Mathew, S.; Baudis, S.; Neffe, A.T.; Behl, M.; Wischke, C.; Lendlein, A.:

Effect of diisocyanate linkers on the degradation characteristics of copolyester urethanes as potential drug carrier matrices

In: European Journal of Pharmaceutics and Biopharmaceutics A (2015)
Elsevier

DOI: 10.1016/j.ejpb.2015.03.025

Effect of diisocyanate linkers on the degradation characteristics of copolyester urethane drug carrier matrices

Simi Mathew, Stefan Baudis, Axel T. Neffe, Marc Behl, Christian Wischke, Andreas Lendlein*

Institute of Biomaterial Science and Berlin-Brandenburg Center for Regenerative Therapies, Helmholtz-Zentrum Geesthacht, Kantstrasse 55, 14153 Teltow, Germany
*Corresponding author: andreas.lendlein@hzg.de, Tel.: +49 (0)3328 352 450; fax: +49 (0)3328 352 452.

Abstract

In this study, the effect of three aliphatic diisocyanate linkers, *L*-lysine diisocyanate ethyl ester (LDI), hexamethylene diisocyanate (HDI), and racemic 2,2,4-/2,4,4-trimethyl hexamethylene diisocyanate (TMDI), on the degradation of oligo[(*rac*-lactide)-*co*-glycolide] (64:36 mol%) based polyester urethanes (PEU) was examined. Samples were characterized for their molecular weight, mass loss, water uptake, sequence structure, and thermal and mechanical properties. Compared to non-segmented PLGA, the PEU showed higher water uptake and generally degraded faster. Interestingly, the rate of degradation was not directly correlating with the hydrophilicity of the diisocyanate moieties; instead, competing intra-/intermolecular hydrogen bonds in between urethane moieties appear to substantially decrease the rate of degradation for LDI-derived PEU. By comparing microparticles (μm) and films (mm) as matrices of different dimensions, it was shown that autocatalysis remains a contributor to degradation of the larger-sized PEU matrices as it is typical for non-segmented lactide/glycolide copolymers. The shown capacity of lactide/glycolide-based multiblock copolymers to degrade faster than PLGA and exhibit improved elastic properties could be of interest for medical implants and drug release systems.

Keywords: polyester urethanes, degradation, PLGA, multiblock copolymer, microparticles

Introduction

Despite polymeric biomaterials being widely accepted as a tool in medical interventions and therapies, the demands set by clinical applications are not fully satisfied by existing materials in many cases. Additionally, further clinical applications may potentially arise from tailored biomaterials, of which, however, each requires the adaption of distinct combinations of properties and functions that match the respective purpose. Accordingly, there is still a need for a variety of new materials with tailorable properties, such as e.g. elastic properties or degradation pattern.

A preferential strategy towards such materials is the generation of polymer systems, i.e. families of polymers, in which small systematically applied changes in the composition give access to a large variation of properties. An example for such systems are multiblock copolymers built from one or more types of (co)polyester blocks [1] [2]. Importantly, the capabilities of polymers do not only base on features like the overall comonomer composition and molecular weight, but also on structural aspects such as the sequence structure and polymer architecture. Accordingly, small structural alterations or the introduction of distinct moieties with a rather low molar content may substantially alter the polymer properties and may allow solving challenges associated with existing materials. An example for such structural elements are urethane bonds as formed by reaction of oligoester diol precursors with diisocyanate linkers during the synthesis of multiblock copolymers [3]. Polyester urethane (PEU) matrices from hydrophobic oligoesterdiols and an aliphatic diisocyanate showed an increased water uptake, i.e. increased hydrophilicity [2], despite the content of these bonds in relation to the ester bonds is rather low in the macromolecule (e.g. 8 mol% in the given example).

While previously the efforts for alteration of material properties were mostly focused on changes in the copolyester segments [4] [5], the observation of increased hydrophilicity suggests that the urethane junction units are substantial contributors and thus may be used to tailor the properties of existing materials. Furthermore, there are indications that also the type of the diisocyanate compounds used to build the junction unit may effect e.g. the mechanical properties of polyurethanes [6]. Even though hydrolytic degradation of PEU mainly proceeds through the cleavage of ester bonds in the oligoester blocks in a multi-step composition-dependent manner [3], it can be expected that the diffusion of water molecules into and out of the matrix again is influenced by the type of urethane component. Therefore, the use of distinct diisocyanate linkers may allow controlling material properties without the need to alter the comonomer types, comonomer ratio or molecular weight.

Specifically, it was assumed that the introduction of hydrophilic urethane microdomains into poly[(*rac*-lactide)-*co*-glycolide] (PLGA), one of the most widely used polymers for drug delivery applications, may be suitable to address issues associated with this model material. Occasionally observed limitations of PLGA include incomplete or biphasic drug release profiles, a sudden loss of mechanical properties during degradation, or the creation of an acidic microclimate due to accumulation of degradation products with free carboxy groups in the matrix. The acid microclimate is known to lead to an autocatalytic enhancement of degradation depending on the matrix structure and size, particularly for non-porous and larger matrices. In addition to enhanced hydrophilicity proposed for PEU possibly allowing exchange and removal of degradation products through diffusion channels, superior elastic properties are often associated with multiblock copolymers. This may be an additional advantage for drug delivery implants subjected to stress during the implantation process or at later stages in the tissue.

In this study, it was hypothesized that the nature and the interactions of the urethane junction units as introduced in between hydrophobic oligo[(*rac*-lactide)-*co*-glycolide] (OLGA) segments via different aliphatic diisocyanate compounds affects the degradation of PEU matrices. More hydrophilic diisocyanates with larger H-bonding capacity were expected to result in faster hydrolysis. In order to explore these assumptions in simulated physiological conditions *in vitro*, OLGA-PEU microparticles and film matrices with dimensions differing by two orders of magnitude and thus differing in diffusion length scales were explored in comparison to non-segmented PLGA. Furthermore, the effect of physical interaction between urethane links on the water uptake and degradation pattern are discussed.

Materials and methods

A detailed description of the applied experimental procedure and used chemicals is provided in the Supporting Information. Important chemicals included: *rac*-dilactide (Bio Invigor, Taiwan, recrystallized from ethyl acetate; the amount of *meso*-dilactide was considered to be neglectable), diglycolide (Boehringer Ingelheim GmbH, Germany), dimethyl carbonate (Alfa Aesar, USA) dibutyl tin dilaurate (Merck Schuhardt, Hohenbrunn, Germany), dibutyl tin (IV)oxide, hexamethylene diisocyanate (HDI), 2,2,4- and 2,4,4- trimethyl hexamethylene diisocyanate isomeric mixture (TMDI), and 1,8-octanediol (all from Fluka Analytical, Sigma-Aldrich), *L*-lysine diisocyanate ethyl ester (LDI; Shanghai Infinite Chemicals, China), and poly(vinyl alcohol) (PVA; Mowiol 4-88, Kuraray Europe GmbH, Frankfurt, Germany).

Polymer synthesis

PLGA and OLGA were synthesized by ring-opening polymerization of *rac*-dilactide and diglycolide in melt at 130 °C for 48 h in dry nitrogen atmosphere using 1,8-octanediol as initiator and dibutyl tin (IV) oxide (0.4 mmol) as catalyst. The yield was 94 wt.%. and 79 wt.% for PLGA

and OLGA, respectively, with a lactide content of 62-64 mol% (see Table 1). OLGA-PU were synthesized by polyaddition of 8.1 mmol OLGA in 93 g of dry dimethyl carbonate and 1.02-1.05 mol equivalent of diisocyanate compounds at 85 °C for 24 h using 0.0105 mmol dibutyl tin dilaurate as catalyst. The yield of the product was 97 wt.%., 91 wt.%, 94 wt.% for OLGA-LDI, OLGA-HDI, and OLGA-TMDI, respectively.

