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Biodegradation and Medical Application of Microbial Poly(3-Hydroxybutyrate)

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This review is designed to be a comprehensive source for biodegradable polymer: poly(3-hydroxybutyrate)—PHB research, including fundamental structure/property relationships and biodegradation kinetics for samples of different geometry. In addition to presenting the scientific framework for the advances in PHB research, this review focuses on applications of PHB in biomedicine and environment with a discussion on commercial applications and health/safety concerns for biodegradable materials.

Keywords Applications; biocompatibility; biodegradation in soil; degradation in animal tissues; enzymatic degradation; microbial poly(3-hydroxybutyrate); nonenzymatic hydrolysis

1. Introduction

Over last decade an intense development of biomedical application of microbial poly((R)-3-hydroxybutyrate) (PHB) in producing of biodegradable polymer implants and controlled drug release systems needs for comprehensive understanding of the PHB biodegradation process [1–3]. Examination of PHB degradation process is also necessary for development of novel friendly environment polymer packaging [4–6]. It is generally accepted that biodegradation of PHB both in living systems and in environment occurs via enzymatic and non-enzymatic processes that take place simultaneously under natural conditions. It is, therefore, important to understand both processes [1,7]. Opposite to PGA and PLGA, PHB is considered to be moderately resistant to degradation in vitro as well as to biodegradation in animal body. The rates of degradation are influenced by the characteristics of the polymer, such as chemical composition, crystallinity, morphology and molecular weight [8,9]. In spite of that PHB application in vitro and in vivo has been intensively investigated, the most of the available data are often incomplete and even contradictory. The presence of conflicting data can be partially explained by the fact that biotechnologically produced

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PHB with standardized properties is relatively rare and is not readily available due to a wide variety of PHB biosynthesis sources and different PHB manufacturing processes.

Above contradictions can be also explained by excess applied trend in PHB degradation research. At most of the papers observed in this review PHB degradation process has been investigated in the narrow framework of development of specific medical device based on PHB. Depending on applied biomedical purposes biodegradation of PHB was investigated under different geometry: films and plates with various thickness [10–13], cylinders [13–16], monofilament threads [17,18] and microspheres [19]. At these experiments PHB was used from various sources, with different molecular weights and crystallinities. Besides, different technologies of PHB devices manufacture affect such important characteristics as polymer porosity and surface structure [11,12]. Reports regarding the complex theoretical research of mechanisms of hydrolysis, enzymatic degradation and biodegradation in vivo of PHB processes are relatively rare [10,11,13,20–22] that confers great value and importance to these investigations. Nevertheless, the effect of thickness, size and geometry of PHB device, molecular weight and crystallinity of PHB on the mechanism of PHB hydrolysis and biodegradation was not well clarified yet.

1.1. Nonenzymatic Hydrolysis of PHB In Vitro

Examination of hydrolytic degradation of natural poly((R)-3-hydroxybutyrate) in vitro is a very important step for understanding of PHB biodegradation. There are several very profound and careful examinations of PHB hydrolysis that have been carried out 10–15 years [20–23]. Hydrolytic degradation of PHB was usually examined under standard experimental conditions simulating internal body fluid: in buffered solutions with pH = 7.4 at 37°C but at some cases the higher temperature (55°C, 70°C and more) and other values of pH (from 2 to 11) were selected.

The classical experiment for examination of PHB hydrolysis in comparison with hydrolysis of other widespread biopolymer, such as polylactic acid (PLA), was carried out by Koyama N. and Doi Y. [20]. They selected films (10 × 10 mm size, 50 μm thickness, 5 mg initial mass) from PHB ($M_n = 300$ kDa, $M_w = 650$ kDa) and polylactic acid ($M_n = 9$ kDa, $M_w = 21$ kDa) prepared by solvent casting and aged for 3 weeks to reach equilibrium crystallinity. It was shown that hydrolytic degradation of natural PHB is very slow process. The mass of PHB film remained unchanged at 37°C in 10 mM phosphate buffer (pH = 7.4) over a period of 150 days, while the mass of the PLA film rapidly decreased in time and reached 17% of the initial mass after 140 days. The rate of decrease in the M_n of the PHB was also much slower than the rate of decrease in the M_n of PLA. The M_n of the PHB decreased approximately to 65% of the initial PHB M_n after 150 days, while the M_n of the PLA decreased to 20% (2 kDa) of the initial PLA M_n at the same time interval. As PLA used at this research was with low molecular weight we should compare this data with the data of investigation of hydrolysis of PLA with the same molecular weight as observed PHB. We examined rates of in vitro mass loss of polymer films with the same thickness (40 μm) from PLA and PHB with the same molecular weight ($M_w = 450$ kDa). It was shown that the mass of PLA film decreased to 87%, whereas the mass of PHB film remained unchanged at 37°C in 25 mM phosphate buffer (pH = 7.4) over a period of 84 days [24,25].

The cleavage of polyester chains is known to be catalysed by the carboxyl end groups, and its rate is proportional to the concentrations of water and ester bonds that may be constant during the hydrolysis due to the presence of a large excess of water molecules and of ester bonds of polymer chains. Thus, the kinetics of nonenzymatic hydrolysis can be

expressed by the following equation [26,27]:

$$\ln M_n = \ln M_n^0 - kt \quad (1)$$

where M_n and M_n^0 are the number-average molecular weights of a polymer component at time t and zero, respectively.

The average number of bond cleavage per original polymer molecule, N , is given by Equation 2:

$$N = (M_n^0/M_n) - 1 = k_d P_n^0 t, \quad (2)$$

where k_d is the rate constant of hydrolytic degradation, and P_n^0 is the number-average degree of polymerization at time zero. Thus, if the chain scission is completely random, the value of N is linearly dependent on time.

The molecular weight decrease with time is the distinguishing feature of mechanism in nonenzymatic hydrolysis conditions in contrast to enzymatic hydrolysis conditions of PHB when M_n values remained almost unchanged. It was supposed also that water-soluble oligomers of PHB may accelerate chain scissions of PHB homopolymer [20]. In contrast, Freier T. et al. [11] showed that PHB hydrolysis was not accelerated by the addition of pre-degraded PHB: the rate of mass and M_w loss of blends (70/30) from high-molecular PHB ($M_w = 641$ kDa) and low-molecular PHB ($M_w = 3$ kDa) was the same with degradation rate of pure high-molecular PHB. Meanwhile, the addition of amorphous atactic PHB (atPHB) ($M_w = 10$ kDa) to blend with high-molecular PHB caused significant acceleration of PHB hydrolysis: the mass loss of PHB/atPHB blends was 7% in comparison with 0% mass loss of pure PHB, the decrease of M_w was 88% in comparison with 48% M_w decrease of pure PHB [11,28]. We have showed that the rate of hydrolysis of PHB films depends on M_w of PHB. The films from PHB of high molecular weight (450 and 1000 kDa) degraded slowly as it was described above whereas films from PHB of low molecular weight (150 and 300 kDa) lost weight relatively gradually and more rapidly [24,25].

To enhance the hydrolysis of PHB a higher temperature was selected for degradation experiments: 55°C, 70°C and more [20]. It was showed by the same research team that the weights of films (12 mm diameter, 65 μm thick) from PHB ($M_n = 768$ and 22 kDa, $M_w = 1460$ and 75 kDa) were unchanged at 55°C in 10 mM phosphate buffer (pH = 7.4) over a period of 58 days. The M_n value decreased from 768 to 245 kDa for 48 days. The film thickness increased from 65 to 75 μm for 48 days, suggesting that water permeated the polymer matrix during the hydrolytic degradation. Examination of the surface and cross-section of PHB films before and after hydrolysis showed that surface after 48 days of hydrolysis was apparently unchanged, while the cross-section of the film exhibited a more porous structure (pore size < 0.5 μm). It was shown also that the rate of hydrolytic degradation is not dependent upon the crystallinity of PHB film. The observed data indicates that the nonenzymatic hydrolysis of PHB in the aqueous media proceeds *via* a random bulk hydrolysis of ester bonds in the polymer chain films and occurs throughout the whole film, since water permeates the polymer matrix [20,21]. Moreover, as the molecular weight distribution was unimodal over the whole degradation time which, together with the observed first-order kinetics, indicates a random chain scission both in the crystalline and the amorphous regions of PHB [11,29]. For synthetic amorphous atactic PHB it was shown that the hydrolysis of PHB is a two-step process. Firstly, the random chain scission proceeds. The scission accompanies by a molecular weight decrease. Then, at a molecular weight of about 10000, mass loss begins [23].

