Poly(caprolactone-*co*-oxo-crown ether)-Based Poly(urethane)urea for Soft Tissue Engineering Applications

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Random copolymers of ϵ-caprolactone and 2-oxo-12-crown-4 ether, poly(CL-*co*-OC), were used as soft segments in the synthesis of a set of poly(urethane)urea thermoplastic elastomers. With increasing OC content, the soft segment crystallinity decreased, which influenced the mechanical properties: strain induced crystallization disappeared upon the introduction of OC into poly(CL). The material therefore became weaker, however, without a reduction in strain at break. All polymers showed mechanical properties that are suitable for soft tissue engineering. Degradation studies of poly(CL-*co*-OC) copolymers revealed a higher intrinsic rate of hydrolysis as compared to poly(CL). When at least two neighboring OC units were present in the soft segment, a jump in the intrinsic hydrolysis rate was observed. From this study we deduced an ideal OC:CL ratio for the thermoplastic elastomer soft segments for soft tissue engineering applications. An in vitro degradation study of these poly(urethane)urea showed an increased weight loss. Combined with the enhanced hydrophilicity and reduced crystallinity, we are confident that this will indeed lead to an increased degradation rate in vivo.

Introduction

The development of new synthetic scaffold materials for soft tissue engineering has received much attention in recent years. Promising candidates for this application are the polyurethanes (PU), due to their good mechanical compatibility with soft tissues.[1] Poly(ϵ-caprolactone) [poly(CL)] is often applied as soft block material.[2−4] However, it is known that the degradation time of poly(CL) can be as long as 2 years in vivo.[5] Albertsson et al. have synthesized 1,5 dioxepan-2-one (DXO), which is known to have a slightly higher hydrolysis rate as compared to CL, in an attempt to solve this problem.[6] Also increasing the hydrophilicity and reducing the crystallinity of the soft block material are ways to increase the degradation rate of poly(CL) in a controllable way.[7] A well-known hydrophilic polymer is poly(ethylene oxide) (PEO). Introducing PEO units into the poly(CL) backbone leads to increased water uptake and accelerated ester hydrolysis.[8−10] Woodhouse et al. have blended poly(CL) and PEO-based polyurethanes for this purpose. Rapid initial degradation was observed in buffer, most probably due to a loss of the PEO based material, followed by a slower degradation of the poly(CL)-based PU. At the same time the blends are weaker and less tough with increasing amount of PEO-based material.[8] Block copolymers of PEO and poly(CL) have been used as soft segments for PUs as well. Again, an increased water uptake results in a faster in vitro degradation[9,10] and reduced mechanical properties.[10]

Recently, we have introduced 2-oxo-12-crown-4 ether (OC) as a novel hydrophilic lactone monomer[11,12] (Scheme 1). Ring-opening polymerization of this strain-free lactone catalyzed by Novozym 435, an immobilized form of the lipase B of *Candida antartica* (CALB), is fast and effective. Poly(OC) is a hydrophilic polyester that is soluble in water. Hydrolysis studies have shown a steady decrease of the molecular weight (40% reduction in weight in 8 days), suggesting that the poly(OC) ester bonds have a higher intrinsic hydrolysis rate compared to poly(CL) ester bonds. Here, we focus on the copolymerization of CL and OC with CALB, with the aim to obtain random copolymers that are more hydrophilic than poly(CL) itself. Copolymers with various CL:OC ratios were studied to select the ideal OC:CL ratio (Table 1). Prepolymers with varying CL:OC ratios were subsequently applied as soft segments in poly(urethane)urea (PUU) aiming at a set of materials with controlled degradation rates. All the resulting polymers were fully characterized by NMR, IR, GPC, and DSC. Moreover, their mechanical properties were evaluated and compared to well-known poly(CL)-based PUUs. Finally, we rationalize the degradation behavior of the three studied PUUs.

Scheme 1. Chemical Structure of 2-Oxo-12-crown-4 Ether (OC) and Its Polymer Poly(OC)

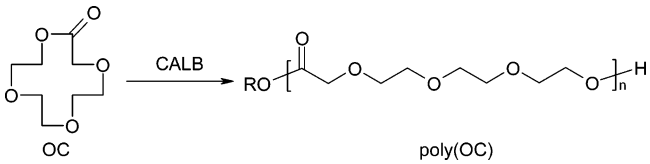

Table 1. Composition and Nomenclature of Prepolymer Diols and Corresponding PUUs

composition CL:OC (obsd molar ratio)	prepolymer diol	poly(urethane)urea
100:0	**P1**	**PUU1**
97:3	**P2**	**PUU2**
86:14	**P3**	**PUU3**

Experimental Section

Materials. Tetrahydrofuran (THF), methanol (MeOH), chloroform ($CHCl_3$), diethyl ether (Et_2O), hexane, hexafluoroisopropanol (HFIP), and heptane were purchased from Biosolve. 1,4-Diisocyanatobutane and ϵ-caprolactone were purchased from Fluka. 1,6-Hexanediol was obtained from Sigma. Novozym 435, CALB immobilized on an acrylic resin, was obtained from Novozymes A/S. All other chemicals were

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purchased from Aldrich and used without further purification unless otherwise noted. Chloroform and DMSO were dried over molecular sieves of 4 and 3 Å, respectively. Toluene was freshly distilled from sodium. Deuterated solvents were purchased from Cambridge Isotope Laboratories. All polymerizations were carried out under a dry argon atmosphere. 2-Oxo-12-crown-4 ether (OC) was synthesized as described before.[12]