Characterization of polymer molecular weight, composition, thermal and mechanical properties

Multidetector GPC analysis was performed in chloroform at 1 $mL \cdot min^{-1}$ at 35 °C using two 300 mm x 8.0 mm linear M columns (Polymer Standards Service GmbH (PSS), Mainz, Germany), a refractive index detector (Shodex RI-101, USA) and a T60A dual detector (Viscotek Corp., USA). Polystyrene standards (PSS; number average molecular weights M_n: 580 - 975,000 $g \cdot mol^{-1}$) were used and data evaluation was performed by universal calibration (initial molecular weight) and standard calibration (degradation study).

$^{1}H/^{13}C$ NMR spectra were recorded on a Bruker Avance 500 spectrometer (500 MHz, Bruker, Karlsuhe, Germany) either in $CDCl_3$ with tetramethyl silane as internal standard or in DMSO-d6 (referenced to DMSO-d6 signal at 39.52 ppm (^{13}C)).

Differential scanning calorimetry (DSC) was performed on a DSC 204 apparatus (Netzsch, Selb, Germany) between -50 and 100 °C with heating and cooling rates of 10 $K \cdot min^{-1}$. The thermal transitions were evaluated from the second heating cycle.

The mechanical properties of film samples were studied using dumbbell-shaped test specimens (15 x 2 mm) on a Zwick tensile tester Z1.0 (Zwick, Ulm, Germany) at 25 °C with a deformation rate of 5 $mm \cdot min^{-1}$.

Preparation and characterization of microparticles and films

Microparticles (~8 μm) were prepared by the emulsion solvent evaporation method [7] by dispersing 15 wt.% polymer solutions in dichloromethane in 2% (w/v) aqueous PVA solution with subsequent solvent evaporation in 25 mL of 0.25% (w/v) aqueous PVA. Films of approximately 1 mm thickness were prepared by compression molding (50-60 °C, 20-30 bar) between spacers in a hot compression press (P200E Collin Press, Ebersberg, Germany).

Particle sizes we determined by laser diffraction (Malvern Mastersizer 2000, Hydro 2000S dispersion unit; Malvern Instruments, Herrenberg, Germany) and the mean of particle distribution d(0.5) by the Fraunhofer model was used as measure of the average particle size.

Scanning electron microscopy (SEM) with a Gemini Supra^{TM} 40 VP SEM (Carl Zeiss NTS GmbH, Oberkochen, Germany) was applied to study sample morphology as native samples (microparticles) or after conductive coating using iridium (polymer films).

Dynamic contact angle analysis of PLGA and OLGA-PU films was conducted by the captive bubble method (DSA 100, KRÜSS, Hamburg, Germany). The advancing contact angle θ_{adv} after 24 h pre-incubation is reported as mean ± standard deviation (SD) (15 measurements per sample on at least two different locations).

Evaluation of polymer degradation

Microparticles and films were incubated in 0.1 mM phosphate buffered saline (PBS) solution (pH 7.4) containing 0.01% (wt./v) Tween 20 to allow proper wetting of particle powder as mediated *in vivo* by presence of various surface active molecules (PBST). Samples were maintained at constant pH at 37 °C under continuous shaking (Certomat IS, B. Braun Biotech International, Melsungen, Germany). Microparticles were recovered by centrifugation at 9400 g for 5 min (Biofuge Stratos, Hereaus, Hanau, Germany), washed with distilled water, and

lyophilized. The films were isolated using forceps, washed by immersion in water, and lyophilized.

The water uptake H (films) and relative mass loss μ_{rel} (films, MP) was monitored by gravimetric analysis. H was calculated from the mass of the incubated sample after drying (m_d) and the mass of swollen samples (m_s) as follows: $H = (m_s\text{-}m_d)\cdot(m_d)^{-1}\cdot 100$. The initial mass m_i and the mass of degraded samples after drying m_d were used to calculate $\mu_{rel} = m_d \cdot m_i^{-1}$.

Results and discussion

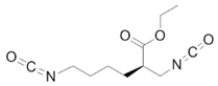
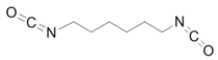
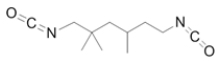
Properties of prepared polymers

A series of well-defined dihydroxy copolyesters were synthesized by ring-opening polymerization of *rac*-dilactide and diglycolide using 1,8-octanediol as initiator and dibutyl tin (IV) oxide as transesterification catalyst. OLGA-diol precursors were prepared with a M_n of 6 kDa (PD = 1.2). For obtaining multiblock copolymers, OLGA was reacted with LDI, HDI, or TMDI to synthesize OLGA-LDI, OLGA-HDI, and OLGA-TMDI (Table 1). With these materials, i) the effect of introducing urethane bonds and ii) specific effects based on the type of diisocyanate compounds used as junction unit on the degradation of lactide/glycolide based copolymers were studied. LDI was considered to result in the most hydrophilic junction unit because of the ethyl ester moiety with hydrogen bonding capacity in its side chain, while HDI and TMDI were considered to show increasing hydrophobicity. As a reference polymer, a non-segmented PLGA with diol end groups was synthesized by ring opening polymerization under comparable conditions.

The comonomer composition of the synthesized polymers/oligomeric telechelics was determined by ^{1}H-NMR spectrometry. PLGA and OLGA macrodiols had a lactide content of 64 mol% as

determined by ^{1}H-NMR (Table 1). In addition to copolymer composition, the sequence structure of copolymers can impact the rate of degradation, particularly when the two comonomers considerably differ in their susceptibility to hydrolysis [3]. In principle, ^{1}H-NMR spectrometry can be used to study the copolyester sequence structure [8], but peak assignment becomes highly complex for repetitive units with stereochemical centers as in case of *rac*-dilactide as a comonomer [9]. In comparison with reference ^{1}H-NMR spectra obtained for poly[(*L*-lactide)-*co*-glycolide] from the literature [10], additional peaks were observed for the poly[(*rac*-lactide)-*co*-glycolide] due to the stereochemistry which impeded a clear assignment (data not shown). Therefore, ^{13}C-NMR experiments were conducted. The average glycolide block length (L_G) was calculated based on the integrals I of the GG and GL peaks at 166.6 and 166.7 ppm using the equation $L_G = 1 + I_{GG} \cdot (I_{GL})^{-1}$ [11]. In all cases, a L_G in the range of 3.0 to 3.5 was determined for PLGA and the different OLGA-PU as synthesized. While an L_G of 1.56 would be expected for an ideal random copolymer of 36 mol% glycolide composition (I_{GG}/I_{GL} = 36/64), the obtained data indicated that the copolymers did not have a perfect random sequence structure but contain larger G- and L-blocks. This may be explained by the lower susceptibility of the secondary ester bonds in lactide-lactide diads to transesterification with the used transesterification catalyst and the fact that by ring-opening of cyclic dimers always a pair of repetitive units is added to the polymer chain [8].

Table 1: Properties of the polymers used in the degradation study.

Polymer	Name and structure of diiso-cyanate linker		GPC [1] standard calibration			GPC [1] universal calibration			Lactide content
			M_n (kDa)	M_w (kDa)	PD	M_n (kDa)	M_w (kDa)	PD	mol%
OLGA-LDI	LDI		36	72	2.0	30	47	1.6	62
OLGA-HDI	HDI		34	71	2.1	25	50	2.0	62
OLGA-TMDI	TMDI [2]		31	69	2.2	26	46	1.8	63
PLGA	not applicable	not applicable	32	46	1.5	20	30	1.8	64

[1]GPC: gel permeation chromatography. [2] TMDI is an isomeric mixture of 2,2,4- and 2,4,4- trimethyl hexamethylene diisocyanate. Here, the structure of the 2,2,4-TMDI isomer is exemplarily depicted.

Preparation of model matrices for degradation studies

In order to address the influence of the different diffusion length scales on the hydrolytic degradation of polymer matrices, films and microparticles were prepared as model matrices that differ in their outer dimensions by two orders of magnitude. The microparticles as obtained by the emulsion solvent evaporation method exhibited an average particle size of d(0.5) = 4-8 μm and a smooth surface morphology irrespective of whether OLGA-PU or PLGA were used (Figure 1a, c). Films with a thickness of 0.98±0.12 mm were prepared by the hot compression method. SEM analysis illustrated a dense film structure without major pores (Figure 2a, c).

