Biodegradable poly(ether-ester) multiblock copolymers for controlled release applications: An *in vivo* evaluation

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Abstract: Multiblock poly(ether-ester)s based on poly(ethylene glycol), butylene terephthalate, and butylene succinate segments were evaluated for their *in vivo* degradation and biocompatibility in order to establish a correlation with previously reported *in vitro* results. Porous polymer sheets were implanted subcutaneously for 32 weeks in rats. The degradation was monitored visually (histology), by molecular weight (GPC), and by copolymer composition (NMR). Substitution of the aromatic terephthalate units by aliphatic succinate units was shown to accelerate the degradation rate of the copolymers. Direct correlation of the *in vivo* and *in vitro* degradation of the porous implants showed a slightly faster initial molecular weight decrease *in vivo*. Besides hydrolysis, oxidation occurs *in vivo* due to the presence of radicals produced by inflammatory cells. In addition, the higher molecular weight plateau of the residue found *in vivo* indicated a higher solubility of the oligomers in the extracellular fluid compared to a phosphate buffer. Minor changes in the poly(ether-ester) compositions were noted due to degradation. Microscopically, fragmentation of the porous implants was observed in time. At later stages of degradation, macrophages were observed phagocytozing small polymer particles. Both *in vitro* cytotoxicity studies and histology on *in vivo* samples proved the biocompatibility of the poly(ether-ester)s. © 2004 Wiley Periodicals, Inc. J Biomed Mater Res 71A: 118–127, 2004

Key words: poly(ether-ester); cytotoxicity; *in vivo* degradation; *in vitro* degradation; biocompatibility

INTRODUCTION

Recently, multiblock poly(ether-ester)s based on poly(ethylene glycol), butylene terephthalate, and butylene succinate segments have been designed as a new series of degradable polymers for controlled release applications.[1] These poly(ether-ester)s are a modification of poly(ethylene glycol) terephthalate (PEGT) / poly(butylene terephthalate) (PBT) copolymers, which have been successfully applied as matrix in controlled release systems.[2–5] However, for controlled release applications requiring frequently repeated injections, the degradation rate of some PEGT/PBT copolymer compositions might be too slow.[6] Substitution of the aromatic terephthalate units by aliphatic succinate units was shown to increase the degradation rate of the copolymers *in vitro*.[1] However, as reported for several polymer systems, the *in vitro* degradation can differ considerably from the *in vivo* degradation. In most cases the *in vivo* degradation is faster than the *in vitro* degradation.[7–13] This is attributed to the presence of certain ions or radicals, produced by inflammatory cells,[12,14] enzymes,[15] and lipids[9] in the body fluid, which may affect the chain cleavage mechanism as well as the dissolution of the oligomers.[16] For poly(lactide-*co*-glycolide) (PLGA) in particular, the *in vivo* degradation is autocatalyzed due to accumulation of acidic degradation products.[10,16] In addition, the extension of both the tissue response and the autocatalytic effect can be affected by the size[16–19] and the shape[20] of the implant. On the other hand, for hydrogel-like polymer systems, a slower *in vivo* degradation has been reported compared to the *in vitro* degradation in phosphate buffer.[21] The presence of less fluid near the implant site retards swelling and subsequently inhibits degradation. For copolymers only degrading by hydrolyses in bulk, the *in vitro* degradation will mimic the *in vivo* degradation.[22–23]

In this article, we evaluate the *in vivo* degradation of

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the novel poly(ether-ester) copolymers in rats and compare it to the *in vitro* degradation. Both the molecular weight and the copolymer composition were monitored. In order to create a large surface area, porous sheets were implanted subcutaneously. Prior to implantation, the cytotoxicity of the poly(ether-ester)s was evaluated.

MATERIALS AND METHODS

Materials

The poly(ethylene glycol) (terephthalate/succinate) poly-[butylene (terephthalate/succinate)] [PEG(T/S)PB(T/S)] copolymers were synthesized at Chienna BV (Bilthoven, The Netherlands). The PEG(T/S)PB(T/S) copolymers varied in PEG segment length (600, 1000, and 4000 g/mol), PEG(T/S)/PB(T/S) weight ratio (62/38–71/29) and T/S molar ratio (0/100–100/0) (composition determined by NMR). The poly(ether-ester)s are indicated as aPEG(T/S)bPB(T/S)c (T/S=d/e), in which a is the PEG molecular weight, b the combined weight percentage PEGT and PEGS, and c is the combined weight percentage of PBT and PBS. d/e is the molar T/S ratio in the whole polymer. Chloroform (analytical grade) was purchased from Fluka Chemie GmbH (Buchs, Switzerland). Merck (Darmstadt, Germany) was the supplier of sodium chloride (NaCl) . Phosphate buffered saline, (pH 7.4) was obtained from Life Technologies Ltd. (Paisley, Scotland).