Instrumentation. NMR spectra were taken with a Varian Mercury 400 spectrometer (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR) or a Varian Gemini (300 MHZ for ^{1}H NMR, 75 MHz for ^{13}C NMR) spectrometer in $CDCl_3$ with the delay time (d1) set at 10 s for ^{1}H NMR. Chemical shifts are reported as parts per million with respect to tetramethylsilane (TMS). The peak assignments are based on ^{1}H NMR studies of the respective homopolymers and poly(CL-*co*-OC) copolymers. The integral ratio of the signals at 4.30 and 4.24 ppm ($CH_2^{(OC)}OC{=}O$) and 2.3 ppm ($CH_2^{(CL)}C{=}O$) were used to determine the composition of the copolymers. Infrared spectra were measured on a Perkin-Elmer 1600 FT-IR. GPC (HFIP, 0.8 mL/min) was measured using a Shimadzu LC-10AD that was equipped with a PSS 2 × PFG-lin-XL column (7 μm, 8 × 300 mm) at 40 °C with a Waters 2487 dual wavelength UV detector, a viscotek 270, a light-scattering (RALS/LALS) detector, and a viscometry Waters 2414 differential refractive index detector at 35 °C. Samples of 4.00 mg mL^{-1} were filtered over a 0.2 μm PTFE filter and injected in volumes of 50 μL. GPC [*N*-methylpyrrolidone (NMP), 1 mL/min] was measured on a PL-GPC 210 high-temperature chromatograph, using a Polymer Laboratories gel 5 μm Mixed-C column with a Shimadzu RID-6A detector at 80 °C. Samples of 100 μL (3 mg/mL concentrations) were injected. GPC (THF, 1 mL/min) was measured on a Shimadzu LC-10ADvp system with a Shimadzu RID-10A detector and a PLgel 5-μm mixed-D and mixed C-column. All molecular weights were relative to polystyrene standards. Differential scanning calorimetry (DSC) measurements were performed on a Perkin-Elmer differential scanning calorimeter Pyris 1 with Pyris 1 DSC autosampler and Perkin-Elmer CCA7 cooling element under a nitrogen atmosphere. Melting and crystallization temperatures were determined in the second heating run at a heating/cooling rate of 10 °C min^{-1}, and glass transition temperatures were determined in a third run at a heating rate of 40 °C min^{-1}. Flow temperatures were determined using a Jeneval microscope equipped with a Linkam THMS 600 heating device.

Determination of the Randomness of HMW Poly(CL-*co*-OC). The fractions of the OC−OC dyad, CL−CL dyad, and OC−CL dyad were derived from the integral of the signals at 4.30, 4.05, and 4.24 ppm in the corresponding ^{1}H NMR spectra. The fraction of the CL−OC dyad was derived from the peak at 4.21−4.12 ppm after correction for overlapping signals. The average sequence lengths of the CL and OC units (L_{CL} and L_{OC}, respectively) were calculated as follows: $L_{CL} = (f_{CL-OC} + f_{CL-CL})/f_{CL-OC}$ and $L_{OC} = (f_{OC-CL} + f_{OC-OC})/f_{OC-CL}$, where *f* represents the observed fraction of the respective dyad.[13,14] The average sequence lengths of the CL and OC units calculated for random copolymers (L_{CL}^R and L_{OC}^R, respectively) were calculated as follows: $L_{OC}^R = k + 1$ and $L_{CL}^R = (k + 1)/k$, where *k* is equal to [OC]/[CL].[14,15] The randomness factor (*R*) was calculated as $R = L_{OC}^R/L_{OC}$.[14,15] Dyad fractions of the copolymer blocks were compared with calculated dyad fractions based on theoretical dyad fractions where random statistical copolymers were assumed.

Hydrolysis Experiments of HMW Poly(CL-*co*-OC). Poly(CL-*co*-OC) was dissolved in a 1/1 (v/v) mixture of THF/water and stirred at 37 °C. Samples were taken at regular time intervals. After removal of water with $MgSO_4$, the molecular weight decrease was analyzed by GPC (THF).

Film Preparation. Films of the various PUUs were prepared by dissolving 1.5 g of polymer in ~26 mL of HFIP under a blanket of argon. The resulting solutions were filtered (5 μm PTFE filter) before solvent casting in a PTFE mold. The solvent was allowed to slowly evaporate overnight in the presence of P_2O_5. Before use the films were thermally treated for approximately 5 h, 50 °C below the observed T_{flow} under reduced pressure and subsequently stored under P_2O_5. The thickness of all films was between 0.2 and 0.3 mm.

Variable Temperature Infrared Spectroscopy. Variable temperature infrared spectra were recorded with an Excalibur FTS 3000 MX from Bio-Rad between 25 and 200 °C. All samples were allowed to reach the set temperature for 5 min before recording the IR spectrum.

Tensile Testing. Tensile properties were measured according to ASTM D 1708-96 on a Zwick Z100 equipped with a 2.5 kN or 100 N load cell applying a crosshead speed of 20 mm/min. Grip to grip separation was sometimes <22 mm; however, samples that failed at the clamps were not included. Yield stresses and strains were determined by determining the intersection point of the two tangents to the initial and final parts of the load elongation curves around the yield point.[15] An indicative Young's modulus was determined by calculating the slope of the stress−strain curve via $E\ [\mathrm{MPa}] = (\sigma_2 - \sigma_1)/(\epsilon_2 - \epsilon_1)$, where $\epsilon_2 = 0.0005$ and $\epsilon_1 = 0.0025$.[16]

Degradation of PUUs in Buffer. Small disks with a diameter of 6 mm were punched from the same films as used for tensile testing. These disks were each placed in 30 mL of PBS buffer (pH 7.4) at 37 °C with gentle shaking. After 50 days, two of each three disks were removed from the buffer, rinsed with water, dried, and weighed, and GPC (HFIP) was measured. Each third disk was removed from the buffer, rinsed, dried, and weighed after 121 days. At this time point, no GPC was measured.

