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In vitro and in vivo degradation studies for development of a biodegradable patch based on poly(3-hydroxybutyrate)

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Abstract

For the development of a resorbable gastrointestinal patch, the in vitro degradation of solution-cast films of poly(3-hydroxybutyrate) (PHB), modifications of PHB expected to influence its degradation time, as well a poly(L-lactide) (PLLA) was examined. The molecular weight of pure PHB decreased by one-half after 1 year in buffer solution (pH 7.4, 37°C). Acceleration in molecular weight decrease was observed by blending with atactic PHB, whereas no influence was found with low-molecular weight PHB. Leaching of a water-soluble additive led to a slight acceleration of PHB degradability. In contrast, a deceleration in degradation rate was observed with the addition of a hydrophobic plasticizer. In vitro tests indicated an accelerating effect of pancreatin on PHB degradation, whereas PLLA degradation remained essentially uninfluenced. In comparison to simple hydrolysis, the degradation rate of PHB was accelerated about threefold.

From the in vitro results, a PHB/atactic PHB blend was selected for repair of a bowel defect in Wistar rats. A patch film was fabricated by a dipping/leaching method. Twenty-six weeks post-implantation, material remnants were found in only one of four animals. The bowel defects were closed in all cases. It could be assessed that the patch material resists the intestinal secretions for a sufficiently long time but that it finally degrades completely.

Keywords: Poly(3-hydroxybutyrate) (PHB); Gastrointestinal patch; Polymer degradation; Enzymatic catalysis

1. Introduction

Poly(3-hydroxybutyrate) (PHB), the simplest and most common member of the group of polyhydroxyalkanoates (PHA) can be considered as a polymer with high potential for applications as a degradable implant material [1,2].

PHB is natural thermoplastic polyester and has many mechanical properties comparable to synthetically produced degradable polyesters such as the polylactides [3]. The relatively high brittleness of crystalline PHB is a disadvantage. The mechanical properties of PHB films can be improved by the addition of plasticizers [4,5]. Blends with other degradable polymers also lead to a higher flexibility and elongation at break [6–9].

PHB is a polymer with excellent biocompatibility as evidence by lack of toxicity [10], compatibility in contact with tissue [11–14] and blood [15,16]. The presence of relatively large amounts of low-molecular weight PHB occurring naturally in human blood as well as the fact that the degradation product, 3-hydroxybutyric acid, is a common metabolite in all higher living beings are further evidence for the nontoxicity of implanted PHB [17].

Besides suitable mechanical properties and biocompatibility, use as a temporary implant material requires degradation within clinically reasonable time periods. In vitro degradation studies on PHB films in buffer solution at 37°C showed no mass loss after 180 days, but a decrease in molecular weight started after an induction period of about 80 days [18]. This induction period was attributed to the time required for water to permeate the polymer matrix. It was concluded that the hydrolysis of microbial polyesters proceeds in two steps. First, there is random chain scission both in the

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amorphous and crystalline regions of the polymer matrix accompanied with a decrease in molecular weight with unimodal distribution and of relatively narrow polydispersity. Mass loss begins below a molecular weight M_n of about 13,000 in the second step.

An acceleration of the PHB degradation rate is possible by the addition of polymers or plasticizers. On the one hand, amorphous or hydrophilic additives lead to higher water adsorption and accelerate hydrolysis. For example, the water content was found to be higher in PHB/PDLLA than in PHB/PCL blend [19]. Amorphous atactic (synthetic) PHB degrades faster in comparison with natural PHB and is also suitable as a blend component [20]. On the other hand, leaching of water-soluble additives leads to an increase in polymer surface area, as discussed for PHB and glycerin derivatives [21].

The in vivo degradation (decrease of molecular weight) or resorption (mass loss) of PHB is a controversial subject in the literature. The main reasons for the controversy are the use of samples made by various processing technologies and the incomparability of different implantation and animal models.

Systematic degradation studies of polyesters, among them PHB, were carried out s.c. in the mouse [12]. The molecular weight of PHB was reduced to 57% of the initial value after six months. In another study, PHB samples implanted s.c. and intraperitoneally (i.p.) in the rat showed a fast initial degradation with deceleration of the degradation rate after four weeks [22]. The molecular weight decreased by half, 1 year post-implantation.