Preparation of porous PEG(T/S)PB(T/S) implants

For the *in vivo* study, porous polymer implants were prepared using a solvent casting / salt leaching procedure. Three grams of PEG(T/S)PB(T/S) copolymer was dissolved in 15 mL chloroform (13.5 wt %). Sodium chloride with a grain size of 212 to 250 μm was added using a NaCl-to-copolymer weight ratio of 7.5:1. The mixture was homogenized and cast on a glass plate using a casting knife (setting 2000 μm). After controlled evaporation of the chloroform, the resulting film was removed from the glass plate. Extensive washings in demineralized water leached the NaCl out. The porous film was dried in air at room temperature and subsequently under vacuum at 50°C for 16 h. Pieces of the porous films (1.6 mm in diameter, 600–800 μm in thickness) were packed under vacuum in aluminum pouches and sterilized by gamma irradiation. A minimum irradiation dose of 25 kGy was applied in a JS6500 Tote Box Irratiator at Gammaster B.V. (Ede, The Netherlands).

To study the effect of the copolymer composition on the biocompatibility and *in vivo* degradation, a series of PEG(T/S)PB(T/S) copolymers were used varying in PEG segment length (600–4000 g/mol) and the terephthalate/succinate ratio (100/0–0/100 mol %). The amount of soft segment [PEG(T/S)] was more or less constant at 66 wt % (62–71 wt %). As it was not possible to obtain porous films from the 600PEGS71PBS29, ground powder (< 600 mm) of this copolymer was used in the *in vivo* study.

Implant characterization

The morphology of the implants was evaluated prior to implantation by using scanning electron microscopy (SEM). A Philips XL 30 Environmental Scanning Electron Microscope was used to study the surface characteristics of the implants after coating the samples with a thin gold layer.

To study the effect of the sterilization process, the molecular weight of the porous implants before and after gamma irradiation was determined using gel permeation chromatography (GPC). Samples were eluted in 0.02 *M* sodiumtrifluoroacetate (NaTFA) in hexafluoroisopropanol (HFIP) through a Polymer Labs HFIP gel guard column (50 × 7.5 mm) and two PL HFIP gel analytical columns (300 × 7.5 mm). Flow rate was 1 mL/min and both an ultraviolet (UV, 254 nm) and a refraction index (RI) detector were used. Column temperature was 40°C and sample concentration was 0.3 mg/mL (50 μL injection). The weight average molecular weights (M_w) and the polydispersity (M_w/M_n) were determined relative to polymethylmethacrylate (PMMA) standards. The GPC analysis was performed in duplicate.

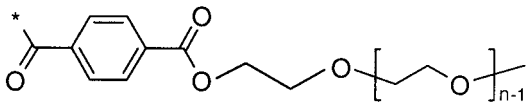
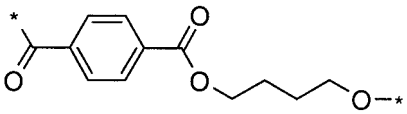
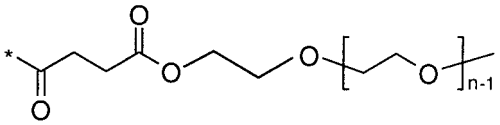
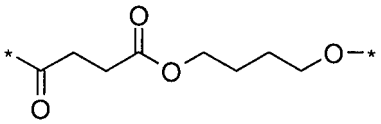
Cytotoxicity

The cytotoxicity of the poly(ether-ester)s towards the growth, morphology, and metabolism of fibroblasts was evaluated according to EN/ISO 10993-5. Powder of 1000PEGS72PBS28 and 1000PEG(T/S)73PB(T/S)27 (T/S=55/45) was extracted at 37°C for 24 h in medium. The medium consisted of minimum essential medium (MEM) supplemented with 10% fetal calf serum. Natural rubber was extracted identically for a positive control. As a negative control material, UHMW polyethylene was used. The extracts were added to cultures of mouse lung fibroblasts (L929) and incubated for 48 h at 37°C. The biological reactivity (cellular degeneration and malformation) was evaluated under a microscope.

Implantation study

Twenty-four Wistar rats (300 g, GDL, Utrecht, The Netherlands) were anesthetized by an intramuscular injection of a mixture containing atropine (0.5 mL, 0.5 mg/mL), xylazine (1.5 mL, 20 mg/mL), and ketamine (1.75 mL, 100 μg/mL). The surgical sites were shaved and cleaned with 70% ethanol and iodine. Subcutaneous pockets (8 per animal) were created along the dorso-medial line, in which the samples were inserted. Subsequently, the pockets were closed using vicryl sutures. The surgery was performed using sterile instruments in a nonsterile environment. At 2, 8, 16, and 32 weeks after implantation, six rats were sacrificed by CO_2. The implants (n = 6 per condition) were removed with

TABLE I
Segments Present in the Poly(ether ester) Copolymers

Formula	Name	Abbreviation
	Poly(ethylene glycol) terephthalate	PEGT
	Butylene terephthalate	BT
	Poly(ethylene glycol) succinate	PEGS
	Butylene succinate	BS

excess surrounding tissue for evaluation. The animal experiment in this study was performed according to the legal guidelines concerning animal welfare ISO 10993 part 2, 1997, European Directive 86/609/EEC, and to the Dutch Laboratory Animal Act.

Characterization of *in vivo* samples

After explantation, the samples remained for 1 day in Karnovsky's fixative. For histological analyses, pieces of the samples were dehydrated in graded series of ethanol and embedded in glycol methacrylate. Subsequently, the samples were sawed using a microtome and stained with hemotoxylin and eosin. The slides were examined under a light microscope (Nikon Eclipse E400) and evaluated for the tissue response.