Syntheses. *Novozym 435 Catalyzed Copolymerization of OC and CL (HMW Copolymers).* 2-Oxo-12-crown-4 ether (OC) (400 mg, 2.11 mmol) and Novozym 435 (5 mg) were dried (separately) in vacuo at 50 °C in the presence of P_2O_5. A 5 M stock solution of CL in distilled toluene was prepared and stirred overnight at 45 °C in the presence of dry molecular sieves (4 Å). After 12 h, 3.2 mL of stock solution was transferred to a 10 mL flask containing dry OC (400 mg), and Nozozym 435 (5 mg) was added. After 7 days, the enzyme was removed from the reaction mixture by filtration and the polymer was precipitated in cold heptane. The yields after precipitation were around 70%. The amount of stock solution was kept constant, the OC feed was varied to obtain CL/OC molar ratios of 93/7, 82/18, 53/47, and 34/66. For the polymerization of CL, no OC was added.

^{1}H NMR for poly(CL-*co*-18 mol % OC) ($CDCl_3$): δ = 4.30 (t, $CH_2^{(OC)}O(C{=}O)$), 4.23 (t, $CH_2^{(OC)}O(C{=}O)$), 4.21−4.12 ($CH_2^{(OC)}(C{=}O)$ and $CH_2^{(CL)}O(C{=}O)$, 4.06 (t, $CH_2^{(CL)}O(C{=}O)$), 3.80−3.60 (CH_2O), 2.30 (dt, $CH_2^{(CL)}(C{=}O)$), 1.63 (m, $CH_2CH_2(C{=}O)$), 1.39 (m, CH_2). ^{1}H NMR for poly(CL) ($CDCl_3$): δ = 4.08 (t, $CH_2O(C{=}O)$), 3.63 (t, CH_2-OH end group), 2.32 (t, $CH_2(C{=}O)O$), 1.68 (m, CH_2) 1.41 (m, CH_2). GPC (THF): poly(CL), M_n = 18.4 × 10^3 kg/mol, M_w = 27.9 × 10^3 kg/mol, M_p = 26.5 × 10^3 kg/mol, PD = 1.5; poly(CL-*co*-7 mol % OC), M_n = 22.8 × 10^3 kg/mol, M_w = 38.7 × 10^3 kg/mol, M_p = 38.0 × 10^3 kg/mol, PD = 1.7; poly(CL-*co*-18 mol % OC), M_n = 8.1 × 10^3 kg/mol, M_w = 17.3 × 10^3 kg/mol, M_p = 17.5 × 10^3 kg/mol, PD = 2.1; poly(CL-*co*-47 mol % OC), M_n = 3.2 × 10^3 kg/mol, M_w = 6.5 × 10^3 kg/mol, M_p = 7.4 × 10^3 kg/mol, PD = 2.0; poly(CL-*co*-66 mol % OC), M_n = 2.5 × 10^3 kg/mol, M_w = 5.0 × 10^3 kg/mol, M_p = 5.7 × 10^3 kg/mol, PD = 2.0.

*Novozym 435 Catalyzed Copolymerization of OC and CL (Prepolymer Diols) (**P1**−**P3**).* OC (2.7 g, 14.21 mmol) and Novozym 435 (97 mg) were dried overnight (separately) in vacuo at 50 °C in the presence of P_2O_5. A solution of ϵ-caprolactone (CL) (6.54 gram, 57.31 mmol) and 1,6-hexanediol (0.55 gram, 4.67 mmol) in 20 mL of distilled toluene was prepared and stirred overnight at 45 °C in the presence of dry molecular sieves (4 Å). After 12 h, the toluene solution was transferred to a 50 mL flask containing the dry OC, and Novozym 435 was added. After stirring at 45 °C for 5 h, the enzyme was removed from the reaction mixture by filtration and the solution was concentrated. The polymer was precipitated in cold heptane. The yields after precipitation were typically around 70%.

^{1}H NMR for poly(CL-*co*-14 mol % OC) diol ($CDCl_3$): δ = 4.30 (t, $CH_2^{(OC)}O(C{=}O)$), 4.23 (t, $CH_2^{(OC)}O(C{=}O)$), 4.21−4.12 ($CH_2^{(OC)}(C{=}O)$ and $CH_2^{(CL)}O(C{=}O)$, 4.06 (t, $CH_2^{(CL)}O(C{=}O)$), 3.80−3.60 (CH_2O),

Table 2. Determination of the Randomness of HMW Poly(CL-*co*-OC) with Various Compositions[a]

obsd CL/OC	f_{OC-OC}		f_{CL-CL}		f_{OC-CL}		f_{CL-OC}						
molar ratio	obsd	calcd	obsd	calcd	obsd	calcd	obsd	calcd	L_{OC}[b]	L_{OC}^R [b]	L_{CL}[b]	L_{CL}^R [b]	R_{OC}[c]
93/7	0.004	0.005	0.866	0.865	0.063	0.065	0.067	0.065	1.06	1.08	13.84	14.29	1.01
82/18	0.030	0.032	0.664	0.672	0.152	0.148	0.154	0.148	1.20	1.22	5.32	5.56	1.02
53/47	0.212	0.185	0.272	0.281	0.252	0.228	0.264	0.228	1.84	1.81	2.03	2.23	0.98
34/66	0.410	0.436	0.110	0.116	0.215	0.224	0.266	0.224	2.90	2.94	1.42	1.52	1.01

[a] Observed from ^{1}H NMR spectra or calculated from a random copolymer chain. [b] L_{OC} and L_{CL} were derived from ^{1}H NMR spectra. L_{OC}^R an L_{CL}^R were calculated from a completely random copolymer chain. [c] Degree of randomness for the OC monomer.