The degradation of microparticles and film matrices was studied for a time period of 8 weeks at 37 °C in pH 7.4 PBST buffer. The pH of the medium was monitored and confirmed to be stable throughout the study. With the progress of degradation, the surface of the microparticles

exhibited pitted structures after three weeks. For the different materials, microparticle surfaces showed similar structure changes during degradation (Figure 1b and d).

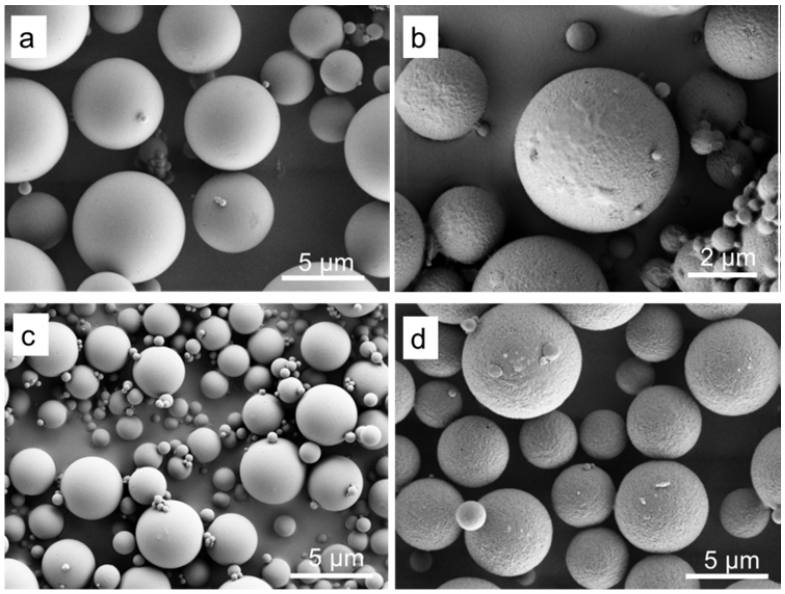


Figure 1: Surface structure of OLGA- TMDI (a, b) and PLGA (c and d) microparticles before (a, c) and after 3 weeks of degradation (b, d).

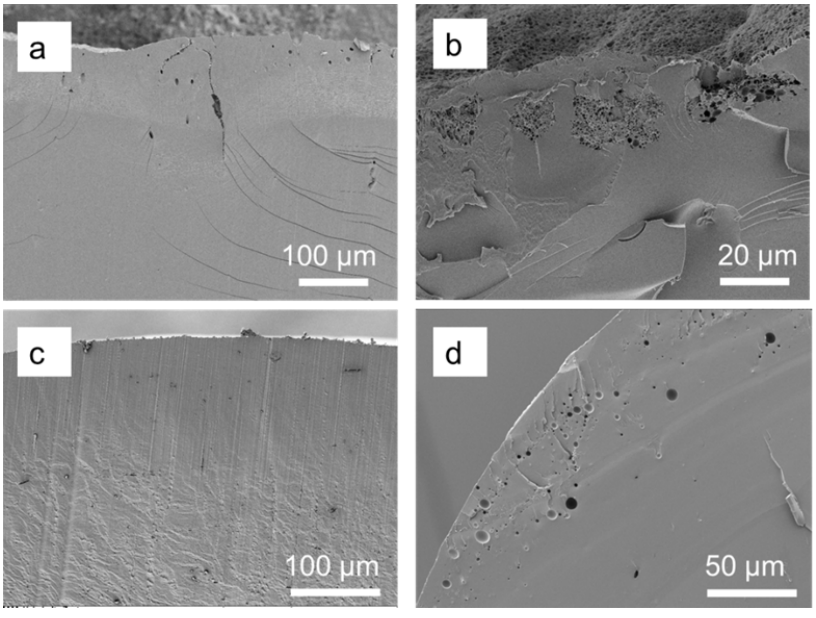


Figure 2: Structure of the cross sections of OLGA- TMDI (a and b) and PLGA (c and d) films before (a, c) and after two weeks of degradation (b, d).

For films, pores were formed at the surface, which slowly progressed into the polymer bulk within two weeks. In particular, cross-sections of films showed high porosity in case of OLGA-TMDI with probably interconnecting pores at surface-near regions. A similar pattern was previously observed for a multiblock copolymer comprising oligo(*p*-dioxanone) and poly(ε-caprolactone) segments, in this case during degradation in enzyme containing media [12]. In contrast, PLGA films showed random formation of only single circular pores in the same time frame.

Water uptake

The uptake of water is a first incident that occurs when exposing the polymers to aqueous media. The extent of *H* depends on the hydrophilic nature of the polymer [13]. For OLGA-PU films, two phases of water absorption were observed (Figure 3). In the first phase up to 7 days, the water uptake was low (~2 wt.%) and was similar for the selected OLGA-PU and PLGA films. In the second phase, the OLGA-PU films showed an increasing *H* depending on the type of the diisocyanate compounds used as linker. For OLGA-HDI films, *H* reached 157±27 wt.% in 21 d, whereas OLGA-TMDI and surprisingly also OLGA-LDI showed at the same time point a lower *H* = 90±20 wt.% and *H* = 13±0.83 wt.%, respectively. The low *H* = 5.1±1.4 wt.% of PLGA films could be attributed to its higher hydrophobicity and presumably slower degradation compare to OLGA-PU due to the absence of urethane bonds. Exemplarily performed analysis of contact angles of polymer films with water confirmed a higher wettability of all OLGA-PU samples (e.g., OLGA-LDI; θ_{adv} = 74.5±3.6) compared to PLGA (θ_{adv} = 85.1±2.2).

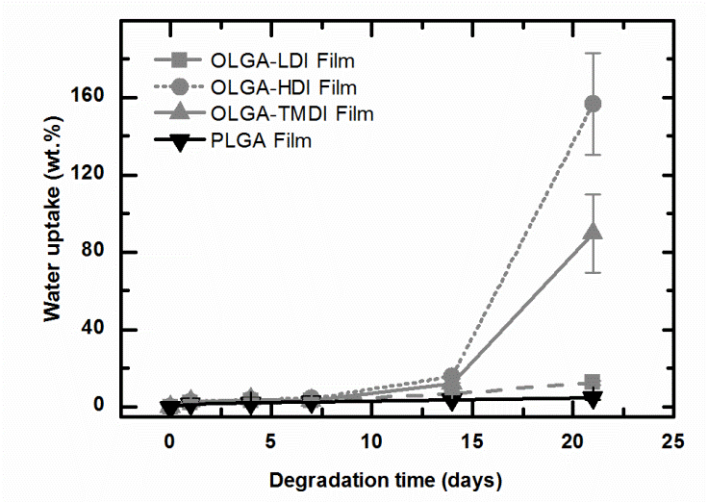


Figure 3: Water uptake *H* of OLGA-PU films and PLGA films depending on degradation time. Samples were incubated in PBST at 37 °C and collected as separate samples at each time point (n = 6, Mean ± SD).

The observed pattern of water uptake of OLGA-PEU materials was unexpected in some cases, particularly for OLGA-LDI. OLGA-TMDI with a higher number of hydrophobic aliphatic groups than OLGA-HDI showed less water uptake as hypothesized. It was further assumed that OLGA-LDI bearing an additional ethyl ester side chain might be capable of absorbing more water than the other OLGA-PU. This, however, could not be confirmed experimentally. Generally, hydrogen bonding with water molecules is a characteristic of PEU [14] and can involve –NH and C=O bonds of the urethane moiety and the carbonyl or ester oxygen of the oligoester segments [15]. The extent of inter/intramolecular hydrogen bond formation between –NH of the urethane moiety and the C=O bond of the oligoester segments may depend on the compatibility between the diisocyanate unit and the polyester segment. The presence of additional hydrogen bonding groups (carbonyl and ester oxygen atoms) in the ethyl ester side group of LDI may make it more hydrophilic and thus, less compatible than other linkers with OLGA segments. The low water uptake of OLGA-LDI might furthermore result from the

existence of stronger intermolecular hydrogen bonding between C=O groups of the polymer ethyl ester side chains and –NH groups of urethane links, which reduce the capacity for interaction with free water.

