DOI: 10.1002/masy.201100059

Degradation Behavior of Porous Copolyester Microparticles in the Light of Dynamic Changes in Their Morphology

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Summary: The pore structures of microparticulate drug carriers are important diffusion pathways, which are not a static property but rather may be changing in the case of degradable matrix polymers such as poly[(*rac*-lactide)-*co*-glycolide] (PLGA). In this study, the mutual impacts of dynamic changes in microparticle porosity and polymer degradation were analyzed for PLGA with different molecular weights and end groups as well as PLGA-based triblock copolymers. In selected cases, particularly for PLGA with hydrophilic end groups and low initial number average molecular weight of 5 kDa, pore opening/pore closing phenomena were detected during incubation in phosphate buffer at 37 °C. Initially, pore closing was induced by water-induced plasticization and the reduction of interfacial tension. The pattern of molecular weight decrease and mass loss suggested that pore closing did not result in undesired autocatalytic acceleration of degradation or delayed mass loss due to trapped acidic degradation products.

Keywords: biomaterials; degradation; microparticles; poly[(*rac*-lactide)-*co*-glycolide]; porosity

Introduction

Degradable synthetic polymers have found widespread use in biomedical applications like tissue engineering and drug delivery, with aliphatic (co)polyesters that undergo hydrolytic degradation being preferentially used particularly as degradable drug carrier.[1] Copolyesters synthesized from hydroxycarboxylic acids, particularly those with short aliphatic chains, are an example of materials, which were designed for biomedical applications and allow tailoring of hydrolytic degradability.

Two major mechanisms of hydrolytic degradation may in principle be applicable for hydrophobic polymers: bulk degradation and erosion.[2] Generally, three processes are necessarily involved in the degradation process, with the individual rates determining the overall mechanism: First, water has to enter the polymer matrix with typically low initial water uptake and low swelling for most (co)polyesters. Second, water uptake leads to hydrolytic cleavage of degradable bonds and, therefore, a shift of the polymer's molecular weight distribution to lower average molecular weights. Third, continuous degradation leads to water soluble oligomers and monomers, which can diffuse out of the matrix and result in mass loss of the sample. Poly(hydroxycarboxylic acids) predominantly undergo bulk degradation, since the diffusion of water into these materials is higher than the rate of hydrolysis.[3] Additionally, carboxylic end groups generated during degradation of poly(hydroxycarboxylic acids) may autocatalyze the polymer hydrolysis.

The degradation pattern of specific polyesters depends on molecular and

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matrix properties as well as environmental conditions.[4] One important factor is, e.g., the different ability of water to permeate into these polymers or the sensitivity of the respective ester bonds between building blocks to be hydrolyzed. Water uptake depends on the density and distribution of polar groups in the polymer chains and is limited by the crystallinity of the material.[5,6] The rates of hydrolytic chain scission depend on both electronic and steric effects, for example resulting in faster hydrolysis of glycolide compared to lactide units.[6] Such weak links,[7] i.e., building blocks undergoing faster degradation can be introduced by copolymerization in order to tailor degradation rates. For poly[(*rac*-lactide)-*co*-glycolide] (PLGA) as one of the most intensively used polymer for parenteral controlled drug release applications, increasing glycolide concentrations in a certain range also enhances the water uptake and reduces the glass transition temperature T_g of the material.

In addition to the composition of the copolyester main chain, the average molecular weight, the nature of end groups, and entrapped residues effects water uptake and swelling.[8,9] Also, sequence structure and the distribution of weak links in copolymers may impact hydrolytic degradation.[7] Polymeric biomaterials with higher diffusivity and water uptake for improved protein release have been established by introducing hydrophilic chain segments in multi-phase materials like poly[(*rac*-lactide)-*co*-glycolide]-*b*-poly(ethylene glycol)-*b*-poly[(*rac*-lactide)-*co*-glycolide] (PLGA-PEG-PLGA).[10] The degradation behavior can also be influenced by crosslinking the copolyester chains.[11] Furthermore, bulk degradation of PLGA was shown to depend on the dimension of dense matrices. Acidic degradation products can promote degradation rates of PLGA due to autocatalysis, particularly for specimen like dense films or beads with diameters >0.5 mm due to poor diffusion pathways. Degradation rates were reduced when changing from macroscopic devices to microparticles.[12,13]

In contrast to dense matrices with diffusion lengths being defined by the overall distance between sample core and outer surface, microparticulate drug carrier loaded with conventional drugs or proteins often are designed at distinct levels of porosity.[14] As per IUPAC, pores can be classified to be macro- (> 50 nm), meso- (2–50 nm), or micro-sized (< 2 nm),[15] with typically macropores being detectable in PLGA microparticles by scanning electron microscopy. Pores at particularly high levels are formed by water absorption and solvent loss in emulsion based microencapsulation techniques such as the water-in-oil-in-water (w/o/w) emulsion method often applied for protein loading.[14] In these matrices with complex morphology, the diffusion pathways and diffusion lengths for acidic degradation products depend on the size, geometry, and dimension as well as the distribution of pores.