2.30 (dt, C$H_2^{(CL)}$(C═O)), 1.63 (m, CH_2CH$_2$(C═O)), 1.39 (m, CH_2). ^{1}H NMR for poly(CL) ($CDCl_3$): δ = 4.08 (t, CH_2O(C═O)), 3.63 (t, CH_2-OH end group), 2.32 (t, CH_2(C═O)O), 1.68 (m, CH_2) 1.41 (m, CH_2). ^{1}H NMR for poly(CL) diol ($CDCl_3$): δ = 4.08 (t, CH_2O(C═O)), 3.63 (t, CH_2OH end group), 2.32 (t, CH_2(C═O)O), 1.68 (m, CH_2) 1.41 (m, CH_2). GPC (NMP): poly(CL) diol, M_n = 3730 g/mol, M_w = 5160 g/mol, PD = 1.4; poly(CL-*co*-3 mol % OC) diol, M_n = 3330 g/mol, M_w = 4600 g/mol, PD = 1.4; poly(CL-*co*-14 mol % OC) diol, M_n = 3320 g/mol, M_w = 4650 g/mol, PD = 1.4.

Prepolymer diols with other OC content were synthesized in the same manner. For the polymerization of CL, no OC was added.

Synthesis of Poly(CL-co-14 mol % OC) Diisocyanate. The poly(CL-*co*-14 mol % OC) diol was dried overnight in the presence of P_2O_5. The experimental setup was flushed with argon; glassware was heated with a heat gun and allowed to cool to room temperature under argon. Poly(CL-*co*-14 mol % OC) diol (10 g, 5.3 mmol) was dissolved in 50 mL of dry chloroform (4 Å molecular sieves) and extra 4 Å molecular sieves were added to remove water under a dry argon atmosphere. Six equivalents of 1,4-diisocyanatobutane (4.0 mL, 32 mmol) and 4 drops of dibutyltin dilaurate were added. After heating under reflux for 22 h under argon, the reaction mixture was allowed to cool down to room temperature and was filtered, precipitated in cold, dry ethyl ether (0 °C) or heptane. The typical yield ~ 85%.

^{1}H NMR for poly(CL-*co*-14 mol % OC) diisocyanate ($CDCl_3$): δ = 4.30 (t, $CH_2^{(OC)}$O(C═O)), 4.22 (t, $CH_2^{(OC)}$O(C═O)), 4.14−4.17 ($CH_2^{(OC)}$(C═O) and $CH_2^{(CL)}$O(C═O)), 4.05 (t, $CH_2^{(CL)}$O(C═O)), 3.65−3.75 (CH_2O), 3.34 (t, CH_2NCO), 3.20 (q, (C═O)NHCH_2), 2.32 (t, O(C═O)CH_2CH$_2$), 2.28 (t, O(C═O)CH_2CH$_2$), 1.56−1.65 (O(C═O)-CH$_2$CH_2, CH_2CH$_2$O(C═O), CH_2CH$_2$NCO), 1.53 (CH_2CH$_2$CH$_2$NCO), 1.21−1.36 (CH$_2$CH_2CH$_2$). IR (ATR): ν = 3374, 2917, 2850, 2266, 1732, 1530, 1464, 1170 cm^{-1}. ^{1}H NMR for poly(CL) diisocyanate ($CDCl_3$): δ = 4.78 (N*H*), 4.06 (t, CH_2O(C═O)), 3.35 (t, CH_2NCO), 3.21 ((C═O)NHCH_2), 2.30 (t, O(C═O)CH_2), 1.52−1.69 (O(C═O)-CH$_2$CH_2, CH_2CH$_2$O(C═O), and CH_2CH$_2$NCO), 1.51 (CH_2CH$_2$NH(C═O)), 1.36−1.42 (CH$_2$CH_2CH$_2$). IR (ATR): ν = 3383, 2944, 2865, 2265, 1732, 1531, 1471, 1175 cm^{-1}.

Diisocyanate prepolymers of other compositions were synthesized in a similar manner, and depending on the amount of OC incorporated, a white powder or a viscous yellow oil was obtained.

*Synthesis of Poly(CL-co-14 mol % OC) (**PUU3**)*. The experimental setup was flushed with argon; glassware was heated with a heat gun and was allowed to cool to room temperature under argon. Poly(CL-*co*-14 mol % OC) diisocyanate (6.3 g, 2.8 mmol) was dissolved in 200 mL of dry chloroform under dry argon atmosphere. 1,4-Diaminobutane (0.28 mL, 2.8 mmol) dissolved in 50 mL of dry chloroform was added dropwise to the reaction mixture until the isocyanate peak had disappeared from the FT-IR spectrum (2265 cm^{-1}). The polymer solution was precipitated in heptane, filtered, and dried overnight under high vacuum at room temperature. The product was obtained as white elastic flakes in an overall yield of 45−87% for the three different compositions. NMR and IR data given for **PUU3** are representative for all three polymers.