Non-woven PHB samples were implanted as transannular patches for enlargement of the right ventricular outflow tract and pulmonary artery [16] and as pericardial patches [11] in sheep. The transannular patches were completely resorbed in 12–24 months postoperatively; the pericardial patches in 24–30 months. In clinical use, PHB pericardial patches showed a significant reduction in size, by an average of 27%, in the 24-month follow-up group [23]. The complete absorption of a PHB pericardial patch, 16 months after implantation in a human, has also been reported [24].

The degradation studies reported here are part of the development of resorbable patches for the gastrointestinal tract [25,26]. Such patches are of interest in covering large open lesions if closure by conventional surgical techniques with sutures or clips is impossible.

The repair of defects in the gastrointestine places some special requirements on the material used for the repair. Besides sealing the tissue defect, the patch material must provide support for tissue regeneration, be resistant to intestinal enzyme attack, not adhere to the surrounding gut and must be flexible and suturable. The material must be able to resist the aggressive secretions of the gastrointestinal tract for the required period of time but degrade in the end. PHB shows promise in fulfulling all these requirements.

The in vitro degradation studies should lead to the choice of a suitable patch material. In addition to pure PHB, PHB with modifications expected to influence its degradation were included in the material screening studies. Such modifications included PHB blended with atactic PHB and low-molecular weight PHB respectively, films with enlarged inner surface area (made by leaching of a water-soluble plasticizer) as well as plasticized PHB. The degradation of these material formulations was compared with PLLA.

The application (defect repair in the bowel) places special requirements on the surface morphology of the patch. One side of the biomaterial should be smooth to prevent cell adhesion, the other side (in contact with the bowel) should have a porous surface in order to support cell adhesion and formation of cicatricial tissue. A new technique for fabrication of these one-sided porous patch materials by a simple dipping/leaching method, without adhesives or thermoplastic fusion, is presented.

2. Materials and methods

2.1. Materials

Poly(3-hydroxybutyrate) (PHB, $M_w = 641,000$, $M_w/M_n = 2.1$) was supplied by Umweltforschungszentrum (Leipzig-Halle, Germany), poly(L-lactide) (PLLA, $M_w = 284,000$, $M_w/M_n = 1.5$) by Boehringer Ingelheim Pharma (Ingelheim, Germany). The plasticizers triethyl citrate (TEC) and butyryltrihexyl citrate (BTHC) were products of Aldrich (Deisenhofen, Germany). Atactic PHB (at-PHB, $M_w = 10,000$, $M_w/M_n = 1.1$) was prepared from β-butyrolactone with potassium acetate as catalyst according to [27], low-molecular weight PHB (dg-PHB, $M_w = 3,000$, $M_w/M_n = 1.5$) was prepared by methanolysis of PHB with sulfuric acid as catalyst according to [28].

2.2. Film fabrication

PHB films were fabricated by dipping a metal core (80 mm length, 32 mm diameter) into a PHB/chloroform (4% w/v) solution. On removal from the solution and drying for a few minutes, the core was inverted and redipped into the solution. This process was repeated to produce film thicknesses up to about 100 μm. The film was stripped from the core and dried in vacuo at 40°C to achieve a chloroform content of $<0.2\%$ as determined by elemental analysis. Films of PHB and at-PHB, dg-PHB, TEC and BTHC, respectively (70/30 in each case) were prepared in a similar fashion from chloroform solutions (5% w/v). The PHB/TEC films were stored in distilled water for 3 days to leach out the plasticizer.

After 3 days in distilled water, TEC was not detectable by NMR analysis (detection limit approximately 0.5% TEC) in the PHB samples. PLLA films were likewise fabricated from chloroform solution (4% w/v). To remove the solvent, the PLLA samples were stored in methanol for one day before drying.

2.3. Patch fabrication

Bioresorbale patch samples with a smooth and a porous surface were prepared from PHB/at-PHB (70/30)–chloroform (5% w/v) solution in two steps. First, a metal core was dipped four times into the solution as described in Section 2.2 to obtain a smooth surface. Then, unsieved NaCl (crystal size 300–500 μm, ten-fold to PHB by weight) was added to the polymer/chloroform solution and the dipping process was repeated six times. The salt crystals provided a rough surface. Finally, the dried PHB film was again removed from the core and incubated in distilled water to dissolve the NaCl leaving a porous surface. Thereafter, the film was dried and analysed as described in Section 2.2. The resultant patch thickness could be modified by the number of dips, the roughness of the porous side by the grain size of the salt crystals to be leached.