Table 1. Nonenzymatic hydrolysis of PHB in vitro

Type of device	Initial M_w of PHB, kDa	Size/ Thickness, μm	Conditions: t, $^{\circ}\text{C}/\text{pH}$	Relative mass loss of PHB, %	Relative decrease of PHB M_w , %	Time, days	Links
film	650	50	37/7.4	0	35	150	20
film	640	100	37 $^{\circ}\text{C}$, pH = 7.4	0	64	730	12
film	640	100	37 $^{\circ}\text{C}$, pH = 7.4	0	45	364	11
film	450	40	37 $^{\circ}\text{C}$, pH = 7.4	0	42	84	24–25
film	150	40	37 $^{\circ}\text{C}$, pH = 7.4	12	63	84	24–25
film	279	—	37 $^{\circ}\text{C}$, pH = 7.4	7.5	—	50	31
plate	—	500	37 $^{\circ}\text{C}$, pH = 7.4	3	—	40	30
plate	380	1000	37 $^{\circ}\text{C}$, pH = 7.4	0	—	28	37
plate	380	2000	37 $^{\circ}\text{C}$, pH = 7.4	0	8	98	32
thread	470	30	37 $^{\circ}\text{C}$, pH = 7.0	0	—	180	18
thread	—	—	37 $^{\circ}\text{C}$, pH = 7.2	0	—	182	17
micro-spheres	50	250–850	37 $^{\circ}\text{C}$, pH = 7.4	0	0	150	36
thread	470	30	37 $^{\circ}\text{C}$, pH = 5.2	0	—	180	17
film	279	—	37 $^{\circ}\text{C}$, pH = 10	100	—	28	31
film	279	—	37 $^{\circ}\text{C}$, pH = 13	100	—	19	31
film	650	50	55 $^{\circ}\text{C}$, pH = 7.4	0	68	150	20
plate	380	2000	55 $^{\circ}\text{C}$, pH = 7.4	0	61	98	32
film	640	100	70 $^{\circ}\text{C}$, pH = 7.4	—	55	28	11
film	150	40	70 $^{\circ}\text{C}$, pH = 7.4	39	96	84	24–25
film	450	40	70 $^{\circ}\text{C}$, pH = 7.4	12	92	84	24–25
micro-spheres	50	250–850	85 $^{\circ}\text{C}$, pH = 7.4	50	68	150	36
micro-spheres	600	250–850	85 $^{\circ}\text{C}$, pH = 7.4	25	—	150	36

The analysis of literature data shows a great spread in rate values of PHB hydrolytic degradation in vitro. It can be explained by different thickness of PHB films or geometry of PHB devices used for experiment as well as by different sources, purity degree and molecular weight of PHB (Table 1). At 37 $^{\circ}\text{C}$ and pH = 7.4 the weight loss of PHB (unknown M_w) films (500 μm thick) was 3% after 40 days incubation [32], 0% after 52 weeks (364 days) and after 2 years (730 days) incubation (640 kDa PHB, 100 μm films) [11,12], 0% after 150 days incubation (650 kDa PHB, 50 μm film) [20], 7.5% after 50 days incubation (279 kDa PHB, unknown thickness of films) [31], 0% after 3 months (84 days) incubation (450 kDa PHB, 40 μm films), 12% after 3 months (84 days) incubation (150 kDa PHB, 40 μm films) [24,25], 0% after 180 days incubation of monofilament threads (30 μm in diameter) from PHB (470 kDa) [17,18]. The molecular weight of PHB dropped to 36% of the initial values after 2 years (730 days) of storage in buffer solution [12], to 87% of the initial values after 98 days [32], to 58% of the initial values after 84 days [24,25] (Table 1).

At acidic or alkaline aqueous media PHB degrades more rapidly: 0% after 20 weeks (140 days) incubation in 0.01 NaOH (pH = 11) (200 kDa PHB, 100 μm films) with surface changing [33], 0% after 180 days incubation of PHB threads in phosphate buffer (pH = 5.2 and 5.9) [18], complete PHB films biodegradation after 19 days (pH = 13) and 28 days (pH = 10) [31]. It was demonstrated that after 20 weeks of exposure to NaOH solution,

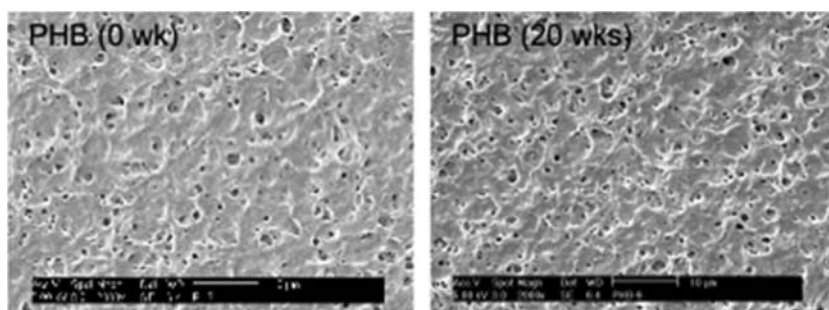


Figure 1. Scanning electron microscopy photographs of PHB films both before (initial sample, panel on the left) and after 20 weeks (panel on the right) of non-enzymatic hydrolysis in 0.01 N NaOH solution (scale bars, 10 μm) [33].

the surfaces of PHB samples became rougher, along with an increased density of whole formation on their surfaces. From these results, it can be supposed that the non-enzymatic degradation of PHAs progresses on their surfaces before noticeable weight loss occurs (Fig. 1) [33].

It was shown also that treatment of PHB film with 1M NaOH caused a reduction in pore size on film surface from 1–5 μm to around 1 μm that indicates a partially surface degradation of PHB in alkaline media [34,35]. At higher temperature no weight loss of PHB films and threads was observed after 98 and 182 days incubation in phosphate buffer (pH = 7.2) at 55°C and 70°C, respectively [17], 12% and 39% of PHB (450 and 150 kDa, respectively) films after 84 days incubation at 70°C [35,40], 50% and 25% after 150 days incubation of microspheres (250–850 μm diameter) from PHB (50 kDa and 600 kDa, respectively) [36].

During degradation of PHB monofilament threads, films and plates in vitro the change of mechanical properties was observed under different conditions [17,37]. It was shown that a number of mechanical indices of threads became worse: load at break lost 36%, strain at break lost 33%, Young's modulus didn't change, tensile strength lost 42% after 182 days incubation in phosphate buffer (pH = 7.2) at 70°C. But at 37°C the changes were more complicated: at first load at break increased from 440 g to 510 g (16%) at 90th day and then decreased to the initial value at 182nd day, strain at break increased rapidly from 60 to 70% (in 17%) at 20th day and then gradually increased to 75% (in 25%) at 182nd day, Young's modulus didn't change [17]. For PHB films it was demonstrated a gradual 32% decrease in Young's modulus and 77% fall in tensile strength during 120 days incubation in phosphate buffer (pH = 7.4) at 37°C [37]. For PHB plates more complicated changes were observed: at first tensile strength dropped in 13% for 1st day and then increased to the initial value at 28th day, Young's modulus dropped in 32% for 1st day and then remain unchanged up to 28th day, stiffness decreased sharply also in 40% for 1st day and then remain unchanged up to 28th day [38].

1.2. Enzymatic Degradation of PHB In Vitro

The examination of enzymatic degradation of PHB in vitro is the next important step for understanding of PHB operation in animal tissues and in environment. The most papers observed degradation of PHB by depolymerases (???) of its own bacterial producers. The degradation of PHB in vitro by depolymerase was thoroughly examined and mechanism

of enzymatic PHB degradation was perfectly clarified by Doi Y. [20,21]. In these early works it was shown that 68–85% and 58% mass loss of PHB ($M_w = 650\text{--}768$ and 22 kDa, respectively) films (50–65 μm thick) occurred for 20 h under incubation at 37°C in phosphate solution (pH = 7.4) with depolymerase (1.5–3 $\mu\text{g/ml}$) isolated from *A. faecalis*. The rate (k_e) of enzymatic degradation of films from PHB ($M_n = 768$ and 22 kDa) was 0.17 and 0.15 mg/h, respectively. The thickness of polymer films dropped from 65 to 22 μm (32% of initial thickness) during incubation. The scanning electron microscopy examination showed that the surface of the PHB film after enzymatic degradation was apparently blemished (???) by the action of PHB depolymerase, while no change was observed inside the film. Moreover, the molecular weight of PHB remained almost unchanged after enzymatic hydrolysis: the M_n of PHB decreased from 768 to 669 kDa or unchanged (22 kDa) [20,21].

The extensive literature data on enzymatic degradation of PHB by specific PHB depolymerases have been collected in detail in review of Sudesh, K., Abe H. and Doi Y. [39]. We summarize some of the most important data. But at first it is necessary to note that PHB depolymerase is very specific enzyme and the hydrolysis of polymer by depolymerase is the unique process. However, in animal tissues and even in environment the enzymatic degradation of PHB is occurred mainly by nonspecific esterases [19,40]. Thus, in the frameworks of this review, it is necessary to observe the fundamental mechanisms of PHB enzymatic degradation.

The rate of enzymatic erosion of PHB by depolymerase is strongly dependent on the concentration of the enzyme. The enzymatic degradation of solid PHB polymer is heterogeneous reaction involving two steps, namely, adsorption and hydrolysis. The first step is adsorption of the enzyme onto the surface of the PHB material by the binding domain of the PHB depolymerase, and the second step is hydrolysis of polyester chains by the active site of the enzyme. The rate of enzymatic erosion for chemosynthetic PHB samples containing both monomeric units of (R)- and (S)-3-hydroxybutyrate is strongly dependent of both the stereocomposition and the tacticity of the sample as well as of substrate specificity of PHB depolymerase. The water-soluble products of random hydrolysis of PHB by enzyme showed a mixture of monomers and oligomers of (R)-3-hydroxybutyrate. The rate of enzymatic hydrolysis for melt-crystallized PHB films by PHB depolymerase decreased with an increase in the crystallinity of the PHB film, while the rate of enzymatic degradation for PHB chains in amorphous state was approximately 20 times higher than the rate for PHB chains in crystalline state. It was suggested that the PHB depolymerase predominantly hydrolyzes polymer chains in the amorphous phase and then, subsequently, erodes the crystalline phase. The surface of the PHB film after enzymatic degradation was apparently blemished by the action of PHB depolymerase, while no change was observed inside the film. Thus, depolymerase hydrolyses of the polyester chains in the surface layer of the film and polymer erosion proceeds in surface layers, while dissolution, the enzymatic degradation of PHB are affected by many factors as monomer composition, molecular weight and crystallinity degree [39].

At the next step it is necessary to observe enzymatic degradation of PHB under the conditions that modeled the animal tissues and body fluids containing nonspecific esterases. In vitro degradation of PHB films in the presence of various lipases as nonspecific esterases was carried out in buffered solutions containing lipases [41,42], in digestive juices (for example, pancreatin) [11], biological media (serum, blood etc.) [18] and crude tissue extracts containing a mixture of enzymes [19] to examine the mechanism of nonspecific enzymatic degradation process. It was noted that a Ser..His..Asp triad constitutes the active center of the catalytic domain of both PHB depolymerase [43] and lipases [44]. The serine

is part of the pentapeptide Gly X1-Ser-X2-Gly, which has been located in all known PHB depolymerases as well as in lipases, esterases and serine proteases [43].