^{1}H NMR of **PUU3** ($CDCl_3$/MeOD (8%)): δ = 4.30 (t, $CH_2^{(OC)}$O-(C═O)), 4.22 (t, $CH_2^{(OC)}$O(C═O)), 4.14−4.17 ($CH_2^{(OC)}$(C═O) and $CH_2^{(CL)}$O(C═O)), 4.05 (t, $CH_2^{(CL)}$O(C═O)), 3.65−3.75 (CH_2O), 3.13 (q, (C═O)NHCH_2), 2.32 (t, O(C═O)CH_2CH$_2$), 2.28 (t, O(C═O)CH_2-CH$_2$), 1.56−1.65 (O(C═O)CH$_2$CH_2 and CH_2CH$_2$O(C═O)), 1.49 ((C═O)NHCH$_2$CH_2), 1.21−1.36 (CH$_2$CH_2CH$_2$). FT-IR (ATR): ν = 3320, 2941, 2866, 1728, 1683, 1620, 1577, 1537, 1464, 1155 cm^{-1}. DSC: **PUU1**, T_g = −54 °C, T_m = 19 °C; **PUU2**, T_g = −51 °C, T_m = 21 °C; **PUU3**, T_g = −52 °C, T_m = −10 °C. GPC (HFIP): **PUU1**, M_n = 33 × 10^3 kg/mol, M_w = 269 × 10^3 kg/mol, PD = 8.1; **PUU2**, M_n = 19 × 10^3 kg/mol, M_w = 76 × 10^3 kg/mol, PD = 4.0; **PUU3**, M_n = 19 × 10^3 kg/mol, M_w = 53 × 10^3 kg/mol, PD = 2.8.

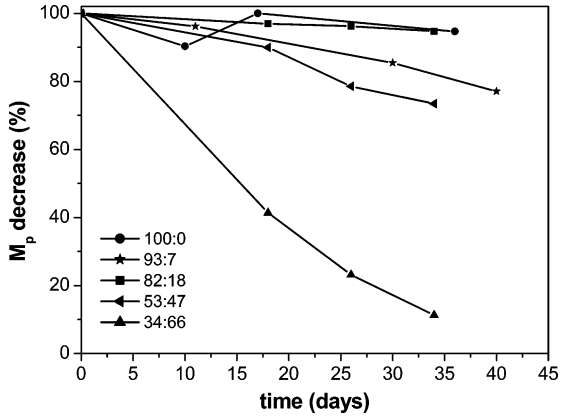

Figure 1. M_p decrease over time for various compositions of poly(CL-*co*-OC).

Results and Discussion

Poly(CL-*co*-OC) Copolymers. Copolymers with various OC:CL ratios (100:0, 93:7, 82:18, 53:47, or 34:66) were obtained by enzymatic ring-opening polymerization of CL and OC by Novozym 435. By varying the ratio of the two lactones, the molecular weight and composition of the prepolymers could be tuned. It was previously found that the use of Novozym 435 in copolymerization results in random copolymers.[12,17] In the characterization of this set of materials, ^{1}H NMR spectroscopy was used to determine the randomness of HMW versions of the copolymers, in analogy to previously reported copolymers of OC and ω-pentadecalactone[12] (see also Experimental Section). The resulting values of the experimental and calculated dyad fractions and the average sequence lengths of OC and CL units, L_{OC} and L_{CL}, are summarized in Table 2. The experimental values are in good agreement with the calculated values for all copolymers analyzed. As expected for a random copolymer, R is always close to 1. These results are also in good agreement with those of the previously reported poly(PDL-*co*-OC) copolymers.[12]

To study the intrinsic hydrolysis rate, these polymers were stirred in a 1/1 THF/water solution (v/v), and GPC data of these polymers were collected as a function of time. Figure 1 shows the decrease of M_p with time (for M_p values, see Experimental Section), and the GPC traces of poly(CL-*co*-18 mol % OC) and poly(CL-*co*-66 mol % OC) are reported in Figure 2. With increasing amounts of OC, a slightly faster decrease in molecular weight was observed. However, a striking difference is observed

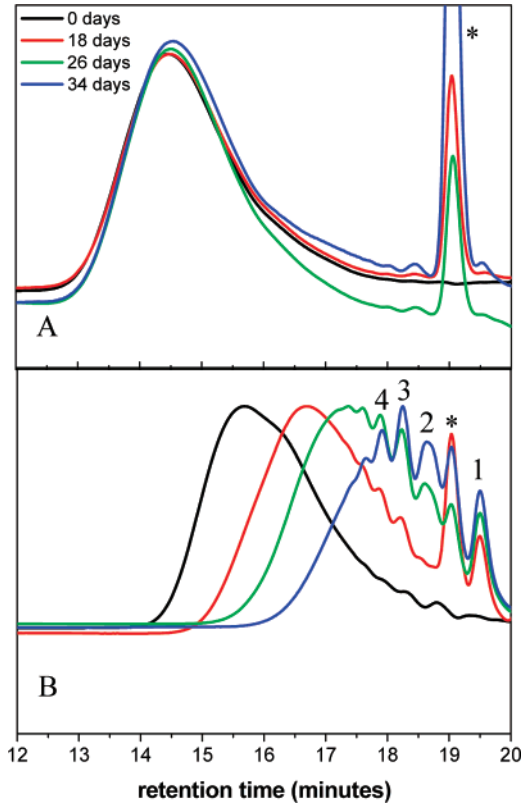

Figure 2. GPC traces of (A) poly(CL-*co*-18 mol % OC) and (B) poly(CL-*co*-66 mol % OC) when stirring in THF/water (1/1, v/v) over time. ∗ denotes the peak corresponding to butylhydroxytoluene (BHT), a stabilizer in THF. The numbers in B denote the amount of repeating CL and/or OC units in the respective oligomers.

when going from poly(CL-*co*- 47 mol % OC) to poly(CL-*co*-66 mol % OC). The latter shows a significantly faster decrease in molecular weight. Furthermore, in the GPC traces beyond 18 days, we can already find monomers and small oligomers. From Table 2, we know that there are most probably at least two neighboring OC units ($L_{OC} > 2$) in those polymers, while that chance is still very small in the former ($L_{OC} < 2$). Apparently, the presence of two connected OC units increases the hydrolysis rate dramatically.