2.4. Mechanical testing

Tensile testing on the films was carried out on a Zwick (Type BZ 2.5/TN1S) at room temperature. The gauge length of all samples was 15 mm and the width was 5 mm. The thickness was measured before testing. The crosshead speed was maintained at 5 mm/min. Tensile properties were calculated from the stress–strain curves as means of five measurements.

2.5. Thermal analysis

Thermal analysis was performed using a Perkin Elmer DSC 7 calorimeter in the temperature range 0–220°C at a heating rate of 20°C/min in a nitrogen atmosphere. The mass crystallinity of sample films was approximated using the heat of fusion originate from the first scan compared with that of totally crystalline PHB (146 J/g [29]) or PLLA (93 J/g [30]), respectively.

2.6. Molecular weight analysis

The molecular weight data were obtained at 35°C by using a TSP GPC system with a Shodex RI 71 detector and three PSS SDV 10 μm columns (10^3, 10^5, and 10^6 Å, respectively). The system was combined with a WGE Dr. Bures η1000 viscosity detector. Chloroform was used as eluant at a flow rate of 1 ml/min. The sample concentration was 1.4 mg/ml, and the injection volume 0.1 ml. The molecular weights were calculated by the method of universal calibration.

2.7. In vitro degradation

Each polymer film (10 × 5 mm, about 5 mg) was placed in a test tube containing 4 ml Sørensen buffer (0.1 M, pH 7.4) and kept at 37°C and 70°C respectively. Samples were periodically removed, washed with distilled water and dried in vacuo before analysis.

Pancreatin from porcine pancreas (Sigma, Deisenhofen, Germany, 8 × USP activity) was used at a concentration of 10 mg/ml in Sørensen buffer to which 100 units/ml penicillin, 100 μg/ml streptomycin and 2.5 μg/ml fungizone (Gibco Life Technologies, Karlsruhe, Germany), were added to suppress microbial activity. The enzyme solution was changed twice weekly.

2.8. In vivo degradation

The abdomen of anesthetized male Wistar rats (initial body weight about 250 g) was opened by median laparotomy. A precise defect, 6 mm in diameter, was created in the bowel with a punch. A formaldehyde gas sterilized PHB/at-PHB patch (10 × 10 mm) was placed over the defect and secured with sutures (polypropylene, Ethicon, USA). The porous side of the patch faced the interior of the bowel. After time periods of 1, 2, 8 and 26 weeks, the rats were sacrificed and the test samples explanted.

3. Results and discussion

3.1. Mechanical properties

The mechanical properties of the polymer films studied are shown in Table 1. Since gas sterilization has a slight affect on the mechanical properties of these polymers, tensile testing was performed after sterilization. The mechanical properties obtained on sterilized samples were used as pre-implant values.

Solution cast PHB films exhibited the expected high brittleness and low flexibility. A plasticizing effect was observed by addition of amorphous atactic PHB (at-PHB) accompanied by a decrease of elastic modulus and an increase in elongation at break. At-PHB could be of interest as a plasticizer for PHB because it has advantages, such as reduced migration in an aqueous environment and on thermal treatment, in comparison with low-molecular weight plasticizers.

The addition of chemically degraded PHB (dg-PHB) had little influence on the mechanical properties of the resultant PHB films. While self-supporting films of the low-molecular weight dg-PHB were not available, PHB films blended with dg-PHB were easily fabricated.

Table 1
Mechanical properties and degree of crystallinity of the polymer films

Sample	Elastic modulus (MPa)	Tensile strength (MPa)	Elongation at break (%)	Degree of crystallinity (%)
PHB	3670±210	36±2	1±0	69
PHB/at-PHB	1500±70	23±1	6±2	49
PHB/dg-PHB	3640±300	29±2	1±0	65
PHB/(TEC)	2990±60	27±0	1±0	65
PHB/BTHC	1060±60	12±1	28±11	52
PLLA	2380±220	77±3	8±4	41

Tensile properties including standard deviations were calculated from the stress–strain curves as means of five measurements. The mass crystallinity of sample films was approximated using DSC measurements (error ±2%).

