T_c), crystallization enthalpy (ΔH_c), and melting enthalpy (ΔH_m) were registered by DSC at the first cooling scan with a rate of 5 °C · min^{-1} or at the second heating scan with a rate of 5 °C · min^{-1}; [b)]thermal decomposition temperature (T_d) (−5 wt.-%) was measured by TGA at a heating rate of 10 °C · min^{-1}.

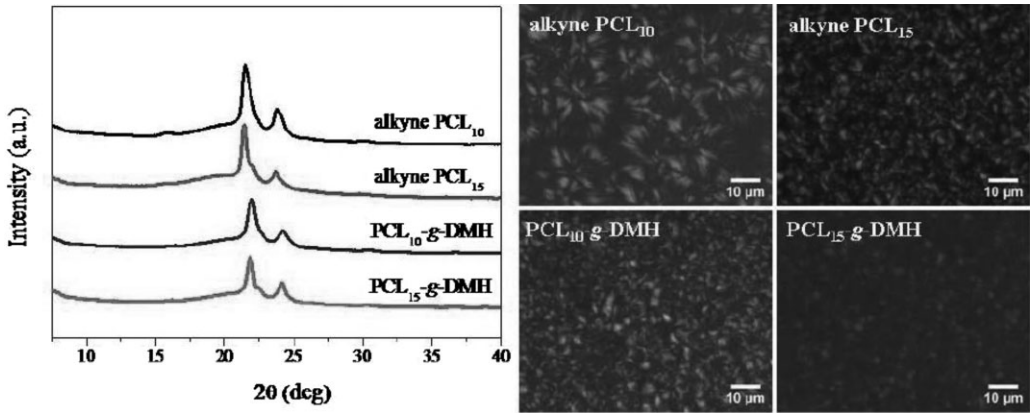

Figure 6. X-ray patterns and POM graphs of alkyne PCL and PCL-*g*-DMH polymers.

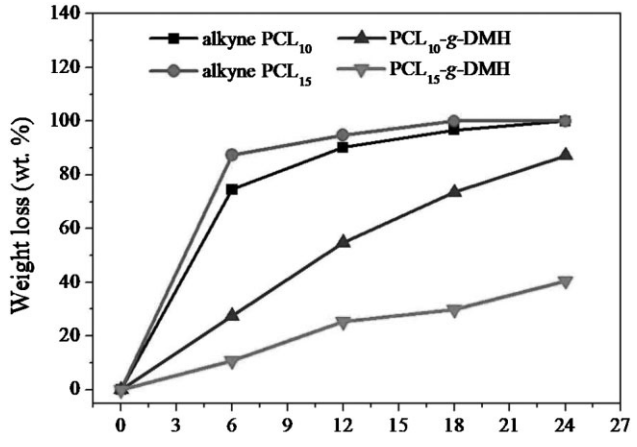

Figure 7. Weight loss versus time for polymer films during enzymatic degradation.

are listed in Table 3. It was observed that the tensile strength of the PCL_{10}-*g*-DMH graft copolymer was higher than alkyne PCL_{10}, which was due to the rigidity of the triazole ring. Furthermore, PCL_{10}-*g*-DMH also showed high elongation-at-break even though the rigid hydantoin moiety was incorporated, indicating the persistence of excellent stretch ability of the graft copolymer, which is a key characteristic of elastomeric materials.

In order to evaluate the antibacterial properties of the PCL_{10}-*g*-DMH copolymers, films cast from chloroform were exposed to the so called sandwich-test, as among others described by Sun and Sun[38] Briefly, 10 μL inoculum were placed between two copolymer films and after given time intervals the number of viable cells was determined via the spread plate method. Two different strains of *E. coli* and one strain of *B. subtilis* were employed for this. In general, PCL_{10}-*g*-DMH showed a higher activity

Table 3. Mechanical properties and antibacterial activities of polymers.

Sample	Tensile strength [MPa]	Elongation at break [%]	*E. coli* (1077) reduction after 5 min	*E. coli* (1058) reduction after 5 min	*B. subtilis* reduction after 5 min
Alkyne PCL_{10}	13.7 ± 0.2	$1\,039 \pm 21$	n.d.	n.d.	n.d.
PCL_{10}-*g*-DMH	29.6 ± 6.3	$1\,664 \pm 328$	99.9%	99.8%	92.5%

toward the Gram-negative organisms with reductions of 99.8 or 99.9%, opposed to killing of 92.5% *B. subtilis* (see Table 3). The killing efficacies did not differ notably among the investigated contact times, which hints to a fast killing of the microorgansism by the grafted PCL surface. Figure 8 depicts the colony formation of *E. coli* 1077 (K12 strain 343/113) after 5 min contact time to PCL_{10}-*g*-DMH along with the respective blank control, represented by the inoculum in the appropriate dilution stage. Therefore it can be deduced, that PCL_{10}-*g*-DMH exhibited a good and fast antibacterial action, especially regarding Gram-negative *E. coli*.

The cell viability test (MTT assay) was performed according to the method of Mosmann[42] using HEK293 cells for checking the biocompatibility of the grafted polymers. Hyperbranched poly(ethylenimine) (hy-PEI25kDa, BASF, Germany) was used as a positive control. The polymer concentration, which inhibits growth of half of the cells relative to non-treated control cells (IC_{50} value) was much more for grafted polymers as compared to hy-PEI25kDa. PCL_{10}-*g*-DMH and PCL_{15}-*g*-DMH showed IC_{50} values of 0.5611 and 0.5608, respectively as compared to IC_{50} value of 0.0101 for hy-PEI25kDa showing significantly less toxicity of the grafted polymers. hy-PEI25kDa with this low IC_{50} value is already an accepted biomaterial for applications like gene transfection.

4. Conclusion

In summary, a series of novel antibacterial and biocompatible poly(ε-caprolactone)-*graft*-hydantoin copolymers have been successfully prepared by a copper(I)-catalyzed click reaction of azide-functionalized hydantoin with PCL bearing pendent alkyne functionalities. FT-IR, NMR, and GPC analyses confirmed the designed structures. The grafting of hydantoin moieties onto the PCL backbone significantly improved thermal stabilities and mechanical properties of the grafted copolymers compared to linear PCL, and retained full degradability of PCL. Moreover, the grafted copolymers showed fast antimicrobial activities regarding both *E. coli* and *B. subtilis*, which suggests that these copolymers possess a considerable potential for application in medical devices, hygienic materials, and the food-processing industry.

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Keywords: antibacterial; click chemistry; degradation; hydantoin; ring-opening polymerization

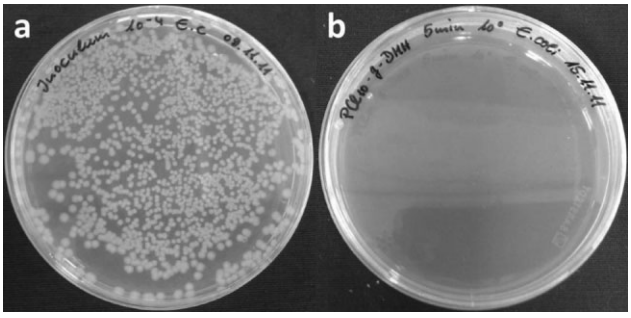

Figure 8. Colony formation of *E. coli* (DSMZ No. 1077, K12 strain 343/113) after incubation for 24 h at 37 °C: (a) blank control (inoculum in the appropriate dilution stage) and (b) after exposure to PCL-*g*-DMH for 5 min.

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