Importantly, the pore structures of PLGA microparticles in some cases may unexpectedly change by the pore closing phenomenon during incubation in aqueous medium.[16] Elevated temperatures and low pH were recently reported to support this phenomenon.[16,17] Apparently, temporary closure of pores alters diffusion pathways and may potentially entrap autocatalytically active degradation products in the microparticle matrix. Therefore, this study aims to identify matrix polymers and microparticle compositions, in which pore closing occurs during incubation under conditions relevant for a physiological environment. Furthermore, potential mutual effects of pore closing and hydrolytic degradation of microparticles should be explored.

Materials and Methods

Materials

PLGA with a glycolide content of 50 mol.-% and different molecular weights and end groups (Resomer® RG 502H, RG 503H, RG 504H, RG 503) as well as PLGA-PEG-PLGA (Resomer® RGPt 50106) were purchased from Boehringer Ingelheim, Ingelheim, Germany. Poly(vinyl alcohol) [Mowiol 4–88] was obtained from Kuraray Europe GmbH, Frankfurt, Germany. PEG with a molecular weight of 10 kDa, ovalbumin, and the BCA assay kit were from Sigma-Aldrich, Taufkirchen, Germany. All chemicals were of analytical grade and were used as received.

Preparation of Microparticles

Microspheres were prepared by the double emulsion solvent evaporation method. Briefly, 75 μl of 4 wt. % ovalbumin solution in water containing 1% (w/v) sodium bicarbonate and 5% (w/v) sucrose were first added to 1 ml of different concentrations of polymer solutions in dichloromethane (compare Table 1). The mixture was homogenized by sonication (SONOPULS HD3080 with MS 72 sonotrode, Bandelin, Germany) at 40% amplitude for 30 s. The so formed w/o primary emulsion was then emulsified in 2 ml of 2% (w/v) aqueous PVA solution containing 5% (w/v) sucrose for 2 min at 24,000 rpm with an Ultra Turrax T25 homogenizer (S25N- 5G disperser; IKA, Staufen, Germany). The resulting w/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 microspheres 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.

Analysis of Particle Size and Surface Morphology

Particle size analysis was performed in aqueous suspension by laser diffraction using Malvern Mastersizer 2000 with Malvern Hydro 2000S dispersion unit (Malvern Instruments, Herrenberg, Germany). The size distribution was calculated by the Fraunhofer model[18] and the mean d(0.5) as well as the span of the size distribution were used.

The surface morphology of lyophilized microparticles was studied using scanning electron microscopy (SEM) with a Gemini Supra™ 40 VP SEM (Carl Zeiss NTS GmbH, Oberkochen, Germany) at 1 kV with a secondary electron detector. Microparticles were analyzed as native samples to avoid sputtering artefacts.

The presence of micropores, the sample surface area, and the average pore diameters were determined by N_2 gas adsorption with liquid nitrogen cooling using the BET and BJH models (ASAP 2010;

Table 1.
Polymers used for particle preparation and degradation study.

Polymer	M_n	M_w	PD	T_g[a]	End group	o-Phase polymer conc.	Particle size		Encapsulation efficiency
							d(0.5)	Span value	
	[kDa]	[kDa]	–	[°C]		[wt.%]	[μm]	–	[wt.%]
PLGA[b]	2	6	3	44	COOH	19	3	0.9	88 ± 1
						30	4	1.3	82 ± 5
	5	16	3.2	47	COOH	11	3	0.8	86 ± 2
						19	6	1.8	82 ± 5
	11	24	2.2	47	Ethyl	11	4	0.8	85 ± 7
						19	6	1.5	80 ± 4
	13	28	2.1	47	COOH	11	4	0.8	83 ± 3
PLGA-PEG- PLGA[c]	36	70	1.9	29	Diol	11	9	1.0	81 ± 2
						15	14	1.5	86 ± 4
						19	14	1.6	84 ± 2

[a] Glass transition temperature T_g of dry samples.

[b] Poly[(*rac*-lactide)-*co*-glycolide] with a glycolide content of 50 mol.-%. The end-group will be indicated as PLGA-COOH or PLGA-Et. For comparing PLGA-COOH of different number average molecular weight M_n, the polymers will subsequently be abbreviated as PLGA2K, PLGA5K, PLGA13K.