For the *in vivo* degradation, the fixed samples were washed with demineralized water. To remove the tissue, the samples were dissolved in chloroform and filtered by using a 0.45 μm filter on a syringe. After evaporation of the solvent the remaining polymer film was analyzed for molecular weight and copolymer composition. The molecular weight as function of degradation time was determined on three samples per polymer composition using GPC as described above. The effect of the *in vitro* degradation on the composition of the copolymers was studied by NMR measurements. In order to remove interfering tissue residues, the polymer samples were first precipitated in hexane. Subsequently, proton NMR spectra were recorded on a Bruker ARX-400 operating at ≥ 200 MHz. $C_2D_2Cl_4$ was used as solvent without internal standard. The copolymer composition was calculated as described elsewhere.[1]

In vitro degradation

To evaluate the *in vitro* degradation, sterilized porous sheets and powder (identical to *in vivo* study) were immersed in 50 mL phosphate buffered saline at 37°C in a shaking bath for several time periods. The buffer was refreshed every 2 weeks. After preselected degradation periods, parts of the samples were washed with purified water and dried using a vacuum oven at 50°C for at least 16 h. The *in vitro* degradation was monitored by analyzing the changes in molecular weights (GPC) and changes in copolymer composition (NMR) as described for the *in vivo* samples.

RESULTS AND DISCUSSION

The poly(ether-ester) copolymers used in this study can be described as being built up of four different repeating segments (Table I). Poly(ethylene glycol) terephthalate (PEGT) and poly(ethylene glycol) succinate (PEGS) form the soft hydrophilic blocks, whereas

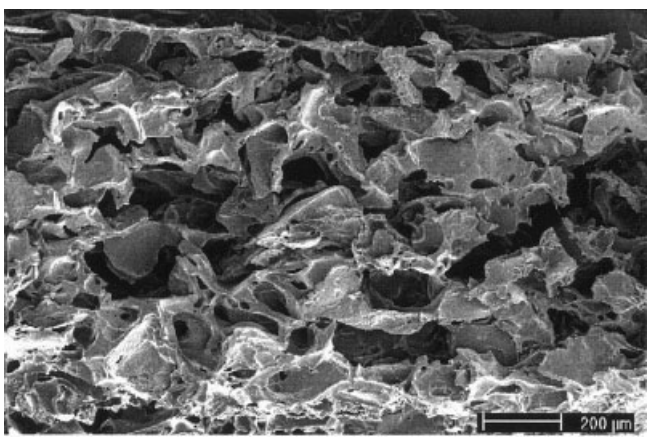


Figure 1. Scanning electron micrograph of porous 1000PEG(T/S)66PB(T/S)34 implant containing 45 mol % succinate in the hard segment.

TABLE II
Weight Average Molecular Weight and Polydispersity of the Poly(ether-ester)s Implants Prior to and After Sterilization as Function of the Copolymer Composition

Polymer Composition (NMR)	Mol % Succinate (NMR)	M_w (10^{-3} g/mol) (GPC) Prior to γ Irradation	M_w/M_n (GPC) Prior to γ Irradation	M_w (10^{-3} g/mol) (GPC) After γ Irradation	M_w/M_n (GPC) After γ Irradation
600PEG(T/S)62PB(T/S)38	46	128	2.0	164	2.8
600PEGS71PBS29	100	123	2.5	160	2.8
1000PEG(T/S)62PB(T/S)38	11	105	2.2	127	2.4
1000PEG(T/S)66PB(T/S)34	45	111	2.2	147	2.7
1000PEGS69PBS31	100	121	2.0	123	2.2
1000PEGT71PBT29	0	107	1.7	118	2.1
4000PEG(T/S)64PB(T/S)36	46	113	2.1	137	2.6
4000PEGS66PBS34	100	124	2.1	146	2.5

the hard hydrophobic blocks contain butylene terephthalate (BT) and butylene succinate(BS).[1]

Implant characterization

Porous poly(ether-ester) implants were prepared using a solvent casting/salt leaching procedure. Figure 1 shows a cross-section of an porous implant of 1000PEG(T/S)66PB(T/S)34 containing 45 mol % succinate in the hard segment. The weight average molecular weights of the implants prior to and after gamma irradiation as determined by GPC analyses are given in Table II (SD $<$ 3%). The M_w prior to gamma irradiation is in the range of 105 to 128 kg/mol. After gamma irradiation, the weight average molecular weights of all copolymer compositions increased (15% on average). A similar increase in molecular weight was observed before for sterilized PEGT/PBT microspheres.[6] This was attributed to crosslinking due to recombination of radicals formed by gamma irradiation. On the other hand, other polymer systems, like PLGA, showed a decrease in molecular weight after sterilization, as a result of chain scission through radical formation.[24] For the PEG(T/S)/PB(T/S) copolymers, the polydispersity increased from ± 2.1 to ± 2.5 by gamma irradation (Table II). This result suggests the occurrence of both chain cleavage and (partial) crosslinking in these poly(ether-ester)s.