Citric acid esters are biocompatible plasticizers [31] and were tested successfully in degradable polymers such as PHB-HV [32]. The addition and leaching of water-soluble triethyl citrate (TEC) was carried out to fabricate PHB films with an enlarged internal surface area. The material thus obtained showed only little differences in mechanical properties in comparison with unmodified PHB. Butyryltrihexyl citrate (BTHC) was selected for plasticization of PHB films. BTHC, a water-insoluble plasticizer should guarantee that the mechanical properties of the PHB/BTHC films do not deteriorate in aqueous environment due to leaching of the plasticizer. As expected however, the BTHC addition led to a decrease of the elastic modulus and to an increase of elongation at break.

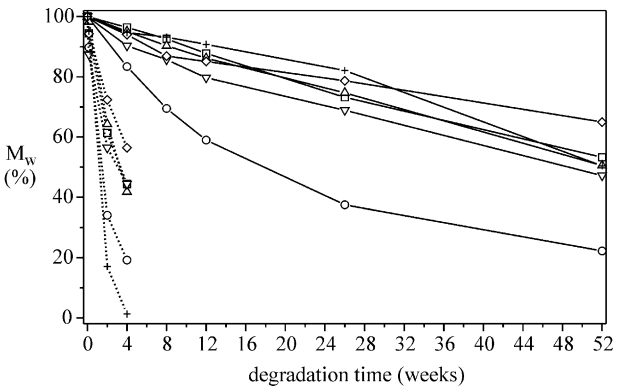

Fig. 1. Changes in molecular weight (M_w, in %) in phosphate buffer (pH 7.4) at 37°C (—) and 70°C (···); (□) PHB, (○) PHB/at-PHB, (△) PHB/dg-PHB, (▽) PHB/(TEC), (◇) PHB/BTHC, (+) PLLA. All data points are means of five measurements. Standard deviations for samples PHB, PHB/at-PHB, PHB/dg-PHB, PHB/(TEC), PHB/BTHC, and PLLA were, respectively, ±1.7%, 1.8%, 3.0%, 3.1%, 3.2%, and 1.4% (4 weeks); ±1.6%, 1.1%, 2.1%, 1.1%, 2.9%, and 1.1% (8 weeks); ±2.8%, 0.7%, 2.3%, 3.3%, 4.4%, and 1.6% (12 weeks); ±2.5%, 0.6%, 1.0%, 1.2%, 1.5%, and 1.0% (26 weeks); and ±1.7%, 0.3%, 0.6%, 1.6%, 1.0%, and 0.5% (52 weeks) in the 37°C test; and ± 3.8%, 3.1%, 2.0%, 3.1%, 1.0%, and 0.2% (1 day); ± 1.0%, 0.8%, 2.4%, 1.3%, 1.6%, and 2.4% (14 days); and ± 2.2%, 1.0%, 0.4%, 1.1%, 1.5%, and 0.1% (28 days) in the 70°C test.

3.2. In vitro degradation

The in vitro degradation tests carried out in phosphate buffer (pH 7.4, 37°C) confirmed the slow degradation of pure PHB (Fig. 1). A continual drop in molecular weight (M_w and M_n respectively) was observed without the characteristic induction period described by Doi et al. [18]. There is, perhaps, a slight acceleration of degradation after about 8–12 weeks which would be in accordance with the reported induction time of about 80 days. The overall changes in M_w followed first-order kinetics with a half-life of about 56 weeks. The molecular weight distribution was unimodal over the whole degradation time (data not shown) which, together with the observed first-order kinetics, indicates a random chain scission both in the crystalline and the amorphous regions of PHB [18].

PHB films with modifications expected to influence film hydrolysis were included in the in vitro studies. Bimodal molecular weight distributions were found for PHB/at-PHB and PHB/dg-PHB, respectively. In the case of PHB/at-PHB the peak of natural PHB was separated by dropping the perpendicular in the minimum of the overlapped curves of natural and atactic parts of PHB. In the case of PHB/dg-PHB the overall peak was analyzed. This blend was intended to represent natural configurated PHB with enriched low-molecular weight part.

The addition of atactic PHB led to an accelerated degradation of natural PHB both at 37°C and 70°C. In PHB/at-PHB blend, an increased water uptake which supports the hydrolysis has to be considered due to the amorphous regions being enlarged and an overall crystallinity decrease (see Table 1) [6,8]. Therefore, addition of at-PHB seems to be an effective method, if acceleration of the degradation of natural PHB is desired. It is known that at-PHB degrades faster than natural PHB [20,33]. The hydrolysis of at-PHB is also a two-step process. First, random chain scission proceeds accompanied by a molecular weight decrease. Then, at a molecular weight of about 10,000, mass loss begins [20]. Unfortunately, molecular weights of the atactic

component in the PHB/at-PHB blend could not be determined in this study with satisfactory precision.