In addition to differences in the hydrophilicity of the polymers, the free volume accessible to water could be another determining factor that controls the water uptake. In a simulation experiment performed for OLGA-PEU degradation, it was reported that the free volume of simulated dry and swollen (2% vs. 7% water) OLGA-PEUs was found to be increased compared to PLGA [16]. This finding supports the increased water uptake in the OLGA-PEU in comparison to non-segmented PLGA. When comparing different OLGA-PEU, the enhancement of free volume was less pronounced for OLGA-LDI [16]. Accordingly, it can be speculated that the differences in free volume and the nature of chemical groups depending on the respective diisocyanate linker contribute to the formation of strong intermolecular hydrogen bonds and thus an enhanced water uptake.

For microparticles, the water uptake was not determined since inter- and intraparticulate water can hardly be distinguished by balancing methods without harsh pretreatment that may create artifacts. Still, it can be expected that molecular binding processes leading to differences in the behavior of film samples from different materials may similarly apply also for microparticles.

Changes in average molecular weight and mass loss

The presence of water uptake in the polymer films should lead to a hydrolysis of susceptible bonds and thus a decrease of the average molecular weight of the polymers. For OLGA-PEU and PLGA, the hydrolysis is expected to occur preferentially at ester linkages. This could lead to abrupt or, preferentially, a gradual decrease of the molecular weight. The reduction of the

relative weight-average molecular weight ($M_{w} = M_{w(t0)} / M_{w(t)}$) in the OLGA- PU films was faster compared to PLGA. The observed decrease of relative M_w of different films was in the following order: OLGA-HDI > OLGA-TMDI >> OLGA-LDI >> PLGA. In the case of OLGA-HDI and OLGA-TMDI, the average molecular weight diminished down to 10% of the initial molecular weight in 14 days. These rapid molecular weight changes of OLGA-HDI and OLGA-TMDI by hydrolysis could be correlated with their relatively high *H*. As will be discussed later in more detail, these polymer films had a glass transition temperature (T_g) similar to the incubation temperature and thus were in the visco-elastic state. The higher chain mobility at temperatures above T_g may have supported enhanced water transport and higher rates of hydrolysis. Differences in the hydrolytic degradation kinetics of PEU depending on the junction units were also observed for materials synthesized either using cycloaliphatic dicyclohexylmethane diisocyanate (H_{12}MDI) or aromatic diphenylmethane diisocyanate (MDI), in this case with slower degradation for materials aromatic junction units [17].

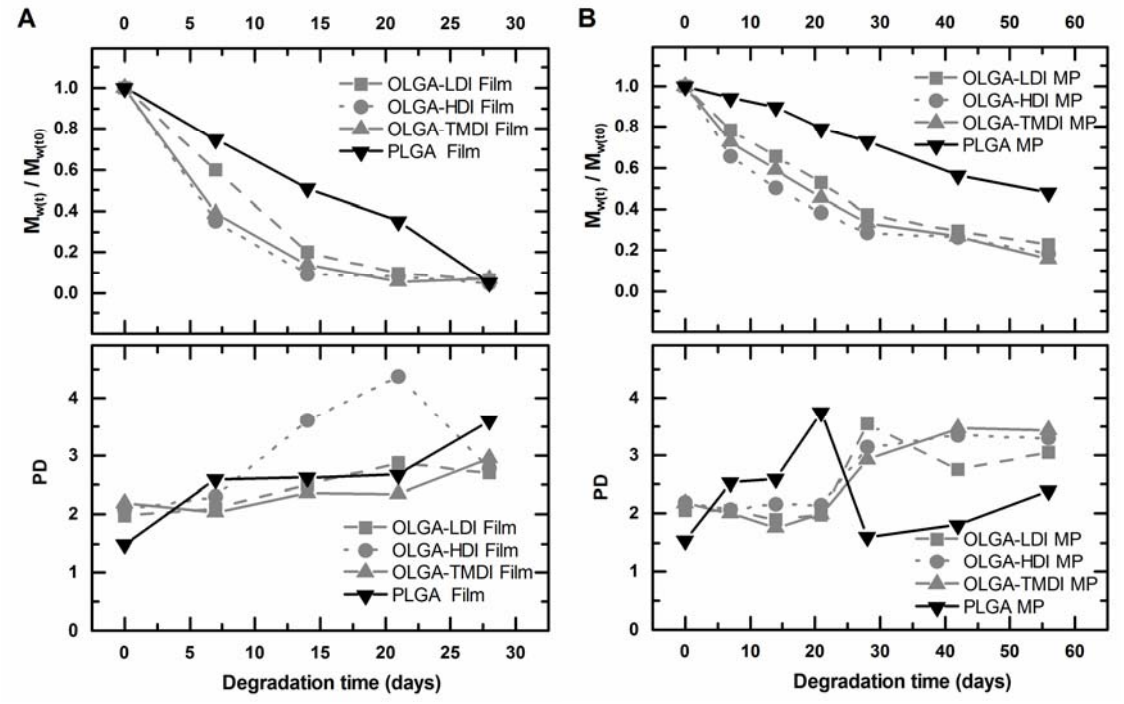


Figure 4: Change in relative M_w and polydispersity (PD) of (A) PLGA-PU films and (B) microparticles (MP) during degradation (PBST, 37 °C) as determined by gel permeation chromatography with standard calibration. The measurements were repeated twice and the graph is plotted using the mean value of the measurements.

The degradation of polyesters was reported to follow a random chain scission [18], whereas kinetic studies of hydrolysis of oligolactides showed a chain end scission with backbiting mechanism. For high molecular weight polylactides, random chain scission should be preferred since there is only a relatively small number of hydroxyl end groups available and accessible to chain end scission at the onset of degradation [19]. Based on the profiles of M_w decrease of the OLGA-PEU studied in here and of lactide-based PEU in previous reports [20], random chain scission can be expected also for OLGA-PEU. Interestingly, an increasing polydispersity PD as typically associated with random chain scission was observed only for PLGA films, where the PD increased from 1.5 to 2.6 within one week of degradation. Apparently, the decrease of relative M_w for microparticles was not as abrupt as for polymer films, but instead showed a more continuous profile in the studied time frame (Figure 4).

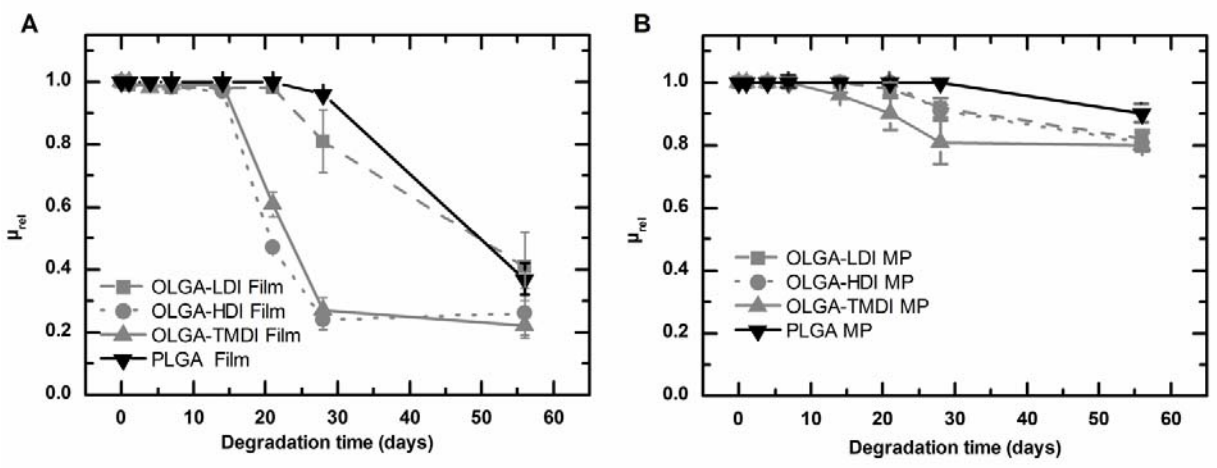


Figure 5: Relative mass remaining (μ_{rel}) of (A) films (n = 6, mean ± SD) and (B) microparticles (MP) (n = 3, mean ± SD) from OLGA-PU and PLGA upon incubation in PBST at 37 °C.