On the one hand, it was shown that PHB was not degraded for 100 days with a quantity of lipases isolated from different bacteria and fungi [41,42]. Moreover, the progressive PHB degradation by lipases was shown [24,25,34,35]. PHB enzymatic biodegradation was studied also in biological media: it was shown that with pancreatin addition no additional mass loss of PHB was observed in comparison with simple hydrolysis [11], the PHB degradation process in serum and blood was demonstrated to be similar to hydrolysis process in buffered solution [24,25], whereas progressive mass loss of PHB sutures was observed in serum and blood: 16% and 25%, respectively, after 180 days incubation [18], crude extracts from liver, muscle, kidney, heart and brain showed the activity to degrade the PHB: from 2% to 18% mass loss of PHB microspheres after 17 h incubation at pH 7.5 and 9.5 [19]. The degradation rate in solution with pancreatin addition, obtained from the decrease in M_w of pure PHB, was accelerated about threefold: 34% decrease in M_w after incubation for 84 days in pancreatin (10 mg/ml in Sorensen buffer) vs. 11% decrease in M_w after incubation in phosphate buffer [11]. The same data were obtained for PHB biodegradation in buffered solutions with porcine lipase addition: 72% decrease in M_w of PHB (450 kDa) after incubation for 84 days with lipase (20 U/mg, 10 mg/ml in Tris-buffer) vs. 39% decrease in M_w after incubation in phosphate buffer [24,25]. This observation is in contrast to enzymatic degradation by PHB depolymerases which was reported to proceed on the surface of the polymer film with an almost unchanged molecular weight [20,21]. 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 [Jesudason J.J. et al., 1993] whereas lipases can penetrate into the polymer matrix through pores in PHB film [34,35]. It was shown that lipase (0.1 g/l in buffer) treatment for 24 h caused significant morphological change in PHB film surface: transferring from native PHB film with many pores ranging from 1 to 5 μm in size into a pore free surface without producing a quantity of hydroxyl groups on the film surface. It was supposed that the pores had a fairly large surface exposed to lipase, thus it was degraded more easily (Fig. 2) [34,35]. It indicates also that lipase can partially penetrate into pores of PHB film but the enzymatic degradation proceeds mainly on the surface of the coarse polymer film achievable for lipase. Two additional effects reported for depolymerases could be of

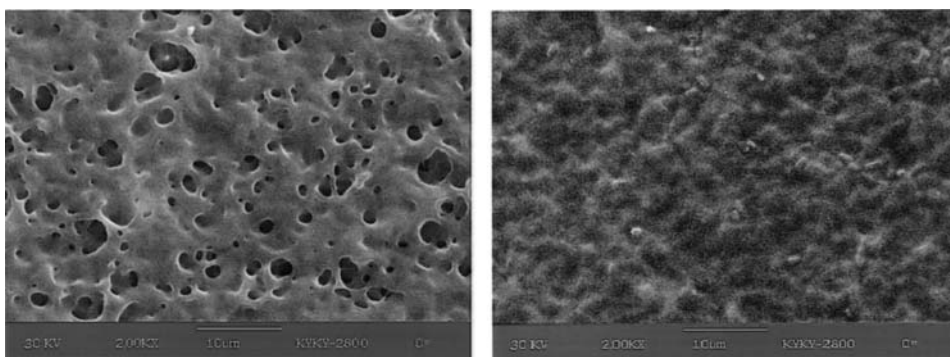


Figure 2. Scanning electron microscopy photographs documented the surface structure of PHB polymer films: (a) PHB film; (b) PHB film treated with lipase (0.1 g/l at 30°C and pH = 7.0 for 24 h) [35].

importance. It was concluded that segmental mobility in amorphous phase and polymer hydrophobicity play an important role in enzymatic PHB degradation by nonspecific esterases [11]. Significant impairment of the tensile strength and other mechanical properties were observed during enzymatic biodegradation of PHB threads in serum and blood. It was shown that load at break lost 29%, Young's modulus lost 20%, and tensile strength did not change after 180 days of threads incubation, the mechanical properties changed gradually [18].

1.3. Biodegradation of PHB by Soil Microorganisms

Polymers exposed to the environment are degraded through their hydrolysis, mechanical, thermal, oxidative, and photochemical destruction, and biodegradation [4,32,45,46]. One of the valuable properties of PHB is its biodegradability, which can be evaluated using various field and laboratory tests. Requirements for the biodegradability of PHB may vary in accordance with its applications. The most attractive property of PHB with respect to ecology is that it can be completely degraded by microorganisms finally to CO_2 and H_2O . This property of PHB allows to manufacture biodegradable polymer objects for various applications (Fig. 3) [2].

The degradation of PHB and its composites in natural ecosystems, such as soil, compost, and bodies of water, was described in a number of publications [2,32,45,46]. Maergaert et al. isolated from soil more than 300 microbial strains capable of degrading PHB in vitro [45]. The bacteria detected on the degraded PHB films were dominated by the genera *Pseudomonas*, *Bacillus*, *Azospirillum*, *Mycobacterium*, and *Streptomyces* etc. The samples of PHB have been tested for fungicidity and resistance to fungi by estimating the growth rate of test fungi from the genera *Aspergillus*, *Aureobasidium*, *Chaetomium*, *Paecilomyces*, *Penicillium*, *Trichoderma* under optimal growth conditions. PHB film did not exhibit neither fungicide properties, nor the resistance to fungal damage, and served as a good substrate for fungal growth [47].



Figure 3. Moulded PHB objects for various applications. In soil burial or composting experiments, such objects biodegrade in about three months [2].

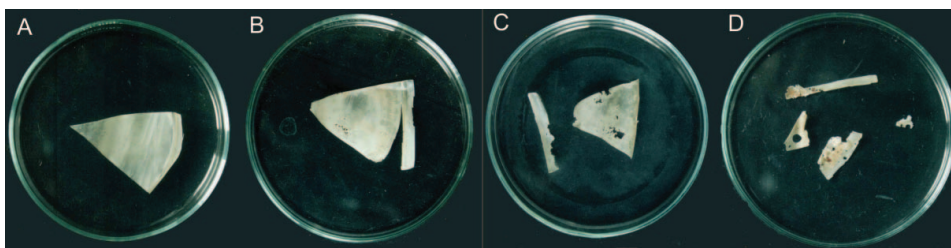


Figure 4. Undegraded PHB film (A) and PHB films with different degrees of degradation after 2 months incubation in soil suspension: anaerobic conditions without nitrate (B), microaerobic conditions without nitrate (C), and microaerobic conditions with nitrate (D) [24,48].

It was studied biodegradability of PHB films under aerobic, microaerobic and anaerobic conditions in the presence and absence of nitrate by microbial populations of soil, sludge from anaerobic and nitrifying/denitrifying reactors, and sediment of a sludge deposit site, as well as to obtain active denitrifying enrichment culture degrading PHB (Fig. 4) [48]. Changes in molecular mass, crystallinity, and mechanical properties of PHB have been studied. A correlation between the PHB degradation degree and the molecular weight of degraded PHB was demonstrated. The most degraded PHB exhibited the highest values of the crystallinity index. As it has been shown by Spyros et al., PHAs contain amorphous and crystalline regions, of which the former are much more susceptible to microbial attack [49]. If so, the microbial degradation of PHB must be associated with a decrease in its molecular weight and an increase in its crystallinity, which was really observed in the experiments. Moreover, microbial degradation of the amorphous regions of PHB films made them more rigid. However, further degradation of the amorphous regions made the structure of the polymer much looser [48].

PHB biodegradation in the enriched culture obtained from soil on the medium used to cultivate denitrifying bacteria (Gil'tai medium) has been also studied. The dominant bacterial species, *Pseudomonas fluorescens* and *Pseudomonas stutzeri*, have been identified in this enrichment culture. Under denitrifying conditions, PHB films were completely degraded for seven days. Both the film weight and M_w of PHB decreased in time. In contrast to the data of Doi et al. [21] who found that M_w of PHB remained unchanged upon enzymatic biodegradation in an aquatic solution of PHB- depolymerase from *Alcaligenes faecalis*, in our experiments, the average viscosity molecular weight of the higher- and lower-molecular polymers decreased gradually from 1540 to 580 kDa and from 890 to 612 kDa, respectively. The “exo”-type cleavage of the polymer chain, i. e. a successive removal of the terminal groups, is known to occur at a higher rate than the “endo”-type cleavage, i. e., a random breakage of the polymer chain at the enzyme-binding sites. Thus, the former type of polymer degradation is primarily responsible for changes in its average molecular weight. However the “endo”-type attack plays the important role at the initiation of biodegradation, because at the beginning, a few polymer chains are oriented so that their ends are accessible to the effect of the enzyme [50]. Biodegradation of the lower-molecular polymer, which contains a higher number of terminal groups, is more active, probably, because the “exo”-type degradation is more active in lower than in higher molecular polymers [48,51].