In contrast, PHB hydrolysis was not accelerated by the addition of pre-degraded PHB. The low-molecular weight polymer (dg-PHB which is made by the methanolysis of natural PHB) prevents water uptake of the film due to its high degree of crystallinity (see Table 1).

A slight increase of the degradation of PHB films at 37°C was observed after leaching of the water-soluble plasticizer TEC. This effect could be explained by an increase of internal surface area. Similar examples are known from the literature [21]. Possibly the effect is only an initial one considering the results of the accelerated test at 70°C (see Fig. 1).

The hydrolysis of PHB was decelerated by plasticization with BTHC. Although BTHC is practically water-insoluble, a partial leaching, possibly due to hydrolysis as described for other citrate esters [32], was observed during incubation in buffer solution. NMR studies showed a BTHC loss of 16% after 12 weeks. This value is almost identical with the mass loss (18%, Table 2). Accordingly, the observed mass loss of PHB/BTHC films can be attributed to the loss of plasticizer. The resulting surface enlargement could have led to the slight initial acceleration of the degradation process observed at 37°C. After the initial decrease, the BTHC content remained essentially constant. It has to be assumed that the hydrophobic character of the remaining BTHC leads to a decreased water uptake of the film and retards hydrolysis.

While PLLA showed a slower initial hydrolysis than PHB samples at 37°C, a much faster degradation was observed at 70°C. This seemingly contradictory behavior may be easily explained by assuming an induction period for PLLA hydrolysis characterized by an initial decelerated rate at 37°C which was not seen in the accelerated test. That would be in accordance with observations on PHB [18] as mentioned above. Delayed molecular weight decrease has been reported for PLLA, as well [34]. On the other hand, orders in degradation times of various polymers or polymer compositions obtained from accelerated tests cannot be reliably transferred to the 37°C state. With first-order kinetics, differences in Eyring activation enthalpies and entropies for the ester hydrolysis could play an important role.

No extensive mass loss of the polymer films was found after 1 year in buffer solution (Table 2). As already mentioned, the mass loss of PHB/BTHC films could be attributed to leaching of plasticizer. Slight mass loss was observed in PHB/at-PHB (7%) and PHB/(TEC) (3%) samples.

3.3. In vitro degradation in presence of pancreatin

In vitro degradation of polymer films in the presence of pancreatin was carried out to examine the possible participation of enzymes of the gastrointestinal tract in the degradation process (Fig. 2). Porcine pancreatin contains a mixture of enzymes, including lipase, amylase, α-chymotrypsin, trypsin and protease. There are only few reports on this topic in the literature. For example, microcapsules made of a PHB-HV/PCL (80/20) blend showed accelerated mass loss with bulk and surface erosion after 30 days in pancreatin solution which was attributed to the effects of enzyme activity over and above simple ester hydrolysis [35]. With microbial lipases, on the other hand, PHB was not degraded [36] and the mass loss of plasticized PHB films was attributed solely to hydrolysis of the glycerin plasticizer [21]. It has been noted that a Ser··His··Asp triad constitutes the active center of the catalytic domain of both PHB depolymerase [37] and pancreatic lipase [38]. The serine is part of the pentapeptide Gly X_1-Ser-X_2-Gly, which has been located in all known PHB depolymerases as well as lipases, esterases and serine proteases [37].