[c] Poly[(*rac*-lactide)-*co*-glycolide]-*b*-poly(ethylene glycol)-*b*-poly[(*rac*-lactide)-*co*-glycolide] with a PEG block of $M_n = 10$ kDa.

Micromeritics, Aachen, Germany). Mercury intrusion porosimetry for characterization of meso- and macropores was performed with Pascal 140 and 440 instruments (Thermo Electron, Milano, Italy).

Encapsulation Efficiency

The encapsulated ovalbumin was degraded to amino acids by basic hydrolysis of microparticles in 1 ml 7.5 N NaOH at 106 °C for 12 hours (PMC Digital dry block heater, Germany). The sample volume was adjusted to 10 ml followed by neutralization and pre-column derivatization with o-phthaldialdehyde (OPA) reagent (50 μl sample, 100 μl borate buffer, 50 μl OPA reagent) with 5 min incubation before HPLC injection. HPLC was performed on a Jasco HSS - 1500 system (Jasco, Hessen, Germany) on a C_{18} column (EC 250/4 Nucleosil 100-5 C_{18} HD) using acetonitrile/water (80/20) as mobile phase at 1 ml/min with fluorescence detection (ex. 335 nm, em. 450 nm).

Evaluation of Polymer Degradation

Fifteen milligrams of microparticles were incubated in 1 ml of 5.8 mM phosphate buffered saline containing 0.01 wt.% Tween 20 (PBST), and maintained at 37 °C under continuous shaking (Certomat IS, B Braun Biotech International, Melsungen, Germany). At defined time intervals, microparticles were recovered by centrifugation (Biofuge Stratos, Hereaus, Hanau, Germany) at 9400 g for 5 min, washed, and lyophilized. The collected microparticles were then subjected to SEM, GPC, and DSC.

Gel Permeation Chromatography Analysis

Multidetector GPC measurements were performed with a flow rate of $1\,\mathrm{ml}\cdot\mathrm{min}^{-1}$ at 35 °C using chloroform as eluent and 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, and an automatic injector AS 2050 (both Jasco, Tokyo, Japan). Two detectors were used: a RI detector Shodex RI-101 (Showa Denko, Japan) and a T60A dual detector (Viscotek Corp., USA), which were connected by a split. The molecular weight distributions were determined by universal calibration with polystyrene standards (PSS) with number average molecular weights M_n between $580\,\mathrm{g}\cdot\mathrm{mol}^{-1}$ and $975{,}000\,\mathrm{g}\cdot\mathrm{mol}^{-1}$ using the SEC software WINGPC 6.2 (PSS).

Gravimetric Analysis – Mass Loss

Gravimetric analysis of mass loss of microparticles was accomplished with an analytical balance (Kern ALT 220-5DAM, Balingen, Germany). Specified amount of microspheres were weighed prior to introduction to the degradation medium as well as after lyophilisation of partially degraded samples. The relative mass loss μ_{rel} was calculated from the initial mass m_i and the mass of degraded samples m_d as follows: $\mu_{rel} = m_d \cdot (m_i)^{-1}$.

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 −100 and 100 °C with heating and cooling rates of 10 K/min. The thermal transitions were evaluated from the second heating cycle.

Results and Discussion

Selection of Copolyesters and Microparticle Preparation

In order to identify matrix polymers and microparticle compositions, in which pore closure occurs at 37 °C in phosphate buffer, different PLGA-based materials should be explored. Pore closing of microparticles has only seldom be reported and few previous reports indicated that pore closing may occur for PLGA with a glycolide content of 50 mol.-%.[16,17] However, the average molecular weight and weight distribution of PLGA as well as the end groups can be expected to potentially impact pore closing.

In order to study easily accessible, medical grade polymers, commercially available materials often used for microparticulate drug carriers were systematically analyzed. Due to its relevance for protein release systems, PLGA with one chain end bearing free carboxyl group was selected and employed at different average molecular weights (Table 1), which were determined by GPC with universal calibration. Additionally, PLGA with ethyl end groups should be explored to potentially distinguish between the effects of average molecular weight and end group hydrophilicity. Finally, because of its relevance in controlled protein release, a PLGA-PEG-PLGA block copolymer with PEG forming hydrophilic domains in the matrix should be explored for its capability to undergo pore closing. Importantly, in contrast to PLGA, which is used for medical devices and is apparently compatible with processing techniques such as hot melt extrusion, PLGA used in controlled drug release can be of rather low average molecular weight (Table 1).