1.4. Biodegradation of PHB In Vivo in Animal Tissues

The first scientific works on biodegradation of PHB in vivo in animal tissues were carried out 15–20 years ago by Miller N.D. et al. and Saito T. et al. [17,19]. They are high-qualitative researches that disclosed many important characteristics of this process. As it was noted above the both enzymatic and non-enzymatic processes of biodegradation of PHB in vivo can occur simultaneously under normal conditions. But it does not mean that polymer biodegradation in vivo is a simple combination of non-enzymatic hydrolysis and enzymatic degradation. Moreover, the in vivo biodegradation (decrease of molecular weight and mass loss) of PHB) is a controversial subject in the literature. As it was noted above for in vitro PHB hydrolysis, the main reason for the controversy is the use of samples made by various processing technologies and the incomparability of different implantation and animal models. Most of researches on PHB biodegradation was carried out with prototypes of various medical devices based on PHB: solid films and plates [10,13,24,52], porous patches [11,12], nonwoven patches consisted of fibers [53–57], screws [24], cylinders as nerve guidance channels and conduits [13,15,16], monofilament sutures [17,18], microspheres [19,58]. In vivo biodegradation researches were carried out on various laboratory animals: rats [11,16–19,24], mice [13,58], rabbits [10,52,59], minipigs [12], cats [15], calves [53], sheep [54–56], patients [57]. It is obviously that these animals differ in level of metabolism very much: for example, only weight of these animals differs from 10–20 g (mice) to 50 kg (calves). The implantation of devices from PHB was carried out through different ways: subcutaneously [10,13,17,18,24,59], intraperitoneally on a bowel [11], subperiostally on the osseus skull [12,52], nerve wrap-around [14–16], intramuscularly [58,59], into the pericardium [54–57], into the atrium [53] and intravenously [19]. The terms of implantation were also different: 2.5 h, 24 h, 13 days, 2 months [19]; 7, 14, 30 days [16], 2, 7, 14, 21, 28, 55, 90, 182 days [17]; 1, 3, 6 months [10,13,14]; 3, 6, 12 months [53]; 6, 12 months [15]; 6, 24 months [57]; 3, 6, 9, 12, 18, 24 months [56] (Table 2).

The most comprehensive study on PHB in vivo biodegradation was fulfilled by Gogolewski S. et al. and Qu X.-H. et al. [10,13]. It was shown that PHB lost about 1.6% (injection-molded film, 1.2 mm thick, M_w of PHB = 130 kDa) [13] and 6% (solvent-casting film, 40 μ m thick, M_w = 534 kDa) [10] of initial weight after 6 months of implantation. But the observed small weight loss was partially due to the leaching out of low molecular weight fractions and impurities present initially in the implants. The M_w of PHB decreased from 130 kDa to 74 kDa (57% of initial M_w) [13] and from 534 kDa to 216 kDa (40% of initial M_w) [10] after 6 months of implantation. The polydispersity of PHB polymers narrowed following implantation. PHB showed a constant increase in crystallinity (from 60.7 to 64.8%) up to 6 months [13] or an increase (from 65.0 to 67.9%) after 1 month and a fall again (to 64.5%) after 6 months of implantation [10] which suggests the degradation process had not affected the crystalline regions. These data are in accordance with data of PHB hydrolysis [20] and enzymatic PHB degradation by lipases in vitro [11] where M_w decrease was observed. The initial biodegradation of amorphous regions of PHB in vivo is similar to PHB degradation by depolymerase [39].

Thus, the observed biodegradation of PHB showed coexistence of two different degradation mechanisms in hydrolysis in the polymer: enzymatically or non-enzymatically catalyzed degradation. Although non-enzymatical catalysis occurred randomly in homopolymer, indicated by M_w loss rate in PHB, at some point in a time, a critical molecular weight is reached where upon enzyme-catalyzed hydrolysis accelerated degradation at the surface because easier enzyme/polymer interaction becomes possible. However considering the low weight loss of PHB, the critical molecular weight appropriate for enzymes predominantly

Table 2. Biodegradation of PHB in vivo (data for comparison)

Type of device	Thickness/ diameter, μm	Animal	Site of implantation/ surgical procedure	Relative mass loss of PHB, %	Relative loss of PHB molecular weight, %	Time, months	Links
film (injection-molded)	1200	mouse	subcutaneously	1.6	43	6	13
film (solvent-casting)	150–200	rabbit	subcutaneously (dorsal side)	6	60	6	10
film (solvent-casting)	50	rat	subcutaneously (ventral side)	100	100	3	24,25
porous PHB/ atactic PHB patch	100	rat	intraperitoneally to repair a bowel defect	>90*	62	6.5	11
porous PHB/ atactic PHB patch	250	minipigs	with contact to bone and dura mater to cover rhinobasal skull defects	>50*	65	6.5	12
Films and plates	100–1000	rabbit	subperiostally on the osseus skull or respectively on cut trough zygomatic arches	100	—	25	52
Films and plates	100 and 500	rabbit	subperiostally on the osseus skull or respectively on cut trough zygomatic arches	<10*	—	20	52
Plates and screws	500 and 1500	rabbit	subperiostally on the osseus skull or respectively trough osseus skull	0	—	12	25
cylinder (nerve conduits)	150 (of wall)	rat	nerve wrap-around to bridge an irreducible nerve gap	0	—	1	16
cylinder (nerve conduits)	150 (of wall)	cat	nerve wrap-around to bridge an irreducible nerve gap	>25*	—	12	15
mono-filament suture	—	rat	subcutaneously (dorsal side)	0	—	6	17
mono-filament suture	30	rat	subcutaneously (fold of neck)	30	—	6	18

thin films and ground particles	—	rabbit	subcutaneously and intramuscularly in the legs	>30*	—	2	59
nonwoven patch (consisted of fibers)	200–600 (of patch) 2–20 (of fibers)	sheep	on the wall of pericardium to close artificial defect and prevent pericardial adhesions	>90*	—	24	55
nonwoven patch (consisted of fibers)	200–600 (of patch) 2–20 (of fibers)	sheep	as transannular patches on the wall of right ventricular outflow tract and pulmonary artery	>99*	—	12	56
nonwoven patch (consisted of fibers)	200–600 (of patch) 2–20 (of fibers)	calve	on the septal of right atrium to close artificial septal defect	>99*	—	12	53
nonwoven patch (consisted of fibers)	200–600 (of patch) 2–20 (of fibers)	patient	on the wall of pericardium to close artificial defect and prevent pericardial adhesions	27	—	24	57
microspheres	0.5–0.8	rat	intravenously	8*	—	2	19
microspheres	100–300	mice	intramuscularly in the legs	0*	—	2	58
rivet-shaped plate	2300	rabbit	intraosseously, into the lateral condyle of femur	<10*	—	6	37

* - indirect data.

does not reach, yet resulting low molecular weight and crystallinity in PHB could provide some sites for the hydrolysis of enzymes to accelerate the degradation of PHB [10,13]. Additional data revealing the mechanism of PHB biodegradation in animal tissues was obtained by Kramp B. et al. in long-term implantation experiments. A very slow, clinically not recordable degradation of films and plates was observed during 20 months (much more than in experiments mentioned above). A drop in the PHB weight loss evidently took place between the 20th and 25th month. Only initial signs of degradation were to be found on the surface of the implant until 20 months after implantation but no more test body could be detected after 25 months [52]. The complete biodegradation in vivo in the wide range from 3 to 30 months of PHB was shown by other researches [53,55–57,60], whereas almost no weight loss and surface changes of PHB during 6 months of biodegradation in vivo was shown [13, 17]. Residual fragments of PHB implants were found after 30 months of the patches implantation [54,56]. A reduction of PHB patch size in 27% was shown in patients after 24 months after surgical procedure on pericardial closure with the patch [57]. Significantly more rapid biodegradation in vivo was shown by other researches [10,15,18,37,53]. It was shown that 30% mass loss of PHB sutures occurred gradually during 180 days of in vivo biodegradation with minor changes in the microstructure on the surface and in volume of sutures [18]. It was shown that PHB nonwoven patches (made to close atrial septal defect in calves) was slowly degraded by polynucleated macrophages, and 12 months postoperatively no PHB device was identifiable but only small particles of polymer were still seen. The absorption time of PHB patches was long enough to permit regeneration of a normal tissue [53]. The PHB sheets progressive biodegradation was demonstrated qualitatively at 2, 6 and 12 months after implantation as weakening of the implant surface, tearing/cracking of the implant, fragmentation and a decrease in the volume of polymer material [15,37,59]. The complete biodegradation of PHB ($M_w = 150\text{--}1000$ kDa) thin films ($10\text{--}50\text{ }\mu\text{m}$) for 3–6 months was shown and degradation process was described. The process of PHB biodegradation consists of several phases. At initial phase PHB films was covered by fibrous capsule. At second phase capsulated PHB films very slowly lost weight with simultaneous increase of crystallinity and decrease of M_w and mechanical properties of PHB. At third phase PHB films were rapidly disintegrated and then completely degraded. At 4th phase empty fibrous capsule resolved (Fig. 5) [24,25]. Interesting data were obtained for biodegradation in vivo of PHB microspheres ($0.5\text{--}0.8\text{ }\mu\text{m}$ in diameter). It was demonstrated indirectly that PHB loss about 8% of weight of microspheres accumulated in liver after 2 months of intravenous injection. It was demonstrated also a presence of several types of PHB degrading enzymes in the animal tissues extracts [19].

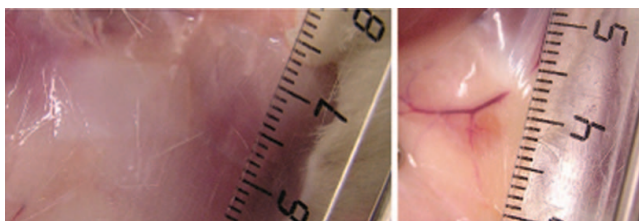


Figure 5. Biodegradation of PHB films in vivo. Connective-tissue capsule with PHB thin films (outlined with broken line) 2 weeks (98% residual weight of the film) (left photograph) and 3 months (0% residual weight of the film) (right photograph) after subcutaneous implantation [24,25].

Some researches studied the biodegradation of PHB threads with a tendency of analysis of its mechanical properties in vivo [17,18]. It was shown that at first load at break index decreased rapidly from 440 g to 390 g (12%) at 15th day and then gradually increased to the initial value at 90th and remain almost unchanged up to 182nd day [17] or gradually decreased in 27% during 180 days [18], strain at break decreased rapidly from 60 to 50% (in 17% of initial value) at 10th day and then gradually increased to 70% (in 17% of initial value) at 182nd day [17] or didn't change significantly during 180 days [18].