This effect of matrix size-dependent degradation pattern well agrees with what was observed before for autocatalysis in PLGA films versus microparticles [21]. Accordingly, the data indicate that acidic degradation products still accumulated and promoted autocatalysis in larger OLGA-PEU matrixes irrespective of the introduced hydrophilic groups and the enhanced water uptake. OLGA-PEU microparticles generally degraded faster than PLGA microparticles, while only a slight difference in relative M_w profiles could be seen when comparing the different OLGA-PEU microparticles. An increase in the PD of all microparticle samples after three weeks of degradation indicated the formation of oligomeric fragments.

Mass loss is a consequence of the formation of water soluble polymer fragments [22]. Specifically, mass loss occurs due to diffusion-driven leakage of short oligomers as well as monomers with sizes below a critical hydrodynamic radius, which is defined by the micro-/macroporosity of the remaining matrix. During degradation of the studied polymers, the mass loss pattern showed three major phases: 1) an induction phase (phase 1), in which the dry state mass remained basically unchanged, 2) a phase 2 that is characterized by accelerated mass loss, and 3) finally a phase 3 showing a declining mass loss. This mass loss behavior coupled with the continuous decrease of relative M_w as observed for both OLGA-PEU and PLGA films (Figure 5) corresponds to the typical mass loss pattern of bulk degradation.

The mass loss of film samples was negligible up to 14 days of aqueous incubation, during which also the water uptake was low. After this, an abrupt onset of mass loss was observed, e.g. for OLGA-HDI and OLGA-TMDI films in the third week of the degradation study. This suggests that the formed fragments reached the critical M_w of solubility/diffusivity. When correlating mass loss and relative decrease of molecular weight (Figure 6), it appears that the critical M_w of

the remaining fragments was below 6 kDa. In comparison, literature reports for critical M_w of mass loss for PLGA vary considerably between 10-25 kDa [23] and 4 kDa [24]. Slide differences could arise from the differences in the hydrophilicity of the oligomeric fragments based on the ratio and sequence structure of lactide/glycolide repetitive units. It may also be noted that mass loss pattern distinguish from other multiblock copolymers with more hydrophobic copolyester segments, for which the onset of mass loss was shifted to late time points >> 100 days and may possibly be less pronounced [3].

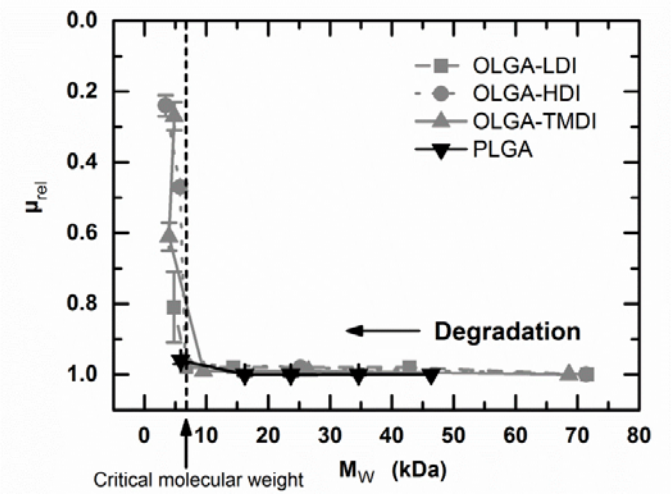


Figure 6: Correlation between decrease in relative M_w and remaining mass in case of films during degradation in PBST at 37 °C.

In case of microparticles, the mass loss was slow with no strong differences for the different materials. By trend, PLGA microparticles appeared to have a slower mass loss than OLGA-PU as would be expected based on the higher hydrophobicity, the lower access of water for hydrolysis and lower availability of aqueous diffusion channels. Autocatalysis of degradation by accumulated acidic degradation products has previously been visualized in copolyester particles ≥ 20 µm. In contrast, this effect was marginal in smaller particles [25] as used in this study. When comparing degradation pattern of films and small sized PLGA and OLGA-PEU

microparticles, it could be concluded that the extent of autocatalytic degradation was low for microparticles, which explains also the lower mass loss rates compared to the much thicker film samples.

Changes of sequence structure and comonomer composition

The changes in the sequence structures of the different polymers during degradation were analyzed for film samples by ^{13}C-NMR [10]. The ^{13}C resonance signals corresponding to the GG diads and GL diads were assigned as indicated in Figure 7A.

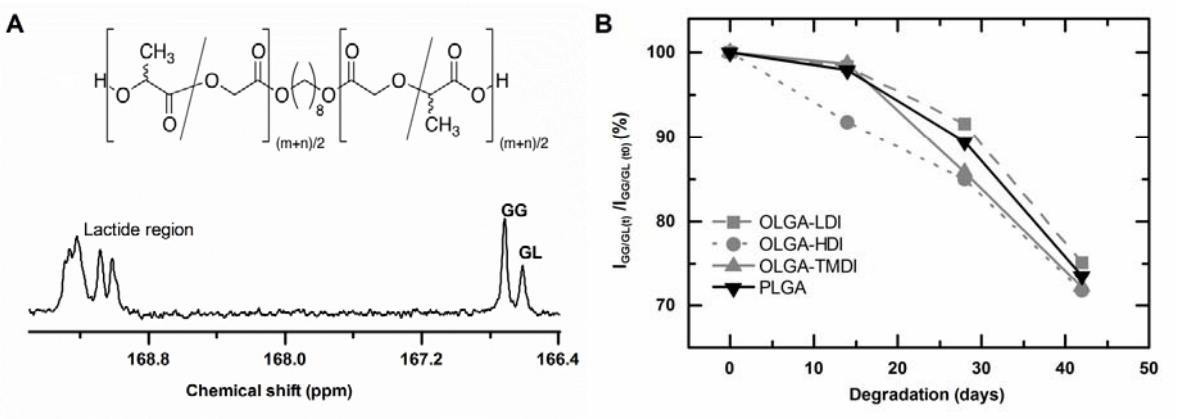


Figure 7: ^{13}C-NMR analysis of degradation effects on copolymer sequence structures. (A) Peak assignment of glycolide-glycolide (GG) and glycolide-lactide (GL) diads. Exemplary ^{13}C NMR spectrum of PLGA recorded in DMSO (d_6). (B) Change in the I_{GG}/I_{GL} ratio of the oligoester segments during degradation in PBST at 37 °C.

During degradation, a decrease in the ratio of the GG/GL integrals was similarly observed for all materials, which suggests a preferential cleavage of GG diads (Figure 7B). This confirmed that the preference of GG hydrolysis (weak links [3]) compared to GL cleavage is not effected by other moieties outside the polyester segment. Additionally, the alteration of chemical composition of OLGA-PEU and PLGA films was studied by ^{1}H-NMR during degradation using the integrals of the methylene protons of glycolide (δ = 4.6-4.9 ppm) and the methine protons of

lactide (δ = 5.1-5.27 ppm). All OLGA-PEU films showed a trend towards an increase of the lactide content from initially 62-64 mol% with degradation particularly in the first 42 d due to preferential hydrolysis and removal of glycolide-rich segments (Table 2). For instance, OLGA-HDI as the most rapidly degrading material (Figure 4A and 5A) exhibited a lactide content of 75 mol% after 42 d of degradation. In case of PLGA films, a relevant change in copolymer composition could be observed only at later time points (56 d).