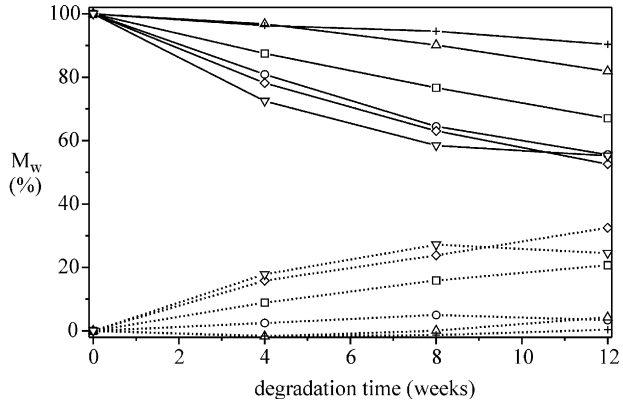

Fig. 2. Changes in molecular weight (M_w, in %) in phosphate buffer (pH 7.4, 37°C) in presence of pancreatin (—); for illustration of the accelerating effect of pancreatin on PHB degradation the difference of the corresponding values of M_w (in %) of hydrolysis in absence and in presence of pancreatin is shown (···); symbols as in Fig. 1. All data points are means of five measurements. Standard deviations for samples PHB, PHB/at-PHB, PHB/dg-PHB, PHB/(TEC), PHB/BTHC, and PLLA were, respectively, ±1.6%, 1.2%, 4.1%, 3.6%, 1.7%, and 1.1% (4 weeks); ±2.5%, 1.2%, 3.2%, 1.7%, 2.5%, and 1.6% (8 weeks); and ±1.5%, 1.2%, 5.5%, 1.5%, 0.3%, and 1.2% (12 weeks).

Generally, with pancreatin addition no additional mass loss was observed in all samples in comparison with simple hydrolysis. The degradation rate, obtained from the decrease in M_w of pure PHB however, was accelerated about threefold. This observation is in contrast to enzymatic degradation by PHB depolymerases which was reported to proceed on the surface of the polymer film accompanied by a decreasing thickness and mass with time, but with an almost unchanged molecular weight of the bulk up to an weight loss of about 70% [39]. Only in the last stage of degradation

Table 2
Changes in film mass (in %) in phosphate buffer (pH 7.4, 37°C)

Sample	Degradation time (weeks)					
	0	4	8	12	26	52
PHB	100	100	99	100	100	99
PHB/at-PHB	100	98	97	97	97	93
PHB/dg-PHB	100	99	99	99	100	99
PHB/(TEC)	100	98	99	97	97	97
PHB/BTHC	100	87	80	82	84	84
PLLA	100	100	100	100	99	100

All data points are means of five measurements. Standard deviations were maximum ±1.0% (PHB, PHB/at-PHB, PHB/dg-PHB, PLLA), ± 2.0% (PHB/(TEC)), and ±3.0% (PHB/BTHC).

does the erosion mechanism change from surface to bulk degradation with more dominantly chemical hydrolysis supported by enhanced porosity and therefore water penetration [40]. However, a simultaneous loss of molecular weight and mass of PHB samples in microbial environment has also been reported [41]. It has been proposed that for depolymerases the relative size of the enzyme compared with the void space in solvent cast films is the limiting factor for diffusion into the polymer matrix [42]. This conclusion can certainly be transferred to nonspecific enzymes. On the other hand, secondary effects caused by enhanced enzymatic activity can play an important role, especially under in vitro conditions [43]. Therefore, it is uncertain if the degradation behavior observed in this study is due to enzymatic catalysis.

In contrast to pure PHB, the molecular weight of PHB/at-PHB blend was essentially unaffected by the presence of enzymes. On the one hand, a higher mobility of the amorphous phase should accelerate enzymatic attack as discussed for PHB depolymerases [44,45]. PHB/at-PHB blends should correspondingly show a faster degradation since the PHB chains are more mobile in the amorphous phase as indicated by lowered glass transition temperatures [8]. On the other hand, the enzymatic degradability of at-PHB could be of importance. Since PHB and at-PHB are miscible and no phase separation occurs [6], in the case of a lower enzymatic degradability of at-PHB the degradation of the blends should be retarded [7]. Unfortunately, the molecular weights of the at-PHB component could not be precisely determined in this study, so that no separation between simple chemical and enzymatic hydrolysis was possible.

Films with addition of low-molecular weight PHB were only slightly influenced by pancreatin within 12 weeks of hydrolysis. The cause could be the high degree of crystallinity and loss of amorphous phase mobility.

PHB films degraded much faster with the addition of plasticizer, with or without subsequently leaching. In TEC leached films it could be assumed that the enlargement of inner surface area supports the enzymatic attack as found for depolymerases [21,46]. In the case of PHB/BTHC films a partial leaching of BTHC was observed which should also have led to an enlarged surface area. NMR investigations yielded a BTHC content of 14% after 12 weeks incubation in pancreatin solution. Two additional effects reported for depolymerases could be of importance. It was concluded that enzymatic PHB degradation is influenced by (a) amorphous phase mobility and (b) polymer hydrophobicity [47]. Accordingly, plasticization should lead to an accelerated polymer degradation [48]. On the other hand, BTHC as hydrophobic additive could affect the overall PHB film hydrophobicity.