It was demonstrated that the primary reason of PHB biodegradation in vivo was a lysosomal and phagocytic activity of polynucleated macrophages and giant cells of foreign body reaction. The activity of tissue macrophages and nonspecific enzymes of body liquids made a main contribution to significantly more rapid rate of PHB biodegradation in vivo in comparison with rate of PHB hydrolysis in vitro. The PHB material was encapsulated by degrading macrophages. Presence of PHB stimulated uniform macrophage infiltration, which is important for not only the degradation process but also the restoration of functional tissue. The long absorption time produced a foreign-body reaction, which was restricted to macrophages forming a peripolymer layer [18,53,56,59]. Very important data that clarifies the tissue response that contributes to biodegradation of PHB was obtained by Lobler M. It was demonstrated a significant increase of expression of two specific lipases after 7 and 14 days of PHB contact with animal tissues. Moreover, liver specific genes were induced with similar results. It is striking that pancreatic enzymes are induced in the gastric wall after contact with biomaterials [40]. Saito T. et al. suggested the presence of at least two types of degradative enzymes in rat tissues: liver serine esterases with the maximum of activity in alkaline media (pH = 9.5) and kidney esterases with the maximum of activity in neutral media [19]. The mechanism of PHB biodegradation by macrophages was demonstrated at cultured macrophages incubated with particles of low-molecular weight PHB [61]. It was shown that macrophages and, to a lesser level, fibroblasts have the ability to take up (phagocytize) PHB particles (1–10 μm). At high concentrations of PHB particles (>10 $\mu\text{g/mL}$) the phagocytosis is accompanied by toxic effects and alteration of the functional status of the macrophages but not the fibroblasts. This process is accompanied by cell damage and cell death. The elevated production of nitric oxide (NO) and tumor necrosis factor alfa (TNF- α) by activated macrophages was observed. It was suggested that the cell damage and cell death may be due to phagocytosis of large amounts of PHB particles; after phagocytosis, polymer particles may fill up the cells, and cause cell damage and cell death. It was demonstrated also that phagocytized PHB particles disappeared in time due to an active PHB biodegradation process (Fig. 6) [61].

2. Application of PHB

2.1. Medical Devices Based on PHB and PHB In Vivo Biocompatibility

The perspective area of PHB application is development of implanted medical devices for dental, cranio-maxillofacial, orthopaedic, cardiovascular, hernioplastic and skin surgery. A number of potential medical devices based on PHB: bioresorbable surgical sutures [17,18,62,63], biodegradable screws and plates for cartilage and bone fixation [24,52], biodegradable membranes for periodontal treatment, surgical meshes with PHB coating for hernioplastic surgery [24], wound coverings [64], patches for repair of a bowel, pericardial and osseous defects [11,12,53–57], nerve guidance channels and conduits [15,16] etc. was developed (Fig. 7).

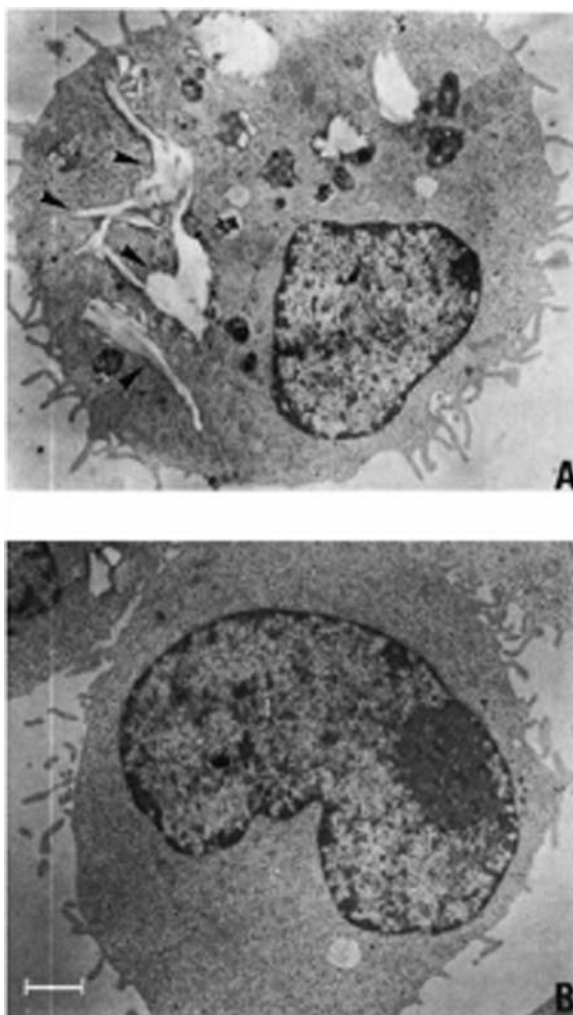


Figure 6. Phagocytosis of microparticles of PHB in macrophages. TEM analysis of cultured macrophages in the presence (A) or absence (B) of 2 μg PHB microparticles/mL for 24 h. Bar in B represents 1 μm , for A and B.

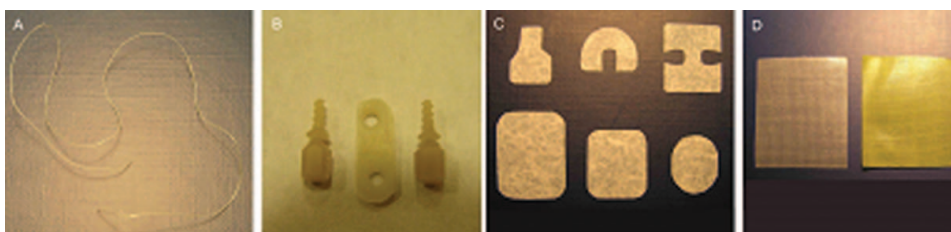


Figure 7. Medical devices based on PHB. (A) bioresorbable surgical suture; (B) biodegradable screws and plate for cartilage and bone fixation; (C) biodegradable membranes for periodontal treatment; (D) surgical meshes with PHB coating for hernioplastic surgery, pure (left) and loaded with antiplatelet drug, dipyridamole (right) [24].

The tissue reaction in vivo to implanted PHB films and medical devices was studied. In most cases a good biocompatibility of PHB was demonstrated. In general, no acute inflammation, abscess formation, or tissue necrosis was observed in tissue surrounding of the implanted PHB materials. In addition, no tissue reactivity or cellular mobilization occurred in areas remote from the implantation site [10,13,24,58]. On the one hand, it was shown that PHB elicited similar mild tissue response as PLA did [13], but on the other hand the use of implants consisting of polylactic acid, polyglycolic acid and their copolymers is not without a number of sequelae related with the chronic inflammatory reactions in tissue [65–69].

Subcutaneous implantation of PHB films for 1 month has shown that the samples were surrounded by a well-developed, homogeneous fibrous capsule of 80–100 μm in thickness. The vascularized capsule consists primarily of connective tissue cells (mainly, round, immature fibroblasts) aligned parallel to the implant surface. A mild inflammatory reaction was manifested by the presence of mononuclear macrophages, foreign body cells, and lymphocytes. Three months after implantation, the fibrous capsule has thickened to 180–200 μm due to the increase in the amount of connective tissue cells and a few collagen fiber deposits. A substantial decrease in inflammatory cells was observed after 3 months, tissues at the interface of the polymer were densely organized to form bundles. After 6 months of implantation, the number of inflammatory cells had decreased and the fibrous capsule, now thinned to about 80–100 μm , consisted mainly of collagen fibers, and a significantly reduced amount of connective tissue cells. A little inflammatory cells effusion was observed in the tissue adherent to the implants after 3 and 6 months of implantation [10,13]. The biocompatibility of PHB has been demonstrated in vivo under subcutaneous implantation of PHB films. Tissue reaction to films from PHB of different molecular weights (300, 450, 1000 kDa) implanted subcutaneously was relatively mild and did not change from tissue reaction to control glass plate [24].

At implantation of PHB with contact to bone the overall tissue response was favorable with a high rate of early healing and new bone formation with some indication of an osteogenic characteristic for PHB compared with other thermoplastics, such as polyethylene. Initially there was a mixture of soft tissue, containing active fibroblasts, and rather loosely woven osteonal bone seen within 100 μm of the interface. There was no evidence of a giant cell response within the soft tissue in the early stages of implantation. With time this tissue became more orientated in the direction parallel to the implant interface. The dependence of the bone growth on the polymer interface is demonstrated by the new bone growing away from the interface rather than towards it after implantation of 3 months. By 6 months post-implantation the implant is closely encased in new bone of normal appearance with no interposed fibrous tissue. Thus, PHB-based materials produce superior bone healing [37].

Regeneration of a neointima and a neomedia, comparable to native arterial tissue, was observed at 3–24 months after implantation of PHB nonwoven patches as transanular patches into the right ventricular outflow tract and pulmonary artery. In the control group, a neointimal layer was present but no neomedia comparable to native arterial tissue. Three layers were identified in the regenerated tissue: neointima with a endothelium-like lining, neomedia with smooth muscle cells, collagenous and elastic tissue, and a layer with polynucleated macrophages surrounding istets of PHB, capillaries and collagen tissue. Lymphocytes were rare. It was concluded that PHB nonwoven patches can be used as a scaffold for tissue regeneration in low-pressure systems. The regenerated vessel had structural and biochemical qualities in common with the native pulmonary artery [56]. Biodegradable PHB patches implanted in atrial septal defects promoted formation of regenerated tissue that macroscopically and microscopically resembled native atrial septal

wall. The regenerated tissue was found to be composed of three layers: monolayer with endothelium-like cells, a layer with fibroblasts and some smooth-muscle cells, collagenous tissue and capillaries, and a third layer with phagocytizing cells isolating and degrading PHB. The neointima contained a complete endothelium-like layer resembling the native endothelial cells. The patch material was encapsulated by degrading macrophages. There was a strict border between the collagenous and the phagocytizing layer. Presence of PHB seems to stimulate uniform macrophage infiltration, which was found to be important for the degradation process and the restoration of functional tissue. Lymphocytic infiltration as foreign-body reaction, which is common after replacement of vessel wall with commercial woven Dacron patch, was wholly absent when PHB. It was suggested that the absorption time of PHB patches was long enough to permit regeneration of a tissue with sufficient strength to prevent development of shunts in the atrial septal position [53]. The prevention of postoperative pericardial adhesions by closure of the pericardium with absorbable PHB patch was demonstrated. The regeneration of mesothelial layer after implantation of PHB pericardial patch was observed. The complete regeneration of mesothelium, with morphology and biochemical activity similar to findings in native mesothelium, may explain the reduction of postoperative pericardial adhesions after operations with insertion of absorbable PHB patches [55]. The regeneration of normal filament structure of restored tissues was observed by immunohistochemical methods after PHB devices implantation [54]. The immunohistochemical demonstration of cytokeratine, an intermediate filament, which is constituent of epithelial and mesodermal cells, agreed with observations on intact mesothelium. Heparan sulfate proteoglycan, a marker of basement membrane, was also identified [54].