Microparticles were prepared from these polymers by a double emulsion solvent evaporation technique. Microparticle porosity is strongly impacted by the saturation and speed of polymer precipitation at the interface of the polymer phase and the external water phase in nascent microparticles.[14] Therefore, different polymer concentrations were employed to impact polymer precipitation and microparticle hardening. Microparticle preparation did not significantly alter the polymer properties, since the values of average molecular weight and PD of samples from the first time point (3 h) of the degradation experiment (as reported below) well corresponded to the initial properties of the employed polymers as given in Table 1.

In order to illustrate the porosity and pore size distribution of microparticles as prepared, one of the most relevant microparticle formulations was produced in the gram-scale in order to allow analysis of initial porosity by gas adsorption and mercury porosity. As illustrated in Figure 1A, the microparticles were characterized by a bimodal pore size distribution with meso- and macropores. Micrometer-sized pore diameters in this plot correspond to the volume between the particles in the sample powder. The total intruded pore volume of the microparticles was $\approx 1100\,\text{mm}^3 \cdot \text{g}$ as determined by mercury porosimetry. Additionally, gas adsorption was performed to conclude on the presence of micropores. Based on the presence of a type 2 adsorption isotherm and a relatively low BET surface area of $10\,\text{m}^2$ per gram, the presence of micropores was excluded. Since larger pores in the

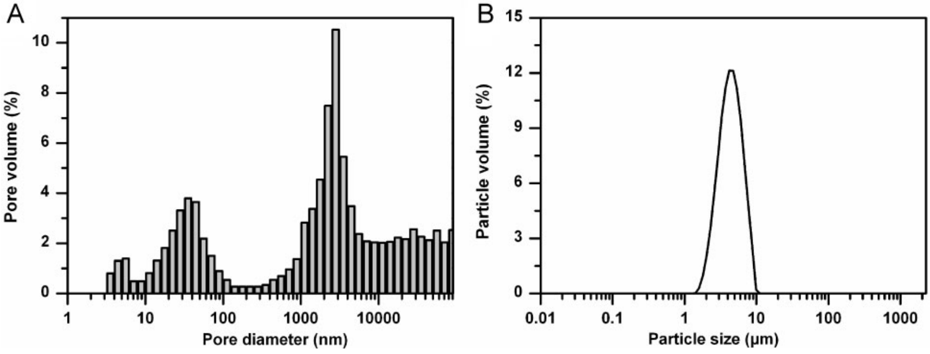

Figure 1.
Characterization of microparticle properties before incubation. A) Pore volume as determined by mercury intrusion porosimetry. B) Volume weighted size distribution of microparticles as determined by laser diffraction. Data correspond to microparticles prepared from PLGA-COOH, initial $M_n = 5\,\text{kDa}$, PD $= 3.2$, at an o-phase concentration of 19 wt.%.

macro-size range are not detected by this technique, an average pore diameter of 16 nm was obtained when applying the BJH model.

The particle sizes of all samples were analyzed by laser diffraction and a very narrow size distribution could be observed (Figure 1B). Even though a substantial increase in the viscosity of the polymer phase could be macroscopically observed, which often results in clearly increased particle sizes,[14] the established preparation technique allowed to provide microparticles with a rather low alteration of particle sizes (Table 1). Also, the protein encapsulation efficiency was similarly high in all cases and ranged from 80 to 88% of the intended payload (Table 1).

Dynamics in the Porosity of PLGA Microparticles During Degradation

For studying the general occurrence of pore closing and the associated morphological changes during degradation, microparticles were incubated in PBST at 37 °C on a horizontal shaker. After isolation by centrifugation and washing for removing salts, the samples were analyzed for mass loss and changes in the average molecular weight. Water uptake measurements could not be included in this study, since water uptake of single microparticles is hard to determine and, for numerous particles collected in centrifuge tubes, the large water-filled inter-particle volume would falsify results as visible, e.g., from the much higher inter-particle volume than pore volume for freshly prepared particles (Figure 1A).

When comparing microparticles prepared from PLGA of similar initial average molecular weight with either hydrophilic carboxyl or more hydrophobic ethyl end groups, a faster decrease in relative M_w of PLGA-COOH was observed than for PLGA-Et (Figure 2A). PLGA-COOH showed a 60% reduction of relative M_w within 28 days of degradation, whereas in case of PLGA-Et, only a 40% reduction of relative M_w occurred in this time period. As a consequence of polymer degradation to water soluble products, a mass loss of 20 wt.% was detected for PLGA-COOH in 28 days, a value being observed for PLGA-Et only after 56 days (Figure 2B). This stresses the contribution of polymer end groups on matrix hydrophilicity and, in consequence, uptake of water,[9] which subsequently is the precondition for polyester hydrolysis and formation of diffusion pathways for degradation products. Additionally, studies of PLGA hydrolysis using the Langmuir-trough method indicated chain-end scission as mechanism of hydrolysis for monolayer films,[19] in which diffusion phenomena may be neglected due to the instantaneous and continuous contact of all building blocks with the water phase. By this technique, ethyl and carboxyl