While the degradation of PHB was accelerated in presence of pancreatin, PLLA remained almost uninfluenced. After 12 weeks the molecular weight of PHB films decreased to 88% in simple buffer and 67% in pancreatin solution, for PLLA films the corresponding values are 91% and 90%, respectively. The differences

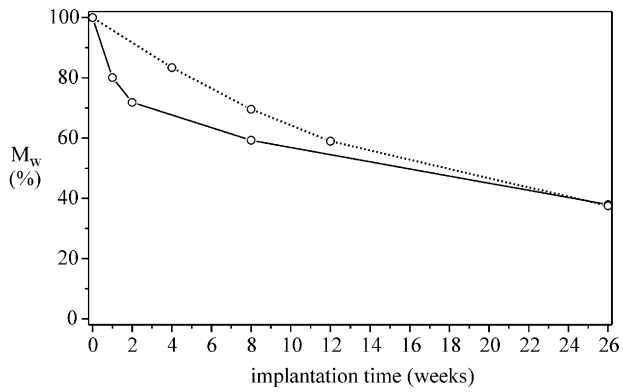

Fig. 3. Changes in molecular weight (M_w, in %) of PHB/at-PHB patches after closure of a rat bowel defect (—) and comparison with in vitro degradation in phosphate buffer (pH 7.4, 37°C) (···). The in vivo data points at 1, 2, and 8 weeks, respectively, are means of two measurements. Standard deviations were, respectively, ±0.3%, 0.8%, and 1.2%. The data point at 26 weeks was obtained from one measurement.

between the two polymers could be explained considering the more rubber-like characteristics of PHB and the glassy state of PLLA as evidenced by their glass transition temperatures [43,45]. Moreover, the individual energetic and steric conditions are also of importance for polymer-enzyme interactions. However, the main reason could be the difference in the origins of PHB and PLLA (biological vs. synthetic).

3.4. In vivo degradation

The in vivo degradation study (Fig. 3) is a part of an ongoing study of defect closure in the rat gastrointestine by a PHB/at-PHB patch. This material combination was selected on the basis of its mechanical properties and degradation behavior. A patch film was fabricated by a dipping/leaching method as described in Section 2.3.

The molecular weight analysis showed a fast initial degradation followed by a deceleration after two weeks. It should be noted that this time period corresponds with the reported observation of mRNA encoding pancreatic enzymes after implantation of PHB patches onto the gastric wall of rats [49]. Twenty-six weeks post-implantation material pieces were found only in one of the four test animals. The bowel defects were closed in all cases. On macroscopic examination, the retrieved patch remnant appeared heavily decomposed with fragmentation and partial dissolution. The explanted remnant material had a molecular weight of about 38% of the initial value. On the basis of this degradation behavior it can be concluded that the temporary patch material based on PHB resists the intestinal secretions for a sufficiently long time but finally does degrade completely.

4. Conclusions

The in vitro degradation of solution-cast films of pure and modified PHB as well as PLLA was examined in buffer solution of pH 7.4. The relatively slow hydrolysis of PHB with a half-life in molecular weight loss of about 1 year at 37°C is accelerated by addition of atactic PHB, whereas no influence on degradability is found with the addition of low-molecular weight PHB. Leaching of water-soluble TEC led to a slight acceleration of PHB degradability, but possibly only at the initial stage of hydrolysis. In contrast, deceleration of degradability is observed with the hydrophobic plasticizer BTHC. PLLA hydrolysis at 37°C starts with a decelerated rate but nevertheless faster degradation in comparison to PHB.

In vitro studies with the presence of pancreatin indicate a participation of enzymes on PHB hydrolysis, whereas the degradation characteristics of PLLA remain essentially the same. In comparison to simple hydrolysis, the degradation rate of PHB is accelerated about threefold. However, enzymatic catalysis is questionable due to the observed degradation behavior with molecular weight loss but constant mass. Possibly, secondary effects caused by enhanced enzymatic activity are of importance.

For repair of bowel defects a patch was developed and implanted in Wistar rats. PHB/atactic PHB was selected as most promising material combination because of its mechanical properties and in vitro degradation behavior. The material is sufficiently flexible to adapt and to be sutured over the defect. Its degradation characteristics are such that the temporary patch material resists the intestinal secretions and closes the bowel defect for a sufficiently long time to allow complete healing of the defect.

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