PHB patches for the gastrointestinal tract were tested using animal model. Patches made from PHB sutured and PHB membranes were implanted to close experimental defects of stomach and bowel wall. The complete regeneration of tissues of stomach and bowel wall was observed at 6 months after patch implantation without strong inflammatory response and fibrosis [11,70].

Recently an application of biodegradable nerve guidance channels (conduits) for nerve repair procedures and nerve regeneration after spinal cord injury was demonstrated. Polymer tubular structures from PHB can be modulated for this purpose. Successful nerve regeneration through a guidance channel was observed as early as after 1 month. Virtually all implanted conduits contained regenerated tissue cables centrally located within the channel lumen and composed of numerous myelinated axons and Schwann cells. The inflammatory reaction had not interfered with the nerve regeneration process. Progressive angiogenesis was present at the nerve ends and through the walls of the conduit. The results demonstrate good-quality nerve regeneration in PHB guidance channels [16,71].

Biocompatibility of PHB was evaluated by implanting microspheres from PHB ($M_w = 450$ kDa) into the femoral muscle of rats. The spheres were surrounded by one or two layers of spindle cells, and infiltration of inflammatory cells and mononuclear cells into these layers was recognized at 1 week after implantation. After 4 weeks, the number of inflammatory cells had decreased and the layers of spindle cells had thickened. No inflammatory cells were seen at 8 weeks, and the spheres were encapsulated by spindle cells. The toxicity of PHB microspheres was evaluated by weight change and survival times in L1210 tumor-bearing mice. No differences were observed in the weight change or survival time compared with those of control. These results suggest that inflammation accompanying microsphere implantation is temporary as well as toxicity to normal tissues is minimal [58].

The levels of tissue factors, inflammatory cytokines, and metabolites of arachidonic acid were evaluated. Growth factors derived from endothelium and from macrophages

were found. These factors most probably stimulate both growth and regeneration occurring when different biodegradable materials were used as grafts [40,53,55,70]. The positive reaction for thrombomodulin, a multifunctional protein with anticoagulant properties was found in both mesothelial and endothelial cells after pericardial PHB patch implantation. Prostacycline production level, which was found to have cytoprotective effect on the pericardium and prevent adhesion formation, in the regenerated tissue was similar to that in native pericardium [53,55]. The PHB patch seems to be highly biocompatible, since no signs of inflammation were observed macroscopically and also the level of inflammation associated cytokine mRNA did not change dramatically, although a transient increase of interleukin-1 β and interleukin-6 mRNA through days 1–7 after PHB patch implantation was detected. In contrast, tumor necrosis factor- α mRNA was hardly detectable throughout the implantation period, which agrees well with a observed moderate fibrotic response [40,70].

2.2. PHB as Tissue Engineering Material and PHB In Vitro Biocompatibility

Biopolymer PHB is promising material in tissue engineering due to high biocompatibility in vitro. Cell cultures of various origins including murine and human fibroblasts [12,34,72–74], human mesenchymal stem cells [75], rabbit bone marrow cells (osteoblasts) [30,73,76], human osteogenic sarcoma cells [77], human epithelial cells [74,77], human endothelial cells [78,79], rabbit articular cartilage chondrocytes [80,81] and rabbit smooth muscle cells [82] in direct contact with PHB when cultured on polymer films and scaffolds exhibited satisfactory levels of cell adhesion, viability and proliferation. Moreover, it was shown that fibroblasts, endothelium cells, and isolated hepatocytes cultured on PHB films exhibited high levels of cell adhesion and growth (Fig. 8) [83].

It was shown also that cultured cells produced collagen II and glycosaminoglycan, the specific structural biopolymers formed the extracellular matrix [77,80,81]. A good viability and proliferation level of macrophages and fibroblasts cell lines was obtained under culturing in presence of particles from short-chain low-molecular PHB [61]. However it was shown that cell growth on the PHB films was relatively poor: the viable cell number ranged from 1×10^3 to 2×10^5 [34,73,81]. An impaired interaction between PHB matrix and cytoskeleton of cultured cells was also demonstrated [77]. It was reported that a number

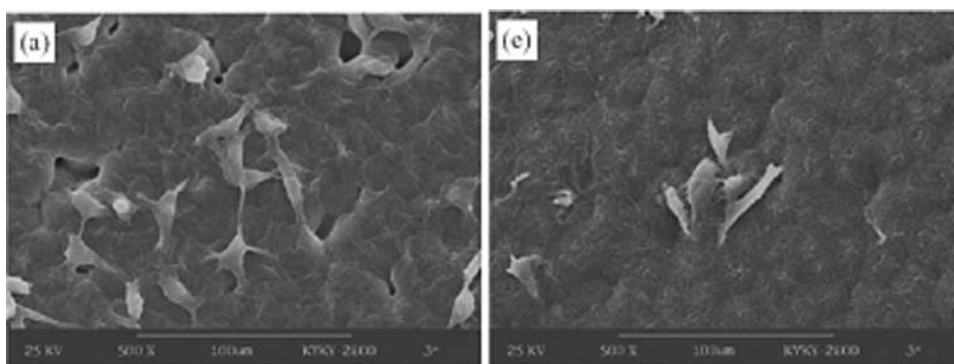


Figure 8. Scanning electron microscopy image of 2 days growth of fibroblast cells on films made of (a) PHB; (e) PLA; (500 x). Cell density of fibroblasts grown on PHB film is significantly higher vs. cell density of fibroblasts grown on PLA film [73].

of polymer properties including chemical composition, surface morphology, surface chemistry, surface energy and hydrophobicity play important roles in regulating cell viability and growth [84]. The investigation showed that this biomaterial can be used to make scaffolds for in vitro proliferous cells [34,76,80].

The most widespread methods to manufacture the PHB scaffolds for tissue engineering by means of improvement of cell adhesion and growth on polymer surface are change of PHB surface properties and microstructure by salt-leaching methods and enzymatic/chemical/physical treatment of polymer surface [34,76,80,85]. Adhesion to polymer substrates is one of the key issues in tissue engineering, because adhesive interactions control cell physiology. One of the most effective techniques to improve adhesion and growth of cells on PHB films is treatment of polymer surface with enzymes, alkali or low pressure plasma [34,85]. Lipase treatment increases the viable cell number on the PHB film from 100 to 200 times as compared to the untreated PHB film. NaOH treatment on PHB film also indicated an increase by 25 times on the viable cell number compared to the untreated PHB film [34]. It was shown that treatment of PHB film surface with low pressure ammonia plasma improved growth of human fibroblasts and epithelial cells of respiratory mucosa due to increased hydrophilicity (but with no change of microstructure) of polymer surface [74]. It was suggested that the improved hydrophilicity of the films after PHB treatment with lipases, alkali and plasma allowed cells in its suspension to easily attach on the polymer films compared to that on the untreated ones. The influence of hydrophilicity of biomaterial surface on cell adhesion was demonstrated earlier [86].

But a microstructure of PHB film surface can be also responsible for cell adhesion and cell growth [87–89]. Therefore, noticed above modification of polymer film surface after enzymatic and chemical treatment (in particular, reduced pore size and a surface smoothing) is expected to play an important role for enhanced cell growth on the polymer films [34]. Different cells prefer different surfaces. For example, osteoblasts preferred rougher surfaces with appropriate size of pores [87,88] while fibroblasts prefer smoother surface, yet epithelial cells only attached to the smoothest surface [89]. This appropriate roughness affects cell attachment as it provides the right space for osteoblast growth, or supplies solid anchors for filopodia. A scaffold with appropriate size of pores provided better surface properties for anchoring type II collagen filaments and for their penetration into internal layers of the scaffolds implanted with chondrocytes. This could be illuminated by the interaction of extracellular matrix proteins with the material surface. The right surface properties may also promote cell attachment and proliferation by providing more spaces for better gas/nutrients exchange or more serum protein adsorption. [30,76,80]. Additionally Sevastianov et al. found that PHB films in contact with blood did not activate the hemostasis system at the level of cell response, but they did activate the coagulation system and the complement reaction [90].