Prior to implantation, the cytotoxicity of the poly(ether-ester)s towards the growth, morphology, and metabolism of fibroblasts was evaluated. For these experiments, the fastest degrading copolymer compositions were selected and a high surface area was created by using milled powder. Table III shows the results for the poly(ether-ester) samples and the positive and negative control. No biological reactivity was observed in the L929 mammalian cells at 48 h post exposure to the polymer extracts. The observed cellular response obtained from the positive control extract and the negative control extract confirmed the suitability of the test system. It was therefore concluded that the poly(ether-ester) copolymers studied were noncytotoxic.

Histological evaluation

The effect of the copolymer composition on the *in vivo* degradation and the tissue response was examined visually by light microscopy. Figure 2 shows the porous sheets after 2 weeks and 15 weeks of implantation with increasing succinate content at a constant PEG segment length (1000 g/mol) and soft segment amount (65 wt %). The higher the degree of succinate substitution in the polymer, the faster the implant fell apart into small polymer fragments. The size of the polymer fragments decreased in time due to degradation. In the absence of succinate [Fig. 2(a, b)], large polymer fragments are visible after 2 weeks of implantation with limited tissue ingrowth, while after 15 weeks the tissue ingrowth was complete around the

TABLE III
Cellular Reactivity in Cytotoxicity Tests as Function of Exposure Time

Sample	Reactivity at 24 h Postexposure	Reactivity at 48 h Postexposure
Negative control	No cell lysis	No cell lysis
1000PEG(T/S)73PB(T/S)27 (T/S 55/45)	No cell lysis	No cell lysis
1000PEGS72PBS28	No cell lysis	No cell lysis
Positive control	Nearly complete destruction of the cell layers	Nearly complete destruction of the cell layers

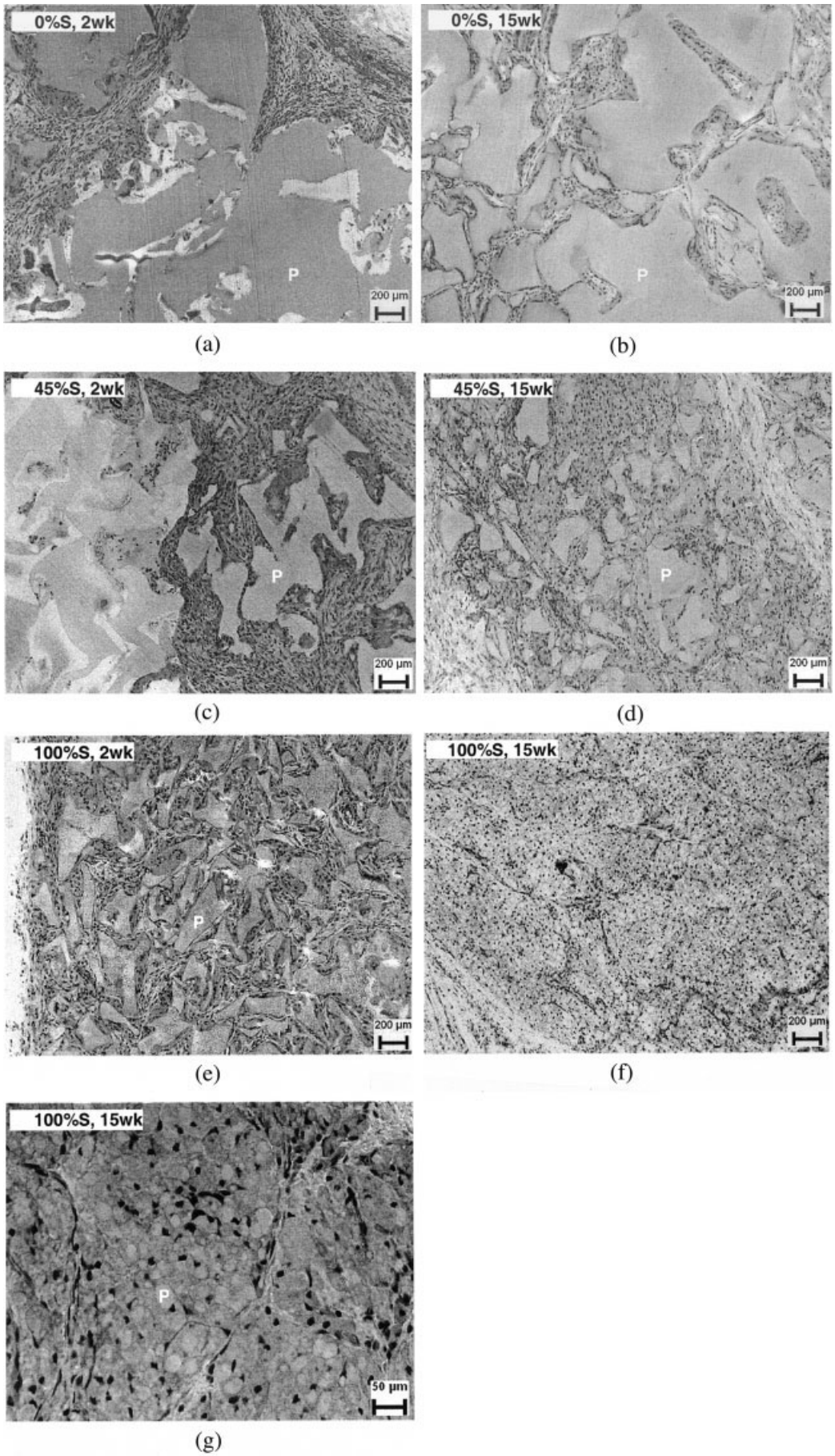


Figure 2. Histology of 1000PEG(T/S)65PB(T/S)35 porous implants containing 0 mol % (a,b), 45 mol % (c,d), and 100 mol % (e,f) of succinate in the hard segment after 2 (a,c,e) and 15 weeks of implantation (b,d,f). Original magnification: 100×. Figure 2 (g) is similar to (f) at a higher magnification (×400). P indicates the polymer material.