Table 2: Change in the copolymer composition of OLGA-PU and PLGA films during degradation as determined by ^{1}H-NMR (PBST, 37 °C)

Degradation time (d)	**Lactide content (mol%)**			
	OLGA-LDI	**OLGA-HDI**	**OLGA-TMDI**	**PLGA**
0	62	62	63	64
21	63	65	67	65
28	67	73	70	64
42	70	75	70	61
56	73	69	72	67

Thermal and mechanical properties

Film samples from OLGA-PEU and PLGA as synthesized exhibited dry state T_g's between 37 °C and 45 °C. The T_g values remained initially constant, subsequently decreased along with degradation due to the formation of oligomeric fragments, and in some cases adapted again higher values at later time points (Figure 8A). Since lactide/glycolide copolymers are known to exhibit mixed T_g's, increasing T_g values at later time points e.g. at 28 d may be linked to the increase of relative lactide content as determined by NMR (Table 2). Similar observations were

reported for PEU networks containing lactide as a comonomer [26]. In case of microparticle samples particularly from OLGA-PU, the initial T_g was higher compared to the corresponding film samples. This effect might be linked to an extraction of low molecular weight components during the water and solvent-based microparticle preparation procedure. A shift to lower T_g during degradation could be observed after 56 d of microparticle degradation, e.g. for OLGA-HDI. No such alterations were detected for PLGA microparticles within 56 d of degradation.

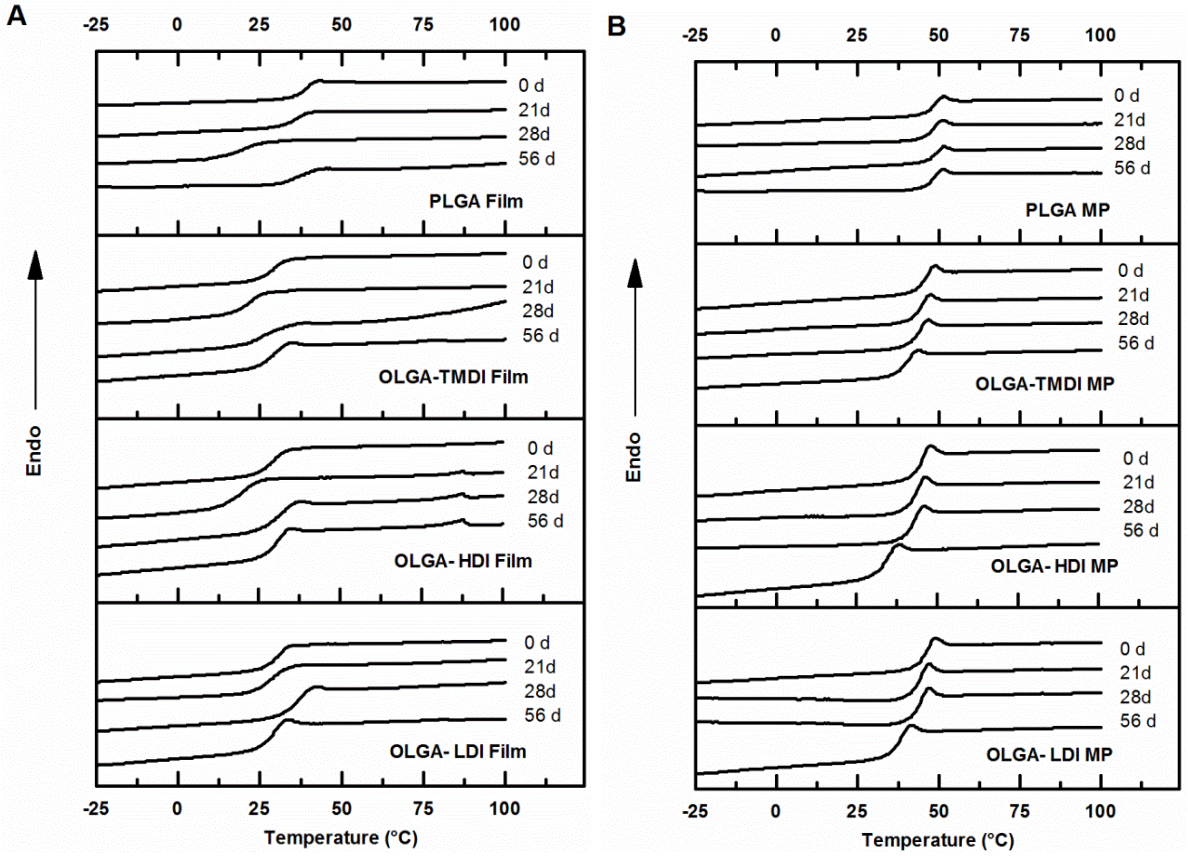


Figure 8: DSC curves showing changes in glass transition of (A) films and (B) microparticles with degradation (PBST, 37 °C).

In the analysis of mechanical properties by tensile test, PLGA films showed to be too brittle to be handled and analyzed particularly after exposure to degradation medium and subsequent drying. OLGA-PU films exhibited a slight increase of the E modulus after incubation in PBST buffer

(Figure 9). This could be assigned to the increase in the stiffness of the materials due to the presence of salts probably originating from PBST buffer entrapped in the film matrix, as confirmed by SEM of film cross sections with EDX detection of sodium.

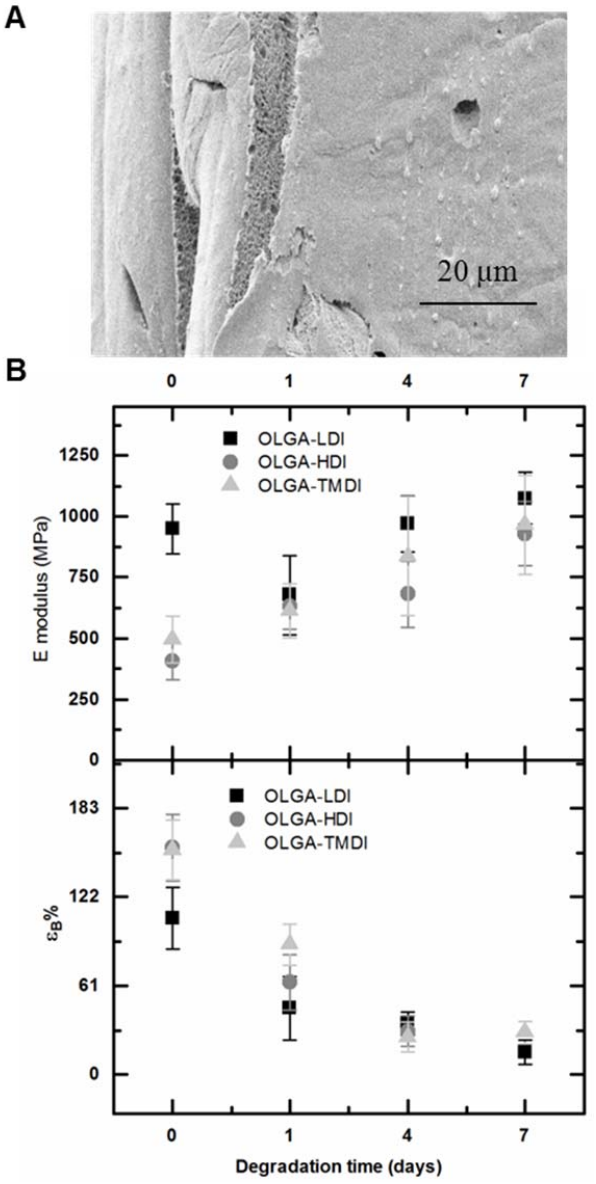


Figure 9: (A) Exemplary SEM of a cross section of OLGA-HDI film after 4 d of incubation in PBST buffer. Mechanical properties of sample as analyzed by tensile tests providing (B) E modulus and (C) elongation at break (degradation in PBST, 37 °C).

Conclusion

In this study, the degradation of OLGA-based PEU was studied for matrix sizes of different length scales. The hypothesis that the nature and interactions of polar urethane bonds, such as hydrogen bonding play a role on PEU degradation was proved to be correct in principle. However, it was shown that not necessarily the most hydrophilic diisocyanate compound used to build junction units in oligoesterdiol-derived multiblock copolymers would result in most rapid degradation. Interestingly, the OLGA-HDI degradation was not only faster than non-segmented PLGA but also than OLGA-LDI, which contains the more hydrophilic LDI with additional ester side chains. This emphasizes the impact of hydrophilic interactions in PEU i) in terms of their typically higher affinity to water than non-segmented polyesters, but ii) also in terms of competing intra-/intermolecular hydrogen bonds involving urethane junctions and moieties of the oligoester segments. The spatial alignment of polymer chains as influenced by their side chains and the free volume in the polymer matrix appear to strongly impact the extent of such interactions. When comparing film and microparticulate matrices as models with different dimensions of diffusion length, it seems that autocatalysis remains a major contributor to degradation of larger sized OLGA-PU matrices as it is typical for lactide/glycolide copolymers. Finally, it should be stated that the OLGA-PU materials exhibited superior mechanical properties, e.g. reduced brittleness, compared to PLGA. Along with the tunable degradation rates depending on the type of employed diisocyanate, OLGA-PU could be an alternative for PLGA in biomedical applications.