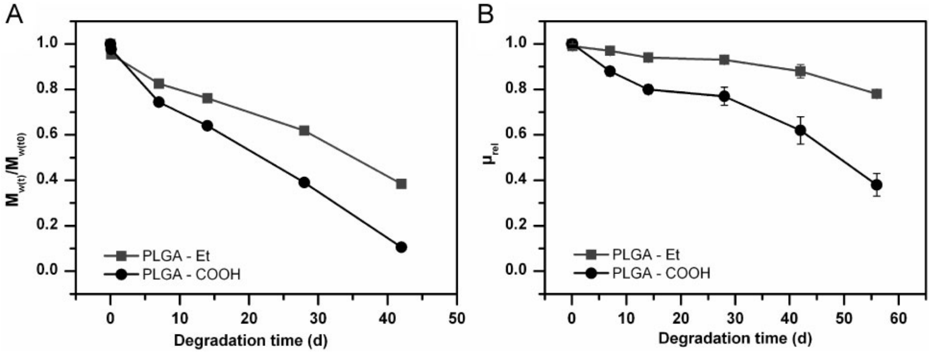

Figure 2.

Effect of polymer end group on A) decrease in relative M_w and B) mass loss of microparticles particles prepared from (■) PLGA-Et; initial M_n = 11 kDa, PD = 2.2 and (●) PLGA-COOH; initial M_n = 13 kDa, PD = 2.1. The o-phase concentration of PLGA during preparation was 11 wt.%.

end groups resulted in identical rates of hydrolysis for poly(*rac*-lactide), suggesting that they behave similarly in terms of hydrolytic attack in chain-end scission. This supports that effects of end groups on degradation pattern for bulk materials rather than monolayer films may be due to differences in diffusion such as water uptake.[19] Despite clear differences in the degradation kinetics for these microparticles, they behaved similar in terms of morphology without prominent changes of pore structure in SEM (data not shown).

The capability for pore closing is assumed to be linked to the increased mobility of polymer chains at temperatures above the T_g of the respective polymeric domain. Plastification of the material is one mechanism to reduce the T_g of PLGA below body temperature. Additionally, reduction of the T_g of PLGA may be achieved by alteration of its molecular weight. Therefore, PLGA-COOH with M_n's of 2, 5, and 13 kDa were compared in parallel studies (Figure 3). The samples showed a similar pattern of relative molecular weight decrease (Figure 3A) without any effects of the polymer concentration used in the o-phase during microparticle preparation on the degradation pattern (data not shown). The samples from PLGA-COOH with the lowest initial average molecular weight exhibited a mass loss of almost 95 wt.% in eight weeks of hydrolytic degradation (Figure 3B). In contrast, PLGA-COOH of the highest initial average molecular weight degraded more slowly with a mass loss of 60 wt.% in eight weeks.

For microparticles from PLGA-COOH with different initial molecular weights, only those being prepared from material with $M_{n.} = 5$ kDa exhibited distinct dynamic changes in the porosity during degradation (Figure 4). The SEM images illustrate the surface morphology to be characterized by pores, which appeared to be filled rather than to be open structured (Figure 4A). Upon incubation, the pores at the surface opened up in three hours (Figure 4B), likely due to the release of proteins and their stabilizers from their lumen. Importantly, pore closure on subsequent incubation led to smooth surfaces as exemplarily shown after two weeks (Figure 4C) and remained like this for an extended period of time (Figure 4D). At later stages of degradation (Figure 4E-F), the pores started reopening and the surface became more and more porous until ultimately particles aggregated and collapsed into a bulky mass.

Mechanistically, pore closure requires the mobility of polymer chains, which had to be acquired at some stage under conditions of incubation. This may be explained by i) water uptake into the