polymer fragments. The implants containing 45 mol % of succinate in the hard segment [Fig. 2(c, d)] also showed partial tissue ingrowth after 2 weeks without capsule formation, whereas after 15 weeks a fibrous capsule was present. The size of polymer fragments of this polymer composition decreased faster in time compared to the copolymer without succinate. In addition, the resulting particles of the partly substituted copolymer were less angular. The implant of the 100% succinate substituted poly (ether-ester) [Fig. 2(e–g)] degraded already in 2 weeks into small particles. The formation of granulation tissue as scaffold for tissue repair was initiated, as expressed by the fibroblast infiltration and the development of blood capillaries.[25] After 15 weeks of implantation, a higher magnification was required to identify the polymer fragments [Fig. 2(g)]. Macrophages started to phagocytose fragments around 10 μm, without the formation of foreign body giant cells, which is in agreement with observation by others.[26,27] All poly(ether-ester) compositions evaluated in this study showed only very mild chronic inflammatory responses followed by a normal foreign body response. In general, the tissue response followed the sequence of local events as described by Anderson and colleagues.[28] Signs of bioincompatibility, like tissue necrosis and changes in tissue morphology, were not observed. From these results, it was concluded that the poly(ether-ester) implants were well tolerated by the surrounding subcutaneous tissue.

In vivo and *in vitro* degradation

The *in vitro* and *in vivo* degradation were evaluated in time by monitoring both molecular weight and copolymer composition by GPC and NMR, respectively. The animal-to-animal variation was less than 5% SD, calculated from molecular weights of three samples per polymer composition per condition, which is comparable to observations by others.[7]

Molecular weight

Figures 3 and 4 show the molecular weight as function of implantation time for poly(ether-ester)s varying in succinate/terephthalate ratio and PEG segment length. The degradation profile — an exponential molecular weight decrease resulting in a plateau value — is independent on the polymer composition. The plateau value reached after 8 to 15 weeks does not mean that degradation has stopped. Further degradation results in soluble low molecular weight oligomers, which are removed from the implantation site, resulting in a more or less constant molecular weight of the residue.

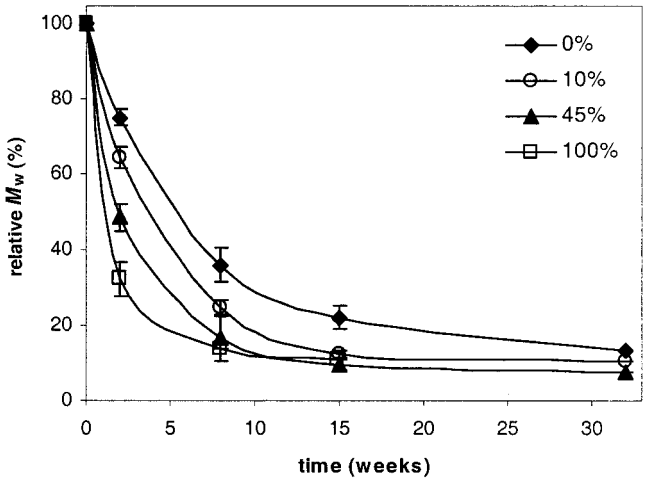


Figure 3. Relative weight average molecular weight of residue as function of degradation time *in vivo* for 1000PEG(T/S)65PB(T/S)35 copolymers containing 0 (♦), 10 (○), 45 (▲) and 100 (□) mol % succinate (n = 3; ± SD).

In agreement to previously reported *in vitro* experiments[1] and the histology results described above, the degree of substitution of the aromatic groups by aliphatic groups showed a clear effect on the *in vivo* degradation. At a constant soft/hard ratio and PEG segment length, the decrease in molecular weight was faster for poly(ether-ester)s with increasing succinate content (Fig. 3). The effect of the PEG segment length on the degradation is shown in Figure 4. For copolymers containing 45 mol % of succinate in the hard segment, the fastest decrease in molecular weight was observed for the copolymers with PEG segments of 1000 g/mol and 600 g/mol, respectively [Fig. 4(a)]. For the copolymer containing 100 mol % of succinate in the hard segment, the fastest degradation was observed for the poly(ether-ester) with 600 g/mol PEG segments, followed by poly(ether-ester)s containing PEG segments of 1000 g/mol and 4000 g/mol [Fig. 4(b)].