On account of the results just described, we decided to use three compositions up to ∼20 mol % of OC in poly(CL-*co*-OC) as prepolymers for the PUU synthesis. We know from previous research that PUUs where PEO had been introduced into the poly(CL) soft segment showed reduced mechanical properties.[10] In the extreme case of only PEO in the soft segment, the resulting polymer is a tacky, semiviscous material, with no mechanical properties at all.[4b,18a] Since PEO and poly(OC) are very similar in chemical structure and properties, a similar trend will hold for the introduction of poly(OC) into the poly(CL) soft segment. We anticipate that using up to ∼20 mol % of OC will not lead to severely decreased mechanical properties. The reduction in mechanical properties is due to (partial) hydrogen bonding of the urea N−H not just to the carbonyl of another urea but also to the ether oxygens in the soft segments,[18b] resulting in reduced phase separation between hard and soft segments. This event would rule out the concept of a modular and supramolecular approach to introduce biofunctionalities into the polymers as we recently introduced.[19] In this approach we functionalize for example peptides with the same hydrogen-bonding unit as present in the polymer hard segment. By simply mixing this peptide with the desired polymer, the peptide is strongly incorporated into the polymer hard segment stacks by specific hydrogen-bonding interactions. Such an approach would lose its specificity when ether units from the soft segment are disrupting the well-defined supramolecular hard segment stacks. Finally, Figure 1 shows a dramatic jump in degradation speed when going from poly(CL-*co*- 47 mol % OC) to poly(CL-*co*-66 mol % OC). And although the in vivo degradation time for soft tissue engineering applications is not exactly known, it is proposed that scaffold degradation should take place within several weeks to months, depending on each specific application.[20] Therefore, the hydrolysis rate of poly(CL-*co*-66 mol % OC) is too fast for tissue engineering applications, especially for load-bearing applications such as cardiovascular tissue engineering. For all these reasons, the choice for PUUs based on poly(CL-*co*-OC) soft segments containing up to ∼20 mol % of OC is regarded as the right choice.

Table 3. Composition and Molecular Weights of Synthesized Poly(CL-*co*-OC) Diol Prepolymers

prepolymers	feed ratio poly(CL_n-*co*-OC_m) (*n*:*m*)	obsd molar ratio (^{1}H NMR) poly(CL_n-*co*-OC_m) (*n*:*m*)	M_n (GPC)[a]	M_w (GPC)[a]
P1	100:0	100:0	3730	5160
P2	95:5	97:3	3330	4600
P3	80:20	86:14	3320	4650

[a] All GPC were measured in NMP relative to polystyrene standards.

Table 4. Thermal Properties of the PUUs

polymer	T_g (°C)	$T_{m,s}$[a] (°C)	$T_{c,s}$[a] (°C)	ΔH (J/g)	T_{flow}[b] (°C)
PUU1	−54	19	2	17.4	160−175
PUU2	−51	21	−9	11.9	140−174
PUU3	−52	−10	−36	6.7	140−160

[a] $T_{m,s}$ and $T_{c,s}$: melting and crystallization temperatures of the soft segment. [b] Determined with optical microscopy.

Poly(CL-co-OC) Poly(urethane)urea. Prepolymer diols **P1**−**P3** were obtained by enzymatic ring opening polymerization of CL and OC by Novozym 435, using 1,6-hexanediol as an initiator. Also, the prepolymers synthesized here showed a random composition (data not shown). The composition of the prepolymers could be determined by ^{1}H NMR spectroscopy and showed almost the same composition as the monomer feed (Table 3). All prepolymer diols showed after precipitation a relatively low polydispersity of around 1.4 relative to polystyrene standards.

The obtained prepolymer diols **P1**−**P3** were end-capped with an excess of diisocyanatobutane using dibutyltin dilaurate as a catalyst. The resulting macro-diisocyanate was subsequently chain extended with 1,4-diaminobutane, resulting in three poly(urethane)ureas, **PUU1**−**PUU3**, respectively (Scheme 2). The structures of all poly(urethane)ureas were confirmed by ^{1}H NMR spectroscopy (see Supporting Information, Figure S1), GPC, and IR.

The thermal properties of **PUU1**−**PUU3** were studied by differential scanning calorimetry (DSC). All polymers were measured between −100 and 180 °C. The second run at 10 °C/min was used to determine the melting and crystallization transitions, while the third heating run at 40 °C/min was used to determine the glass transition temperature. The results are presented in Table 4 and the DSC curve of **PUU1** is shown in Figure 3.