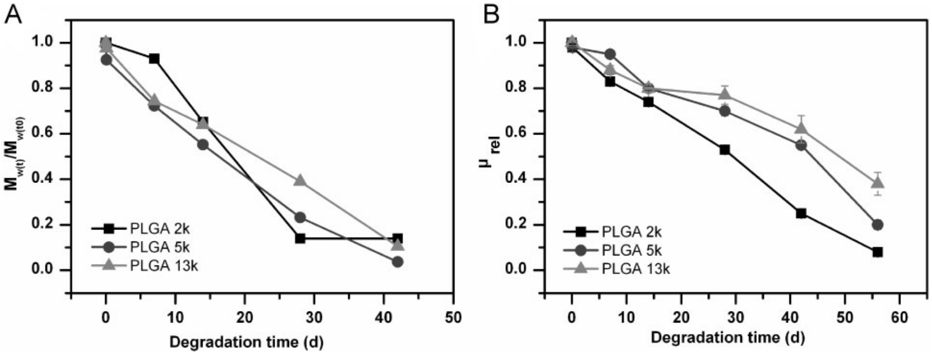

Figure 3.
Effect of initial average molecular weight of the polymer on microparticle degradation as determined by A) decrease in relative M_w and B) mass loss of microparticles prepared from (■) PLGA-COOH; $M_{n.} = 2$ kDa, PD = 3 (o-phase conc. 19 wt.%), (●) PLGA-COOH; $M_{n.} = 5$ kDa, PD = 3.2, (o-phase conc. 19 wt.%) (▲) PLGA-COOH; $M_{n.} = 13$ kDa, PD = 2.1 (o-phase conc. 11 wt.%).

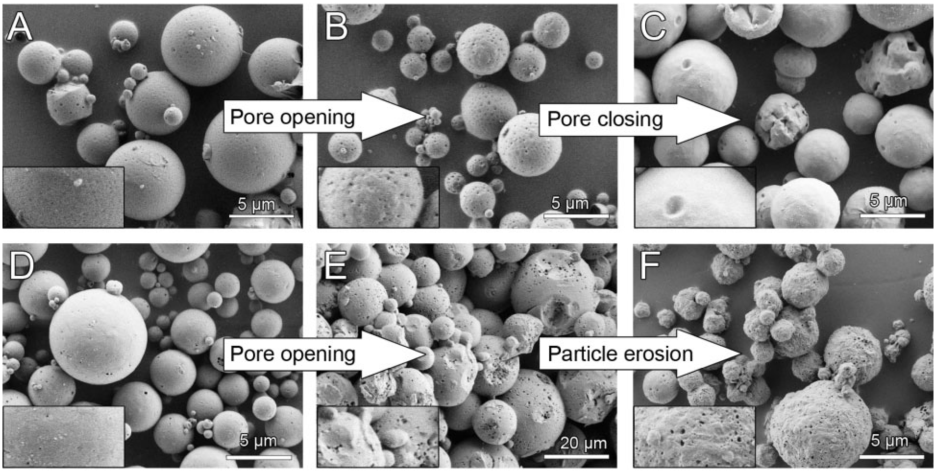

Figure 4.
Dynamic changes in pore structure of microparticles from PLGA-COOH, initial $M_n = 5$ kDa, PD = 3.2 during degradation as illustrated by SEM pictures A) before the incubation as well as after B) 3 hours, C) 2 weeks, D) 4 weeks, E) 6 weeks, and F) 8 weeks. The insets show a detailed view at a fourfold zoom. The o-phase concentration of PLGA during preparation was 19 wt.%.

matrix resulting in swelling, i.e., volume increase and occlusion of pores and/or ii) 'self-healing' because of interfacial tension driven reduction of interface area after water-induced plasticization and decrease of T_g below the incubation temperature.[16,17] Additionally, as postulated for low local pH conditions, iii) less electrostatic repulsion of charged carboxyl end groups as created during degradation may support hydrophobic polymer-polymer interactions as alternative pore closing mechanism.[17]

On the one hand, the dynamic changes in porosity can be discussed in the context of hydrolytic degradation, i.e., changes in the properties of the matrix polymer. The initial opening of pores at the particle surface certainly is driven by the dissolution of compounds located in the preformed pores and may not be strongly depending on polymer degradation. Subsequent pore closing, however, timely overlapped with a strong reduction in relative molecular weight (Figure 5A). For amorphous polymers, degradation is linked to an exponential decrease of T_g when reaching a certain critical molecular weight.[20] The dry state T_g, however, does only slowly decrease (Figure 5B), which only occurred after pore closing was already initiated. In contrast, the wet state T_g of particles as prepared was 32 °C and thus clearly below the incubation temperature. This indicates that in the present study, pore closing was initiated by interfacial tension-driven self-healing after plasticization by the initial water uptake. The pH of the degradation medium decreased during the study, however, potential contributions of hydrophobic polymer-polymer interactions to pore closing upon pH decrease as recently suggested[17] may only be speculated on.