Degradation of the polymer and subsequent dissolution of the oligomers is based on a combination of swelling[29–31] and average block length of the copolymer's hard segment.[29,32] A higher swelling makes the ester bonds more accessible for hydrolysis.[33,34] Longer average block lengths of the hard PB(T/S) segments reduces the number of easy accessible hydrolysable ether-ester bonds. At a constant soft/hard ratio and PEG segment length, the swelling of the poly(ether-ester)s increases with increasing amount of succinate.[1] Therefore, the faster degradation observed in poly(ether-ester)s with higher aliphatic succinate substitution can be explained by the increase in swelling (Fig. 3). Besides the higher accessibility of the ester bonds due to the higher swelling, the aliphatic esters are

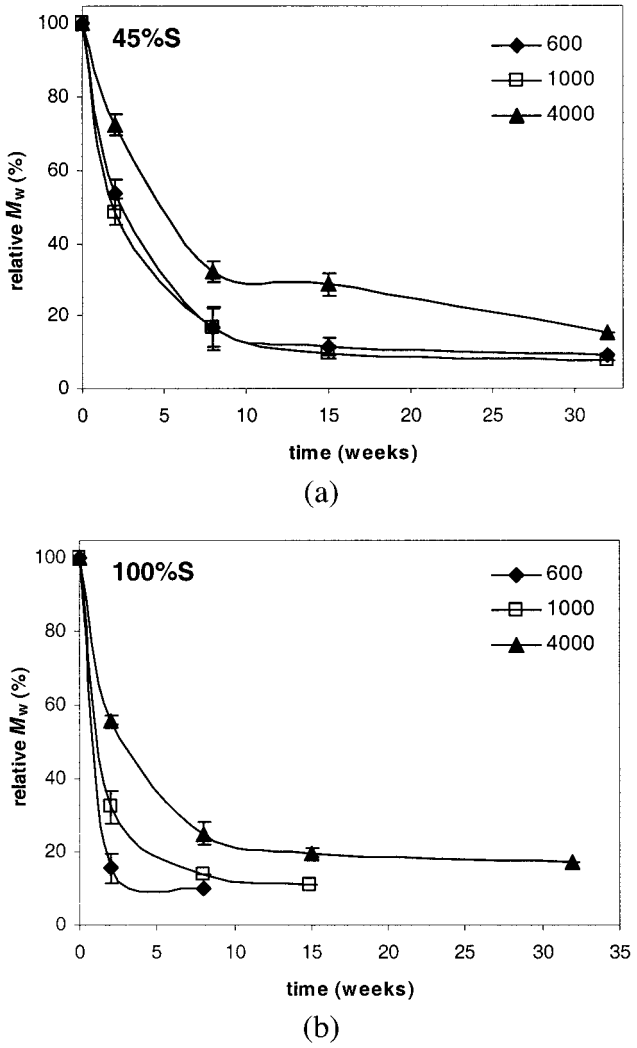
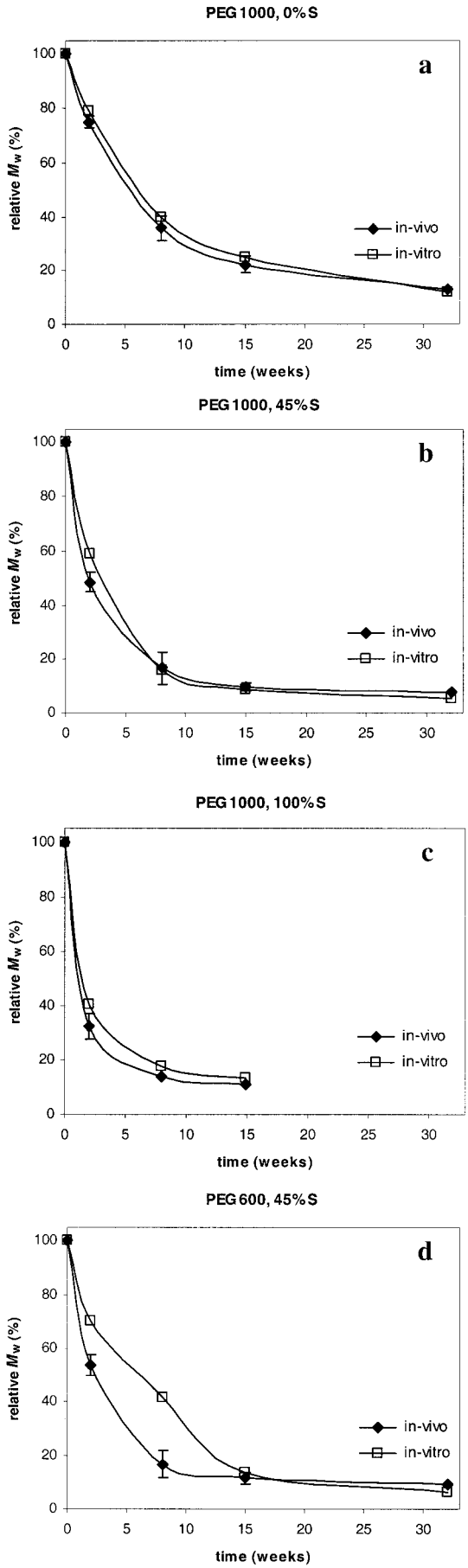