PUU1 has a glass transition at −54 °C, close to the T_g of high molecular weight poly(CL) (T_g = −60 °C).[21] When OC

Scheme 2. Chemical Structure of Poly(CL-*co*-OC) Prepolymer (top) and the Corresponding PUU (bottom)

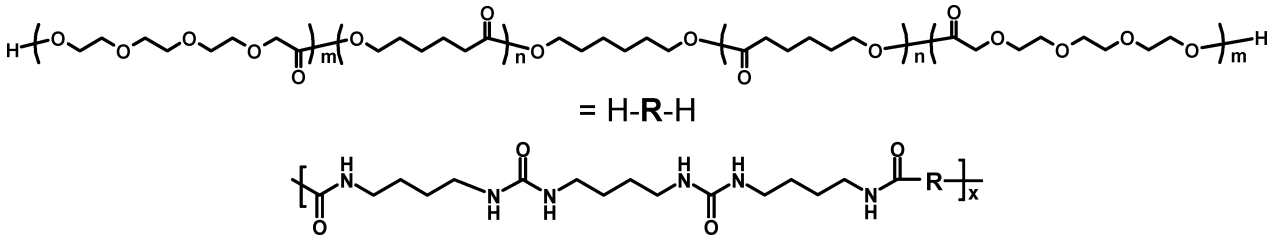

Table 5. Mechanical Properties for **PUU1**−**PUU3**

polymer (no. of evaluated samples)	*E* (MPa)	σ_{yield} (MPa)	ϵ_{yield} (%)	tensile strength (MPa)	$\epsilon_{at\ break}$ (%)	toughness (MPa)
PUU1 ($n = 8$)	14.4 ± 4.1	2.9 ± 0.1	17.6 ± 1.0	36.9 ± 5.7	910 ± 29	174 ± 18
PUU2 ($n = 5$)	33.2 ± 1.2	4.4 ± 0.1	14.3 ± 0.5	28.1 ± 0.3	1030 ± 14	173 ± 3
PUU3 ($n = 6$)	25.5 ± 0.5	3.9 ± 0.1	16.1 ± 0.4	18.4 ± 0.3	970 ± 30	109 ± 4

was introduced in **PUU2** and **PUU3**, a single T_g between the T_g of the respective homopolymers, poly(CL) and poly(OC) ($T_g = -40$ °C),[12] confirmed the random distribution of OC and CL. All traces showed only one melting transition that was attributed to melting of the poly(CL-*co*-OC) soft segment. Poly(CL) is known to be semicrystalline. However, in the copolymer, OC can strongly reduce the crystallinity of poly(CL), as can be seen from the decreasing trend of the melting and crystallization temperatures as well as the melting enthalpy, when incorporating more OC.

For the hard segments, we never observed a melting or crystallization transition by DSC. Optical microscopy was therefore used to determine the T_{flow}, by applying a heating rate of 1 °C/min. We observed a gradual melting transition between 140 and 175 °C. The onset of flow (locally in the sample) and the temperature at which the entire sample was flowing are reported in Table 4. Variable temperature IR, as performed before for similar poly(urethane)urea and polyurea,[18] confirmed a gradual transition and revealed the presence of hydrogen bonds even in the melt (see Supporting Information Figure S2).

For soft tissue engineering, flexible materials are needed that are sufficiently strong and elastic. To be suitable for cardiovascular applications, for example, a soft tissue with relatively high demands, the scaffolds should have a Young's modulus in the range of a few MPa, purely elastic behavior up to at least 10% strain, and sufficiently high strain at break and tensile strength to prevent in vivo failure, as was also discussed in a previous paper.[19] We studied the mechanical properties of our materials by unidirectional tensile testing under ambient conditions. Representative tensile curves are plotted in Figure 4, and the mechanical properties are listed in Table 5.

All polymers **PUU1**−**PUU3** are flexible and elastic, properties typical for thermoplastic elastomers. The stress−strain curve of **PUU1** shows a different shape as compared to the other two curves, most probably caused by the semicrystallinity of the poly(CL) soft segment. Also **PUU1** showed some strain-induced crystallization from approximately 500% onward. **PUU2** became more opaque during tensile testing; however, the stress−strain curve shows no evidence for strain-induced crystallization. As a result, the tensile strength and toughness (defined as the area under the stress−strain curve) are reduced. **PUU3** stayed transparent during the whole tensile test. The tensile strength and toughness were further reduced, while strain at break was not changed. This trend is very similar, as observed by others.[8,10] The material seems to become a little more stiff (increased Young's modulus) when OC was present in the soft segments, but no clear trend could be derived. Despite their differences, the polymers also have an important communality: mechanical properties that are suitable for soft tissue engineering. After processing these materials into porous structures, we anticipate that the Young's modulus will decrease and come to the appropriate range for soft tissues. The materials are more than

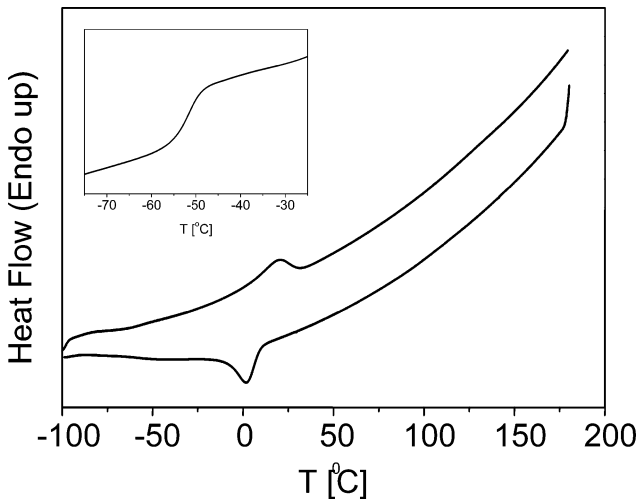

Figure 3. Second DSC heating and cooling curves at 10 °C/min of **PUU1**. Inset: Zoom in on T_g region.