On the other hand, rather than considering the contribution of degradation on pore closing, an interesting point would be on whether pore closing affects the degradation pattern. Clearly, pore closing can create diffusion barriers, which could have wide implications on the drug/protein controlled release properties of microparticles by trapping macromolecules.[16] In addition to the drug payload, also other molecules may be hindered in their diffusion, namely water and PLGA degradation products. During the initial pore opening phase, limited diffusion barriers exist for water-soluble oligomers from lactide and

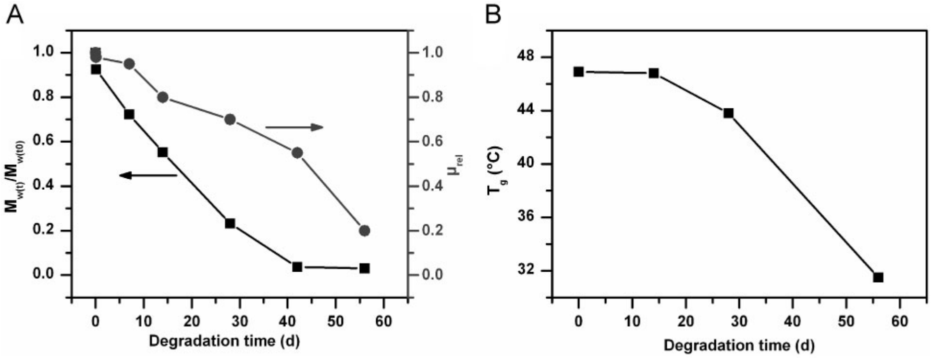

Figure 5.
Degradation pattern of PLGA microparticles, which show the pore closing phenomenon (PLGA-COOH, $M_n = 5$ kDa, PD = 3.2 A) Mass loss (●) and decrease in relative M_w (■) as summarized from Figure 3, B) Changes in dry state glass transition temperature. The o-phase concentration of PLGA during preparation was 19 wt.%.

glycolide. However, subsequent pore closure might retard the diffusion of acidic degradation products out of the matrix, which could support autocatalysis by acidic oligomers and decrease the mass loss. As illustrated in Figure 5, there was no unexpected or step-like degradation pattern, with neither the decrease on relative M_w being accelerated nor the mass loss being delayed during pore closing. Therefore, in contrast to proteins, which were previously reported to be non-continuously released,[16] the diffusional exchange of water and low molecular weight products of polymer degradation may not be effected by pore closing. A reason for that could be that rather thin polymer layers are covering the pores and that those small molecules can migrate through the polymer free volume, particularly in the case of plasticized amorphous polymers being in their rubbery state.

In contrary to the expectation that PLGA-COOH with an even lower initial $M_n = 2$ kDa should also be plasticized and show pore closing, such behaviour was not clearly visible in SEM studies. Possibly, the degradation of this material was so fast that the pore closure and pore opening might have occurred in between the sampling time points of this study. Also, osmotic flow induced by the degradation products may have kept pores open.

Morphological Changes in Phase-Separated Matrices Containing Hydrophilic Polyether Segments

Matrix hydrophilicity and water uptake appears to correlate with the occurrence of pore closing. However, decreasing the PLGA molecular weight to increase the number of hydrophilic end groups was shown to be not necessarily a successful strategy. An alternative approach to increase the water uptake of microparticles could be the introduction of hydrophilic domains. With the aim to create hydrophilic diffusion pathways for proteins, PLGA-PEG-PLGA triblock copolymers have been designed in the past,[10] which contain hydrophilic polyether segments and therefore should here be studied for pore closing. Additionally, a physical mixture of PEG and PLGA-COOH was employed in order to conclude on the necessity of covalent attachment of the chain segments.

In SEM analysis, PLGA-PEG-PLGA microparticles as prepared appeared to be rather irregularly structured (Figure 6). Upon incubation in aqueous environment, a smoother surface morphology was observed already after day 1 (Figure 6C). Even though the particles were not perfectly spherical, the pores of the particles were closed. After two weeks of degradation (Figure 6D), a strong increase in particle diameter, i.e., a swelling by water