Figure 4. Relative weight average molecular weight of residue as function of degradation time *in vivo* for PEG(T/S)65PB(T/S)35 copolymers containing 45 mol % (a) and 100 mol % (b)of succinate with PEG segments of 600 (♦), 1000 (□), and 4000 (▲) g/mol (n = 3; ± SD).

more susceptible to hydrolysis than aromatic ester, as a result of steric effects[30,31] and flexibility of the chain backbone.[32,35] The order of degradation rates for copolymers containing various PEG segment lengths is explained by both the swelling and the average block length (Fig. 4). Based on the swelling, the highest degradation rate would be expected for the 4000 g/mol PEG segment containing poly(ether-ester)s as, at a constant amount of succinate, the swelling of the poly(ether-ester) increases with increasing PEG segment length.[1] On the other hand, increasing the molecular weight of the PEG segments at a constant soft/hard ratio will increase the average block length of the hard PB(T/S) segments, resulting in a slower degradation. Apparently, the combination of swelling and hard block length of copolymers with 600 g/mol and 1000 g/mol PEG segments favors degradation compared to copolymers containing 4000 g/mol PEG segments. The order of degradation rates for these copolymers was also observed *in vitro* for dense sheets,[1] although the *in vivo* degradation was faster than expected for the 600 g/mol PEG segment containing copolymer with 100 mol % succinate [Fig. 4(b)]. Being implanted as a powder, the difference in shape,[20] surface area, and size[17,18,36] may increase the sensitivity of the 600PEGS71PBS29 copolymer towards the tissue response.

The relative molecular weights of the porous implants during the *in vitro* and *in vivo* degradation time are compared in Figure 5 for a selection of poly(ether-ester) compositions. Examination of the plots indicated that, for the 1000PEG(T/S)66PB(T/S)34 copolymers, the *in vitro* and *in vivo* curves were very similar in profile and timecourse, independent on the degree of succinate substitution [Fig. 5(a–c)]. The same high correlation was observed for the 4000 g/mol PEG segment containing copolymers (data not shown). Greater variability was observed between *in vitro* and *in vivo* curves for the poly(ether-ester)s with 600 g/mol PEG segments [Fig. 5(d) for 45 mol % succinate]. For most compositions studied, only at early stages of degradation ($t \leq 2$ weeks) was a significant deviation observed between the *in vitro* and *in vivo* curves. The molecular weights of the *in vivo* samples were lower than the values for the *in vitro* samples. After 8 to 15 weeks of degradation, the molecular weights reached a plateau value. In general, the plateau value of the *in vivo* residue was slightly higher than the *in vitro* plateau after 32 weeks of degradation.

The lower molecular weights of the *in vivo* samples at early stages of degradation indicated a slightly faster initial degradation. The faster *in vivo* degradation can be attributed to the tissue response.[12,14] Acute inflammation, due to the injury of implantation, and the foreign body reaction, due to the presence of the implant, induce the migration of cells, like polymorphonuclear leucocytes and macrophages to the implant site.[26,28] Reactive species, like super oxide and hydrogen peroxide, produced by these inflammatory cells can oxidize the poly(ether-ester)s.[37,38] Therefore, the *in vivo* decrease in molecular weight values is not only due to hydrolytic degradation caused by the extracellular fluid, but probably also by the influence of the of the oxygen free radicals and other species generated by the inflammatory cells.[14] In our *in vitro* study in phosphate buffered saline, however, only hydrolysis of the ester bonds occurs resulting in a slightly slower degradation compared to the *in vivo* degradation. For the 600 g/mol PEG segment containing copolymers a relatively fast *in vivo* degradation was observed compared to the *in vitro* results. This might be explained by a higher sensitivity towards chain scission by oxidation of poly(ether-ester)s con-



taining short PEG segments as reported by Deschamps and colleagues.[39]

When the plateau value for the molecular weight has been reached, further degradation results in oligomers, which dissolve and diffuse out of the sample. The higher plateau value of the *in vivo* residue compared to the *in vitro* plateau after 32 weeks of degradation indicates that segments of higher molecular weight are dissolved in body fluids compared to phosphate buffered saline.[16]

Copolymer composition

Table IV gives an overview of the poly(ether-ester) compositions, initially and after 32 weeks of *in vitro* and *in vivo* degradation (SD $<$ 3%). For 1000PEGS67PBS33, no polymer could be retrieved after 32 weeks. The effect of the PEG segment length on the amount of soft segment and the molar percentage of succinate in the hard segment is shown in Figure 6 for copolymers with 65 wt % soft segment and 45 mol % succinate as initial composition. Regarding the soft segment content, no significant difference was observed between the *in vitro* and *in vivo* results. Independent on the PEG segment length or the degree of succinate substitution, the weight percentage of soft segment decreased from approximately 65 wt % to 45 wt % after 32 weeks (Table IV, Fig. 6). The preferential loss of PEG-rich segments during degradation has also been reported for other polymer systems.[33,34,36,40] This phenomenon was ascribed to the primary cleavage of the ester linkages between the PEG[33,34] and the hard segments and subsequently the higher solubility of the PEG containing oligomers.[40,41] Compared to the 50% decrease of the initial PEG content shown for PEG-PLGA-PEG triblock copolymers,[36] however, only a minor decrease in soft segment content was observed in our polymer system. Taking into account the mass loss, as reported elsewhere,[1] later stages of the degradation will most likely not result in an inert residue containing only hard segment.