The high biocompatibility of PHB may be due to several reasons. First of all, PHB is a natural biopolymer involved in important physiological functions both prokaryotes and eukaryotes. PHB from bacterial origin has property of stereospecificity that is inherent to biomolecules of all living things and consists only from residues of D(-)-3-hydroxybutyric acid [91]. Low molecular weight PHB (up to 150 residues of 3-hydroxybutyric acid), complexed to other macromolecules (cPHB), was found to be a ubiquitous constituent of both prokaryotic and eukaryotic organisms of nearly all phyla [92–96]. Complexed cPHB was found in a wide variety of tissues and organs of mammals (including human): blood, kidney, vessels, nerves, eye, brain, as well as in organelles, membrane proteins, lipoproteins, and plaques. cPHB concentration ranged from 3–4 $\mu\text{g/g}$ wet tissue weight in nerves and brain to 12 $\mu\text{g/g}$ in blood plasma [97,98]. In humans, total plasma cPHB ranged from

0.60 to 18.2 mg/l, with a mean of 3.5 mg/L. [98]. It was shown that cPHB is a functional part of ion channels of erythrocyte plasma membrane and hepatocyte mitochondria membrane [99,100]. The singular ability of cPHB to dissolve salts and facilitate their transfer across hydrophobic barriers defines a potential physiological niche for cPHB in cell metabolism [94]. However a mechanism of PHB synthesis in eukaryotic organisms is not well clarified that requires additional studies. Nevertheless, it could be suggested that cPHB is one of products of symbiotic interaction between animals and gut microorganisms. It was shown, for example, that *E.coli* is able to synthesize low molecular weight PHB and cPHB plays various physiological roles in bacteria cell [96,101].

Intermediate product of PHB biodegradation, D(-)-3-hydroxybutyric acid is also a normal constituent of blood at concentrations between 0.3 and 1.3 mM and it is contained in all animal tissues [102,103]. As it was noted above PHB has a rather low degradation rate in the body in comparison to, e.g., poly(lactic-co-glycolic) acids, that results from increase of 3-hydroxybutyric acid concentration in surrounding tissues [10,13], whereas polylactic acid release, following local pH decrease in implantation area and acidic chronic irritation of surrounding tissues is a serious problem in application of medical devices on the base of poly(lactic-co-glycolic) acids [104,105]. Moreover, chronic inflammatory response to polylactic and polyglycolic acids that was observed in a number of cases may be induced by immune response to water-soluble oligomers that are released during degradation of synthetic polymers [105–107].

2.3. Novel Drug Dosage Forms on the Base of PHB

An improvement of medical devices based on biopolymers by encapsulating different drugs opens up the wide prospects in applications of these new devices with pharmacological activity in medicine. The design of injection systems for sustained drug delivery in the forms of microparticles (microspheres, microcapsules) prepared on the base of biodegradable polymers is extremely challenging in the modern pharmacology. The fixation of pharmacologically active components with the biopolymer and following slow drug release from the microparticles provides an optimal level of drug concentration in local target organ during long-term period (up to several months), that provides effective pharmaceutical action. At curative dose the prolonged delivery of drugs from the systems into organism permits to eliminate the shortcomings in peroral, injectable, aerosol, and the other traditional methods of drug administration. Among those shortcomings hypertoxicity, instability, pulsative character of rate delivery, ineffective expenditure of drugs should be pointed out. Alternatively, applications of therapeutical polymer systems provide orderly and purposefully the deliverance for an optimal dose of agent that is very important at therapy of acute or chronic diseases [108]. An ideal biodegradable microsphere formulation would consist of a free-flowing powder of uniform-sized microspheres less than 125 μm in diameter and with a high drug loading. In addition, the drug must be released in its active form with an optimized release profile. The manufacturing method should produce such microspheres in a process that is reproducible, scalable, and benign to some often delicate drugs, with a high encapsulation efficiency [109,110].

PHB is a promising biodegradable and biocompatible material for producing of polymer systems for controlled drug release. A number of drugs with various pharmacological activities were used for development of PHA polymer controlled release systems, mainly based on poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and poly(3-hydroxybutyrate-co-4-hydroxybutyrate) copolymers: model drugs (2,7-dichlorofluorescein [111], dextran-FITC [112], methyl red [113,114], 7-hydroxyethylthiophylline [115,116]),

antibiotics and antibacterial drugs (rifampicin [117,118], tetracycline [119], cefoperazone and gentamicin [120], sulperazone and duocid [121–124], sulbactam and cefoperazone [125]), anticancer drugs (5-fluorouracil [126], 2',3'-diacyl-5-fluoro-2'-deoxyuridine [58]), anti-inflammatory drug (indomethacin [127]), analgesics (tramadol [128], vasodilator and antithrombotic drugs (dipyridamole [24,127,129], nitric oxide donor [130,131]). The biocompatibility and pharmacological activity of some of these systems was studied [24,58,117,123–125,128,131]. But only a few drugs were used for production of drug controlled release systems based on PHB homopolymer: 7-hydroxethyltheophylline, methyl red, 2',3'-diacyl-5-fluoro-2'-deoxyuridine, rifampicin, tramadol, indomethacin and dipyridamole [58,113–118,127–131].

The first drug sustained PHB delivery system was developed by Korsatko W. et al., who observed a rapid release of encapsulated drug, 7-hydroxethyltheophylline, from tablets of PHB ($M_w = 2000$ kDa), as well as weight losses of PHB tablets containing the drug after subcutaneous implantation. It was suggested that PHB with molecular weight greater than 100 kDa was undesirable for long-term medication dosage [115].

Pouton C.W. and Akhtar S. describing the release of low molecular drugs from PHB matrices reported that the latter have the tendency of enhanced water penetration and pore formation [132]. The entrapment and release of model drug, methyl red, from melt-crystallized PHB matrices was found to be a function of polymer crystallization kinetics and morphology whereas overall degree of crystallinity was shown to cause no effect on drug release kinetics. Methyl red released from PHB films for more than 7 days with initial phase of rapid release ("burst effect") and second phase with relatively uniform release. Release profiles of PHB films crystallized at 110°C exhibited a greater burst effect when compared to those crystallized at 60°C. This was explained by better trapping of drug within polymeric spherulites with the more rapid rates of PHB crystallization at 110°C [113,114].

Kawaguchi T. et al showed that chemical properties of drug and polymer molecular weight had a great impact on drug delivery kinetics from PHB matrix. Microspheres (100–300 μm in diameter) from PHB of different molecular weight (65, 135, and 450 kDa) were loaded with prodrugs of 5-fluoro-2'-deoxyuridine (FUdR) synthesized by esterification with aliphatic acids (propionate, butyrate, and pentanoate). Prodrugs have different physicochemical properties, in particular, solubility in water (from 70 mg/ml for FUdR to 0.1 mg/ml for butyryl-FUdR). The release rates from the spheres depended on both the lipophilicity of the prodrug and the molecular weight of the polymer. Regardless the polymer, the relative release rates were propionyle-FUdR > butyryl-FUdR > pentanoyl-FUdR. The release of butyryl-FUdR and pentanoyl-FUdR from the spheres consisting of low-molecular-weight polymer ($M_w = 65$ kDa) was faster than that from the spheres of higher molecular weight ($M_w = 135$ or 450 kDa). The effect of drug content on the release rate was also studied. The higher the drug content in the PHB microspheres, the faster was the drug release. The release of FUdR continued for more than 5 days [58].

Kassab A.C. developed a well-managed technique for manufacture of PHB microspheres loaded with drugs. Microspheres were obtained within a size of 5–100 μm using a solvent evaporation method by changing the initial polymer/solvent ratio, emulsifier concentration, stirring rate, and initial drug concentration. Very high drug loading of up to 408 g rifampicin/g PHB were achieved. Drug release rates were rapid: the maximal duration of rifampicin delivery was 5 days. Both the size and drug content of PHB microspheres were found to be effective in controlling the drug release from polymer microspheres [118].

The sustained release of analgesic drug, tramadol, from PHB microspheres was demonstrated by Salman M.A. et al. It was shown that 58% of the tramadol (the initial drug content in PHB matrix = 18%) was released from the microspheres (7.5 μm in diameter) in the first

24 h. Drug release decreased in time. From 2 to 7 days the drug release was with zero-order rate. The entire amount of tramadol was released after 7 days [128].

The kinetics of different drug releases from PHB films and microspheres have been studied by our team [24,127]. It was found that the release occurs *via* two mechanisms, diffusion and degradation, operating simultaneously. In case of vasodilator and antithrombotic drug, such as dipyridamole, and anti-inflammatory drug, indomethacin, diffusion processes determine the rate of the release at the early stages of the contact of the system with the environment (the first 6–8 days). The coefficient of the release diffusion of a drug depends on its nature, the thickness of the PHB films containing the drug, the weight ratio of dipyridamole and indomethacin in polymer, and the molecular weight of PHB. Thus, it is possible to regulate the rate of drug release by changing of molecular weight of PHB, for example. A number of other drugs have been also used to develop polymeric systems of sustained drug delivery: antibiotics (rifampicin, metronidazole, ciprofloxacin, levofloxacin), anti-inflammatory drugs (flurbiprofen, dexamethasone, prednisolone), and antitumor drugs (paclitaxel) [127]. The biodegradable microspheres based on PHB designed for controlled release of dipyridamole were kinetically studied. The profiles of release from the microspheres with different diameters 4, 9, 63, and 92 μm present the progression of nonlinear and linear stages. Diffusion kinetic equation describing both linear (PHB hydrolysis) and nonlinear (diffusion) stages of the dipyridamole release profiles from the spherical subjects has been written down as the sum of two terms: desorption from the homogeneous sphere in accordance with diffusion mechanism and the zero-order release. In contrast to the diffusivity dependence on microsphere size, the constant characteristics of linearity are scarcely affected by the diameter of PHB microparticles. The view of the kinetic profiles as well as the low rate of dipyridamole release are in satisfactory agreement with kinetics of weight loss measured *in vitro* for the PHB films and observed qualitatively for PHB microspheres. Taking into account kinetic results, it was supposed that the degradation of both films and PHB microspheres is responsible for the linear stage of dipyridamole release profiles. Thus, a good method for production of systems with sustained drug release was demonstrated. The sustained invariable drug release is an essential property of injectable therapeutic polymer systems that allows to keep constant the adjusted drug dosing. PHB films and microspheres with sustained uniform drug release for more that 1 month were developed (Fig. 9) [24,127,129].