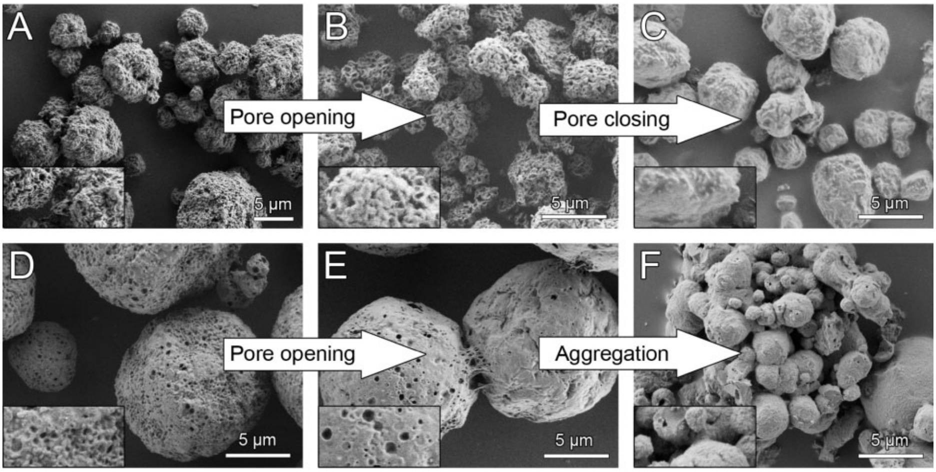

Figure 6.
Dynamic changes in the pore structure of microparticles from PLGA-PEG-PLGA block copolymer during degradation as illustrated by SEM pictures A) before the incubation as well as after B) 3 hours, C) 1 day, D) 2 weeks, E) 4 weeks, and F) 8 weeks. The insets show a detailed view at a fourfold zoom. The o-phase concentration of PLGA during preparation was 19 wt.%.

uptake was observed. However, swelling did not resulted in an occlusion of pores. Unexpectedly, the pores were reopening, possibly due to leakage of cleaved chains from PEG rich domains driven by osmotic pressure (Figure 6E). Subsequently, the particles started to aggregate into a bulky mass (Figure 6F). For the triblock copolymer, pore closing occurred faster than for PLGA-COOH. This might be because of the difference in hydrophilicity as well as due to low T_g of PLGA-PEG-PLGA of 25 °C. This may have allowed faster rearrangements of polymer chains and pore closing. In the case of the physical mixture, a similar pattern of pore closing phenomenon was observed.

When comparing the degradation pattern of the different microparticles, a faster initial decrease of relative M_w was observed (Figure 7A). This may be due to the phase-separated structure with water predomi-

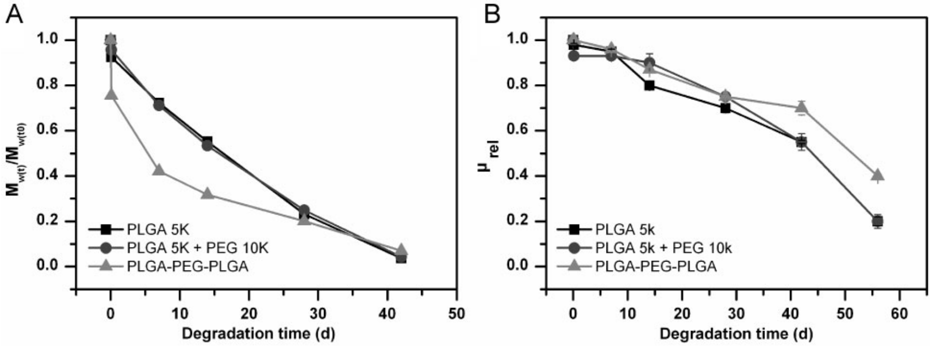

Figure 7.
Effect of the inclusion of hydrophilic domains on degradation pattern of copolyester microparticles. A) Decrease of relative M_w, B) mass loss for microparticles prepared from (■) PLGA-COOH ($M_n = 5$ kDa, PD = 3.2), (●) a mixture of this PLGA-COOH and PEG 10 kDa (19:1, w/w), and (▲) PLGA-PEG-PLGA. The o-phase concentration of PLGA during preparation was 19 wt.%.

nately entering into the PEG domains. Possibly, hydrolysis in this case occurred not only as chain end scission as for PLGA.[19] It is expected that water preferential attacks ester bonds being located close to the hydrophilic PEG block, (i.e., close to the middle of the molecule) rather than at the chain ends in the less hydrophilic PLGA rich domain.[21] This may result in the release of PEG with attached short oligoester segments once they become water soluble.[22] Mass loss of PLGA-PEG-PLGA was slightly slower than for PLGA-COOH, probably due to the higher initial average molecular weight of the PLGA blocks. Interestingly, the physical mixture of PEG and PLGA showed a fast initial mass loss most likely associated to PEG, and afterwards showed pattern similar to pure PLGA-COOH.

Conclusion

Significant changes in the morphology of (co)polyester-based microparticles were observed during their incubation in aqueous media, which include the pore closing phenomenon for selected matrix polymers and microparticle compositions at 37 °C in PBS. The pore closing of PLGA with carboxyl end groups and a relatively low molecular weight was not primarily initiated by degradation, but mainly driven by the initial water uptake and subsequent plasticization of the polymer. Pore closing, on the other hand, could not be observed to cause unexpected or undesired changes in degradation pattern such as acceleration of molecular weight decrease by autocatalysis from trapped acidic oligomers.

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