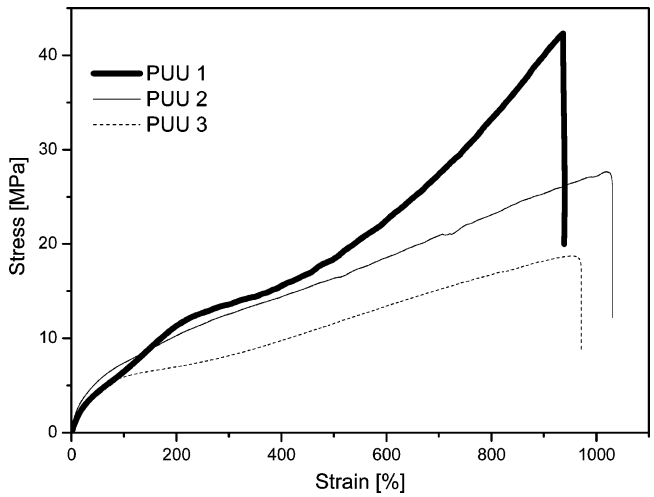

Figure 4. Representative engineering stress−strain curves for **PUU1**−**PUU3**.

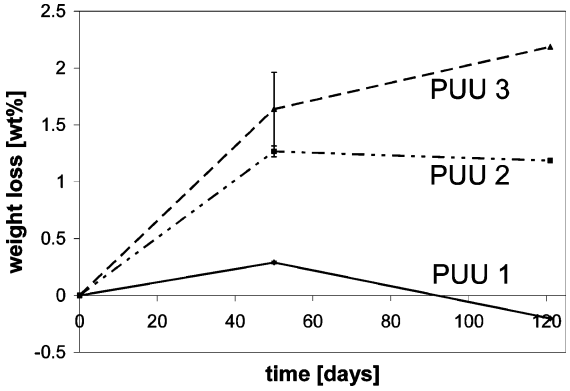

Figure 5. Weight loss over time of poly(CL-*co*-OC) PUUs. For $t =$ 50 days, average and standard deviation of two measurements; for $t =$ 121 days, single measurement.

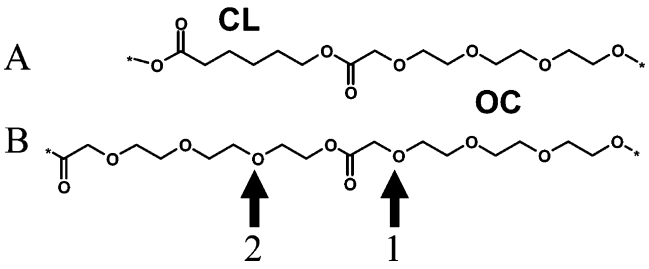

Figure 6. More labile ester bond in the case of an OC−OC repeat.

sufficiently strong and tough to prevent in vivo failure. Finally, the yield point is around 15−20% strain, which is more than most soft tissues will experience in vivo.

Small disks with a diameter of 6 mm were punched from the same films as used for tensile testing. These disks ($n = 3$ for each polymer) were then placed in 30 mL of PBS buffer (pH 7.4) at 37 °C with gentle shaking. After 50 days, the weight loss of two of the three disks was determined, and the third disk was weighed after 121 days (Figure 5). The more OC that was introduced into the poly(CL) backbone, the more weight loss that was observed over 4 months. The intrinsic hydrolysis rate has indeed increased as anticipated. However, a maximum weight loss of little over 2% is still relatively low. Yet, the in vivo degradation behavior is not only determined by the hydrolysis rate of the polyester but also by morphological parameters, that is, the hydrophilicity and crystallinity of the polymers. Since we increased also those parameters, the in vivo degradation behavior is expected to be relevant for soft tissue engineering purposes.

Conclusions

Random poly(CL-*co*-OC) copolymers with varying molecular weights were synthesized by varying the amount of OC and CL monomers, and the amount of initiator. The intrinsic hydrolysis rate increased when more OC was introduced and made an unexpected jump in the case of at least two neighboring OC units (situation B in Figure 6). This can be attributed to oxygen 1, which makes the ester more labile, in combination with oxygen 2, which provides a better leaving group as compared to situation A.

On the basis of these initial results, short diol prepolymers of three different compositions (CL:OC = 100:0, 97:3, and 86:14) were synthesized and used as a soft segment in poly(urethane)urea polymers. The hard bisurea−bisurethane segments do not cause a melting transition in DSC. From previous work we already expected a more gradual melting transition.[18] This observation was also made for **PUU1**−**PUU3** by VTIR and optical microscopy. **PUU1** displays a T_g at −54 °C, close to the T_g of pure poly(CL). When OC is introduced, still a single T_g is observed at slightly higher temperatures. This finding confirms that OC is randomly incorporated in the PCL backbone of the soft segment, as a block type copolymer would result in two separate glass transitions. This random incorporation of OC into poly(CL) also reduces the crystallinity of the soft segment. The presence of OC units hinders the CL units in their crystallization process. This could also explain the reduction in strength of these materials as compared to **PUU1**. However, all polymers have mechanical properties that are suitable for soft tissue engineering applications. Due to the increased hydrophilicity and reduced crystallinity, relevant in vivo degradation of the synthesized **PUU2** and **PUU3** is anticipated, with a faster degradation rate as compared to poly(CL) based PUUs.

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Supporting Information Available. ^{1}H NMR spectrum of **PUU3** and representative variable-temperature IR data of **PUU2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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