Acknowledgement

The authors acknowledge Alexander Battig for provision of some polymers and Monique Hannemann, Dr. Hans Joerg Ziegler, Yvonne Pieper, Susanne Schwanz, and Dr. Hans-Juergen

Kosmella for technical support. The authors thank the Helmholtz Association for funding of this work through Helmholtz-Portfolio Topic "Technology and Medicine".

References

[1] J.M. Bezemer, R. Radersma, D.W. Grijpma, P.J. Dijkstra, C.A. van Blitterswijk, J. Feijen, Microspheres for protein delivery prepared from amphiphilic multiblock copolymers: 1. Influence of preparation techniques on particle characteristics and protein delivery, J. Controlled Release, 67 (2000) 233-248. DOI: 10.1016/S0168-3659(00)00213-3
[2] C. Wischke, M. Schossig, A. Lendlein, Shape-Memory Effect of Micro-/Nanoparticles from Thermoplastic Multiblock Copolymers, Small, 10 (2014) 83-87. DOI: 10.1002/smll.201202213
[3] A. Lendlein, M. Colussi, P. Neuenschwander, U.W. Suter, Hydrolytic degradation of phase-segregated multiblock copoly(ester urethane)s containing weak links, Macromol. Chem. Phys., 202 (2001) 2702-2711. DOI: 10.1002/1521-3935(20010901)202:13<2702::Aid-Macp2702>3.0.Co;2-I
[4] C. Mattu, M. Boffito, S. Sartori, E. Ranzato, E. Bernardi, M.P. Sassi, A.M. Di Rienzo, G. Ciardelli, Therapeutic nanoparticles from novel multiblock engineered polyesterurethanes, J. Nanopart. Res., 14 (2012) 1306. DOI: 10.1007/s11051-012-1306-6
[5] J.Y. Cherng, T.Y. Hou, M.F. Shih, H. Talsma, W.E. Hennink, Polyurethane-based drug delivery systems, Int. J. Pharm., 450 (2013) 145-162. DOI: 10.1016/j.ijpharm.2013.04.063
[6] M. Barikani, M.V. Ebrahimi, S.M.S. Mohaghegh, Influence of diisocyanate structure on the synthesis and properties of ionic polyurethane dispersions, Polym.-Plast. Technol. Eng., 46 (2007) 1087-1092. DOI: 10.1080/03602550701525214
[7] C. Wischke, S. Mathew, T. Roch, M. Frentsch, A. Lendlein, Potential of NOD receptor ligands as immunomodulators in particulate vaccine carriers, J. Controlled Release, 164 (2012) 299-306. DOI: 10.1016/j.jconrel.2012.06.034
[8] A. Lendlein, P. Neuenschwander, U.W. Suter, Hydroxy-telechelic copolyesters with well defined sequence structure through ring-opening polymerization, Macromol. Chem. Phys., 201 (2000) 1067-1076. DOI: 10.1002/1521-3935(20000701)201:11<1067::aid-macp1067>3.0.co;2-y
[9] R.M. Stayshich, T.Y. Meyer, New Insights into Poly(lactic-co-glycolic acid) Microstructure: Using Repeating Sequence Copolymers To Decipher Complex NMR and Thermal Behavior, J. Am. Chem. Soc., 132 (2010) 10920-10934. DOI: 10.1021/ja102670n
[10] J. Kasperczyk, Microstructural analysis of poly[(L,L-lactide)-co-(glycolide)] by 1h and 13C n.m.r. spectroscopy, Polymer, 37 (1996) 201-203. DOI: 10.1016/0032-3861(96)81088-3
[11] D.W. Grijpma, A.J. Nijenhuis, A.J. Pennings, Synthesis and hydrolytic DEGRADATION behavior of high-molecular-weight L-lactide and glycolide copolymers, Polymer, 31 (1990) 2201-2206. DOI: 10.1016/0032-3861(90)90096-h
[12] A. Kulkarni, J. Reiche, J. Hartmann, K. Kratz, A. Lendlein, Selective enzymatic degradation of poly(epsilon-caprolactone) containing multiblock copolymers, Eur. J. Pharm. Biopharm., 68 (2008) 46-56. DOI: 10.1016/j.ejpb.2007.05.021
[13] A. Kulkarni, J. Reiche, A. Lendlein, Hydrolytic degradation of poly(rac-lactide) and poly[(rac-lactide)-co-glycolide] at the air-water interface, Surf. Interface Anal., 39 (2007) 740-746. DOI: 10.1002/sia.2580

[14] E. Ghobadi, M. Heuchel, K. Kratz, A. Lendlein, Atomistic Simulation of the Shape-Memory Effect in Dry and Water Swollen Poly (rac-lactide)-co-glycolide and Copolyester Urethanes Thereof, Macromol. Chem. Phys., 215 (2014) 65-75. DOI: 10.1002/macp.201300507
[15] H. Ishihara, I. Kimura, K. Saito, H. Ono, Infrared studies on segmented polyurethane-urea elastomers, J. Macromol. Sci., Phys., B 10 (1974) 591-618. DOI: 10.1080/00222347408219408
[16] E. Ghobadi, M. Heuchel, K. Kratz, A. Lendlein, Simulation of volumetric swelling of degradable poly[(rac-lactide)-co-glycolide] based polyesterurethanes containing different urethane-linkers, J. Appl. Biomater. Funct. Mater., 10 (2012) 293-301. DOI: 10.5301/jabfm.2012.10432
[17] I. Rehman, E.H. Andrews, R. Smith, In vitro degradation of poly(ester-urethanes) for biomedical applications, J. Mater. Sci. Mater. Med., 7 (1996) 17-20. DOI: 10.1007/bf00121184
[18] F. Alexis, S. Venkatraman, S. Kumar Rath, L.-H. Gan, Some insight into hydrolytic scission mechanisms in bioerodible polyesters, J. Appl. Polym. Sci., 102 (2006) 3111-3117. DOI: 10.1002/app.23888
[19] C.F. van Nostrum, T.F.J. Veldhuis, G.W. Bos, W.E. Hennink, Hydrolytic degradation of oligo(lactic acid): a kinetic and mechanistic study, Polymer, 45 (2004) 6779-6787. DOI: 10.1016/j.polymer.2004.08.001
[20] K. Hiltunen, J.V. Seppala, M. Itavaara, M. Harkonen, The biodegradation of lactic acid-based poly(ester-urethanes), J. Environ. Polymer Degradation, 5 (1997) 167-173. DOI: 10.1007/BF02763660
[21] I. Grizzi, H. Garreau, S. Li, M. Vert, Hydrolytic degradation of devices based on Poly(DL-Lactic Acid) size -dependence, Biomaterials, 16 (1995) 305-311. DOI: 10.1016/0142-9612(95)93258-f
[22] S.K. Saha, H. Tsuji, Hydrolytic degradation of amorphous films of L-lactide copolymers with glycolide and D-lactide, Macromol. Mater. Eng., 291 (2006) 357-368. DOI: 10.1002/mame.200500386
[23] M. Husmann, S. Schenderlein, M. Luck, H. Lindner, P. Kleinebudde, Polymer erosion in PLGA microparticles produced by phase separation method, Int. J. Pharm., 242 (2002) 277-280. DOI: 10.1016/S0378-5173(02)00187-4
[24] R. Bhardwaj, J. Blanchard, In vitro characterization and in vivo release profile of a poly (d,l-lactide-co-glycolide)-based implant delivery system for the α-MSH analog, melanotan-I, Int. J. Pharm., 170 (1998) 109-117. DOI: 10.1016/S0378-5173(98)00149-5
[25] Y. Liu, A.H. Ghassemi, W.E. Hennink, S.P. Schwendeman, The microclimate pH in poly(D,L-lactide-co-hydroxymethyl glycolide) microspheres during biodegradation, Biomaterials, 33 (2012) 7584-7593. DOI: 10.1016/j.biomaterials.2012.06.013
[26] A.T. Neffe, G. Tronci, A. Alteheld, A. Lendlein, Controlled Change of Mechanical Properties during Hydrolytic Degradation of Polyester Urethane Networks, Macromol. Chem. Phys., 211 (2010) 182-194. DOI: 10.1002/macp.200900441