The succinate content in the hard segment decreased slightly during degradation for copolymers initially containing 45 mol % of succinate [Fig. 6(b)]. Succinic acid is soluble in water (77 g/L), whereas terephthalic acid is practically insoluble in water.[42] It is therefore expected that succinate-containing degradation products show a higher solubility and diffusivity, resulting in a lower residual succinate content. This effect was more pronounced *in vivo*. Preferential

Figure 5. Relative weight average molecular weight of residue as function of *in vitro* and *in vivo* degradation time for 1000PEG(T/S)65PB(T/S)35 copolymers containing 0 (a), 45 (b), and 100 (c) mol % succinate and 600PEG(T/S)62PB(T/S)38 containing 45 mol % succinate (d) (n = 3; ± SD).

TABLE IV
Composition of the Poly(ether-ester)s Implants as Function of Degradation Time

PEG Length (g/mol)	$t = 0$		$t = 32$ Weeks *in vitro*		$t = 32$ Weeks *in vivo*	
	Wt % PEG(T/S)	Mol % Succinate	Wt % PEG(T/S)	Mol % Succinate	Wt % PEG(T/S)	Mol % Succinate
600	62	46	47	44	43	33
1000	62	11	48	14	48	15
1000	66	45	43	40	44	33
1000	69	100	58*	100*	43*	100*
1000	71	0	61	0	62	0
4000	64	46	45	46	48	44

*$t = 15$ weeks.

degradation of glycolic acid units observed in PLGA was also ascribed to difference in hydrophilicity of the monomers and was faster *in vivo*.[16] As discussed before, the solubility in body fluid is apparently higher than in phosphate buffer. The change in hard segment composition is marginal compared to the mass loss[1] and therefore no hard segment will remain in the residue containing terephthalate groups only.

CONCLUSION

The *in vivo* degradation, monitored by the molecular weight and copolymer composition, or porous poly-(ether-ester) implants confirmed the trends as observed before for *in vitro* degradation of dense sheets. Most important, substitution of the aromatic terephthalate units by aliphatic succinate units accelerated the degradation rate of the copolymers. Direct correlation of the *in vivo* and *in vitro* degradation of the porous implants showed a faster initial degradation *in vivo*. Besides hydrolysis, oxidation occurs *in vivo* due to the presence of radicals, produced by inflammatory cells. In addition, the higher molecular weight plateau of the residue found *in vivo* indicated a higher solubility of the oligomers in the extracellular fluid compared to a phosphate buffer. Minor changes in the poly(ether-ester) compositions were noted due to degradation. The amount of soft segment decreased both *in vitro* and *in vivo* due to preferential loss of PEG-rich segments. Microscopically, fragmentation of the porous implants was observed in time. At later stages of degradation, macrophages were observed phagocytozing small polymer particles. In addition, both *in vitro* cytotoxicity studies and histology on *in vivo* samples proved the biocompatibility of the poly(ether-ester)s based on poly(ethylene glycol), butylene terephthalate, and butylene succinate segments.

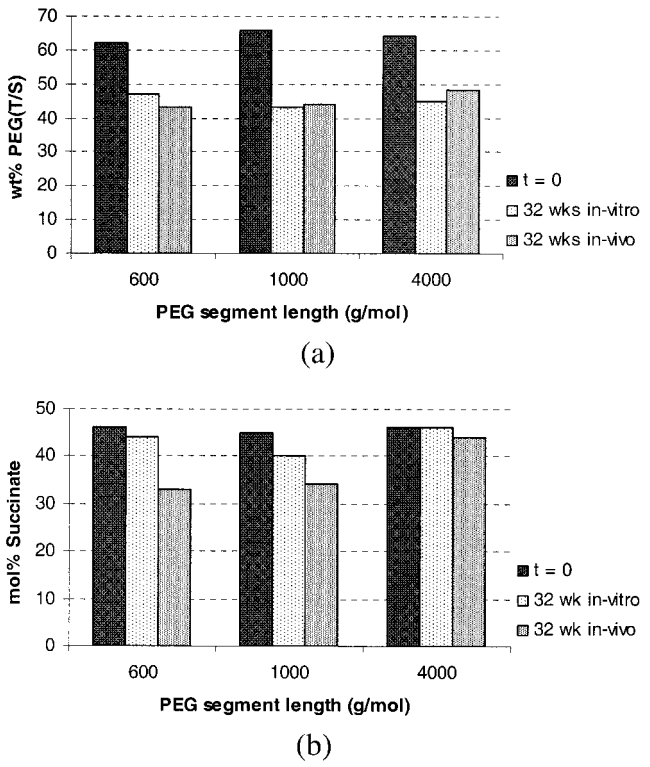


Figure 6. Weight percentage of soft segment (a) and molar percentage of succinate in the hard segment (b) after 32 weeks of degradation *in vitro* and *in vivo* for copolymers with different PEG segment lengths having PEG(T/S)65PB(T/S)35 and 45 mol % of succinate as initial composition.

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