Supporting Information

Materials and methods

rac-Dilactide (Bio Invigor, Taiwan) and diglycolide (Boehringer Ingelheim GmbH, Germany) were recrystallized from ethyl acetate solution prior to use. Dimethyl carbonate was obtained from Alfa Aesar, USA, and dibutyl tin dilaurate was from Merck Schuhardt (Hohenbrunn, Germany). Dibutyl tin (IV)-oxide, hexamethylene diisocyanate (HDI), 2,2,4- and 2,4,4- trimethyl hexamethylene diisocyanate isomeric mixture (TMDI), and 1,8-octanediol were procured from Fluka Analytical (Sigma Aldrich). *L*-lysine diisocyanate ethyl ester (LDI) was obtained from Shanghai Infinite Chemicals, China. HDI and LDI were distilled prior to use. Poly(vinyl alcohol) [Mowiol 4-88] (PVA) was obtained from Kuraray Europe GmbH, Frankfurt, Germany. All the other chemicals were of analytical grade and used as received.

Polymer synthesis

PLGA was synthesized by ring-opening polymerization of *rac*-dilactide and diglycolide using 1,8-octanediol as initiator and dibutyl tin (IV) oxide (0.4 mmol) as catalyst. The reaction was performed in melt at 130 °C for 48 h in dry nitrogen atmosphere, followed by cooling to 50 °C to terminate the reaction. The product was dissolved in chloroform and precipitated in methanol. OLGA was synthesized under similar conditions with altered amounts of monomers and initiator. The yield was 94 wt.%. and 79 wt.% for PLGA and OLGA, respectively, .

OLGA-PU were synthesized by polyaddition of OLGA and diisocyanate compounds. Briefly, 8.1 mmol OLGA were dissolved in 1.03 mol of dry dimethyl carbonate under stirring at 85 °C, followed by the addition of 0.0105 mmol dibutyl tin dilaurate and reaction for 15 minutes. Then, 1.02-1.05 mol equivalent of the reactive diisocyanate compound (LDI, HDI, or TMDI) was

added and the mixture was stirred for 24 h. After this, more solvent was added for dissolving the product and the mixture was precipitated in liquid nitrogen. The obtained product was then dried under vaccum. The yield of the product was 97 wt.%, 91 wt.%, 94 wt.% for OLGA-LDI, OLGA-HDI, and OLGA-TMDI, respectively.

Preparation of microparticles and films

Microparticles (~8 μm) were prepared by the emulsion solvent evaporation method[7]. Briefly, 15 wt.% polymer solutions in dichloromethane were emulsified in 2% (w/v) aqueous PVA solution for 2 min at 24,000 rpm with an Ultra Turrax T25 homogenizer (S25N- 5G disperser; IKA, Staufen, Germany). The resulting o/w emulsion was poured into 25 mL of 0.25% (w/v) aqueous PVA solution and stirred for 3 h at ambient conditions. The hardened microparticles were collected by centrifugation for 5 min at 9400 g (Biofuge Stratos, Hereaus, Hanau, Germany) and washed three times with 5 mL deionized water, lyophilized, and stored at 4 °C.

Films of approximately 1 mm thickness were prepared by compression molding (50-60 °C, 20-30 bar) between spacers in a hot compression press (P200E Collin Press, Ebersberg, Germany).

Analysis of particle size

Particle size analysis in water was performed by laser diffraction using a Malvern Mastersizer 2000 with Malvern Hydro 2000S dispersion unit (Malvern Instruments, Herrenberg, Germany). The size distribution was calculated by the Fraunhofer model and the mean of particle distribution d(0.5) was used as measure of the average particle size.

Structure of microparticles and films

The surface morphology of lyophilized MP and cross-sections of films were studied using scanning electron microscopy (SEM) with a Gemini Supra^{TM} 40 VP SEM (Carl Zeiss NTS

GmbH, Oberkochen, Germany) at 1 kV with a secondary electron detector. MP could be analyzed as native samples, whereas films were examined after conductive coating using iridium.

Gel permeation chromatography analysis (GPC)

Multidetector GPC analysis was performed in chloroform at 1 $mL \cdot min^{-1}$ at 35 °C using 0.2 wt.% toluene as internal standard. The system was equipped with a precolumn, two 300 mm x 8.0 mm linear M columns (Polymer Standards Service GmbH (PSS), Mainz, Germany), an isocratic pump 2080, an automatic injector AS 2050 (both Jasco, Tokyo, Japan), and a refractive index detector (Shodex RI-101, USA) and T60A dual detector (Viscotek Corp., USA). The molecular weight distributions were determined using polystyrene standards (PSS) with number average molecular weights M_n between 580 $g \cdot mol^{-1}$ and 975,000 $g \cdot mol^{-1}$ using the SEC software WINGPC 6.2 (PSS). For the degradation study, only standard calibration could be applied.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed on a DSC 204 apparatus (Netzsch, Selb, Germany) with 5-10 mg samples in aluminium pans with pierced lids. Samples were analyzed between -50 and 100 °C with heating and cooling rates of 10 $K \cdot min^{-1}$. The thermal transitions were evaluated from the second heating cycle.

Nuclear magnetic resonance spectroscopy

$^{1}H/^{13}C$ NMR spectra of the different samples before and after degradation were recorded on a Bruker Avance 500 spectrometer (500 MHz, Bruker, Karlsuhe, Germany) either in $CDCl_3$ with tetramethyl silane as internal standard or in DMSO (d6).

Tensile tests

The mechanical properties of film samples were studied using dumbbell-shaped test specimens (15 mm x 2 mm) on a Zwick tensile tester Z1.0 (Zwick, Ulm, Germany) at 25 °C with a deformation rate of 5 mm·min^{-1}.

Contact angle measurement

The wettability of the PLGA and OLGA-PU films was examined by dynamic contact angle measurements at room temperature using the captive bubble method with a Drop Shape analysis System, DSA 100 (KRÜSS, Hamburg, Germany). The samples were incubated in water for 24 h before the measurement. The advancing contact angle θ_{adv} is reported as mean ± standard deviation (SD) (15 measurements per sample on at least two different locations).

Evaluation of polymer degradation

Microparticles and films were incubated in 0.1 mM phosphate buffered saline (PBST, pH 7.4) containing 0.01% (w/v) Tween 20 to allow proper wetting of particle powder as mediated *in vivo* by presence of various surface active molecules. Samples were maintained at 37 °C under continuous shaking (Certomat IS, B. Braun Biotech International, Melsungen, Germany). The pH of the degrading medium was monitored to be constant. Individual samples were collected at each time point. Microparticles were recovered by centrifugation at 9400 g for 5 min (Biofuge Stratos, Hereaus, Hanau, Germany), washed with distilled water, and lyophilized. The films were isolated using forceps, washed by immersion in water, and lyophilized.

Gravimetric analysis of water absorption and mass loss

The water uptake H (films) and relative mass loss μ_{rel} (films, MP) was monitored by gravimetric analysis using an analytical balance (Kern ALT 220-5DAM, Balingen, Germany). H was

calculated from the mass of the degraded sample after drying (m_d) and the mass of swollen samples (m_s) as follows: $H = (m_s\text{-}m_d)\cdot(m_d)^{-1}\cdot 100$. The initial mass m_i and the mass of degraded samples after drying m_d were used to calculate $\mu_{rel} = m_d \cdot m_i^{-1}$.