The biocompatibility and pharmacological activity of advanced drug delivery systems based on PHB was studied [24,58,117,128]. It was shown that implanted PHB films loaded

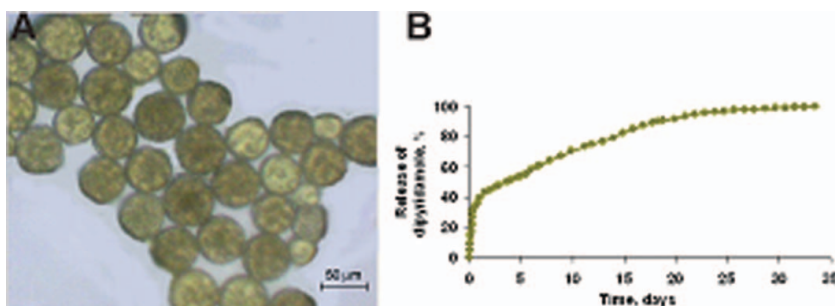


Figure 9. PHB microspheres for sustained delivery of drugs. (A) PHB microspheres (average diameter = 63 μm , PHB M_w = 1000 kDa) loaded with dipyridamole (10% w/w); (B) Sustained delivery of dipyridamole from PHB microspheres for more than 1 month [24,131].

with dipyridamole and indomethacin caused the mild tissue reaction. The inflammation accompanying implantation of PHB matrices is temporary and additionally toxicity relative to normal tissues is minimal [24]. No signs of toxicity were observed after administration of PHB microspheres loaded with analgesic, tramadol, [128]. A single intraperitoneal injection of PHB ($M_w = 450$ kDa) microspheres containing anticancer prodrugs, butyryl-FUDR and pentanoyl-FUDR, resulted in high antitumor effects against P388 leukemia in mice over a period of five days [58]. Embolization with PHB microspheres in vivo at dogs as test animals has been studied by Kasab et al. Renal angiograms obtained before and after embolization and also the histopathological observations showed the feasibility of using these microspheres as an alternative chemoembolization agent [117]. Epidural analgesic effects of tramadol released from PHB microspheres were observed for 21 h, whereas an equal dose of free tramadol was effective for less than 5 h. It was suggested that controlled release of tramadol from PHB microspheres in vivo is possible, and pain relief during epidural analgesia is prolonged by this drug formulation compared with free tramadol [128].

The observed data indicate the wide prospects in applications of drug-loaded medical devices and microspheres based on PHB as implantable and injectable therapeutic systems in medicine for treatment of various diseases: cancer, cardio-vascular diseases, tuberculosis, osteomyelitis, arthritis etc. Besides application of PHB for producing of medical devices and systems of sustained drug delivery, PHB can be used for production of systems for controlled release of activators or inhibitors of enzymes. The use of these systems allows the development of the physiological models for prolonged local activation or inhibition of enzymes in vivo. PHB is a perspective tool in design of novel physiological models due to minimal adverse inflammatory tissue reaction to PHB matrices implantation or PHB microspheres administration. A system of sustained nitric oxide (NO) donor delivery based on PHB was developed. This system can be used for study of prolonged NO action on normal tissues of blood vessels in vivo. The development of in vivo model of prolonged NO local action on vascular tissues is a difficult problem, because NO donors deliver NO at most only for a few minutes. We have developed a model of prolonged local NO action on appropriate artery based on PHB loaded with a new effective NO donor, FPTO [133]. It has been shown that FPTO-loaded PHB cylinders can release FPTO (and consequently NO) for up to 1 month with relatively constant rate. FPTO-loaded PHB cylinders with sustained FPTO delivery were implanted around left carotid artery of Wistar rats, pure PHB cylinders were implanted around right carotid artery as control. At 1st, 4th and 10th days after implantation arteries and cylinders have been isolated. The elevated levels of the main metabolic products of NO, nitrites and nitrates, in arterial tissues were observed that indicates the possibility of application of this system for production of physiological model of NO prolonged action on arterial tissues in vivo [24,130,131].

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References

- [1] Chen, G. Q., & Wu, Q. (2005). The application of polyhydroxyalkanoates as tissue engineering materials. *Biomaterials*, 26(33), 6565–6578.

- [2] Lenz, R. W., & Marchessault, R. H. (2005). Bacterial polyesters: biosynthesis, biodegradable plastics and biotechnology. *Biomacromolecules*, 6(1), 1–8.
- [3] Anderson, A. J., & Dawes, E. A. (1990). Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates. *Microbiological Reviews*, 54(4), 450–472.
- [4] Jendrossek, D., & Handrick, R. (2002). Microbial degradation of polyhydroxyalkanoates. *Annu. Rev. Microbiol.*, 56, 403–432.
- [5] Kim, D. Y., & Rhee, Y. H. (2003). Biodegradation of microbial and synthetic polyesters by fungi. *Appl. Microbiol. Biotechnol.*, 61, 300–308.
- [6] Steinbüchel, A., & Lutke-Eversloh, T. (2003). Metabolic engineering and pathway construction for biotechnological production of relevant polyhydroxyalkanoates in microorganisms. *Biochem. Eng. J.*, 16, 81–96.
- [7] Marois, Y., Zhang, Z., Vert, M., Deng, X., Lenz, R., & Guidoin, R. (1999). Hydrolytic and enzymatic incubation of polyhydroxyoctanoate (PHO): a short-term in vitro study of a degradable bacterial polyester. *J. Biomater. Sci. Polym. Ed.*, 10, 483–499.
- [8] Abe, H., & Doi, Y. (2002). Side-chain effect of second monomer units on crystalline morphology, thermal properties, and enzymatic degradability for random copolyesters of (R)-3-hydroxybutyric acid with (R)-3-hydroxyalkanoic acids. *Biomacromolecules*, 3(1), 133–138.
- [9] Renstad, R., Karlsson, S., & Albertsson, A. C. (1999). The influence of processing induced differences in molecular structure on the biological and non-biological degradation of poly(3-hydroxybutyrate-co-3-hydroxyvalerate), P(3-HB-co-3-HV). *Polym. Degrad. Stab.*, 63, 201–211.
- [10] Qu, X. H., Wu, Q., Zhang, K. Y., & Chen, G. Q. (2006). In vivo studies of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) based polymers: biodegradation and tissue reactions. *Biomaterials*, 27(19), 3540–3548 (c).
- [11] Freier, T., Kunze, C., Nischan, C., Kramer, S., Sternberg, K., Sass, M., Hopt, U. T., & Schmitz, K. P. (2002). In vitro and in vivo degradation studies for development of a biodegradable patch based on poly(3-hydroxybutyrate). *Biomaterials*, 23(13), 2649–2657.
- [12] Kunze, C., Edgar Bernd, H., Androsch, R., Nischan, C., Freier, T., Kramer, S., Kramp, B., & Schmitz, K. P. (2006). In vitro and in vivo studies on blends of isotactic and atactic poly(3-hydroxybutyrate) for development of a dura substitute material. *Biomaterials*, 27(2), 192–201 (a).
- [13] Gogolewski, S., Jovanovic, M., Perren, S. M., Dillon, J. G., & Hughes, M. K. (1993). Tissue response and in vivo degradation of selected polyhydroxyacids: polylactides (PLA), poly(3-hydroxybutyrate) (PHB), and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHB/VA). *J. Biomed. Mater. Res.*, 27(9), 1135–1148.
- [14] Borkenhagen, M., Stoll, R. C., Neuenschwander, P., Suter, U. W., & Aebischer, P. (1998). In vivo performance of a new biodegradable polyester urethane system used as a nerve guidance channel. *Biomaterials*, 19(23), 2155–2165.
- [15] Hazari, A., Johansson-Ruden, G., Junemo-Bostrom, K., Ljungberg, C., Terenghi, G., Green, C., & Wiberg, M. (1999). A new resorbable wrap-around implant as an alternative nerve repair technique. *Journal of Hand Surgery (British and European Volume)*, 24B(3), 291–295 (a).
- [16] Hazari, A., Wiberg, M., Johansson-Rudén, G., Green, C., & Terenghi, G. (1999). A resorbable nerve conduit as an alternative to nerve autograft. *British J. Plastic Surgery*, 52, 653–657 (b).
- [17] Miller, N.D., Williams, D. F. (1987). On the biodegradation of poly-beta-hydroxybutyrate (PHB) homopolymer and poly-beta-hydroxybutyrate-hydroxyvalerate copolymers. *Biomaterials*, 8(2), 129–137.
- [18] Shishatskaya, E. I., Volova, T. G., Gordeev, S. A., & Puzyr, A. P. (2005). Degradation of P(3HB) and P(3HB-co-3HV) in biological media. *J. Biomater. Sci. Polym. Ed.*, 16(5), 643–657.
- [19] Saito, T., Tomita, K., Juni, K., & Ooba, K. (1991). In vivo and in vitro degradation of poly(3-hydroxybutyrate) in rat. *Biomaterials*, 12(3), 309–312.
- [20] Koyama, N., & Doi, Y. (1995). Morphology and biodegradability of a binary blend of poly((R)-3-hydroxybutyric acid) and poly((R,S)-lactic acid). *Can. J. Microbiol.*, 41(Suppl. 1), 316–322.

- [21] Doi, Y., Kanesawa, Y., Kunioka, M., & Saito, T. (1990a). Biodegradation of microbial copolyesters: poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and poly(3-hydroxybutyrate-co-4-hydroxybutyrate). *Macromolecules*, 23, 26–31.
- [22] Holland, S. J., Jolly, A. M., Yasin, M., & Tighe, B. J. (1987). Polymers for biodegradable medical devices. II. Hydroxybutyrate-hydroxyvalerate copolymers: hydrolytic degradation studies. *Biomaterials*, 8(4), 289–295.
- [23] Kurcok, P., Kowalczyk, M., Adamus, G., Jedlinski, Z., & Lenz, R. W. (1995). Degradability of poly (b-hydroxybutyrate)s. Correlation with chemical microstructure. *JMS-Pure Appl. Chem.*, A32, 875–880.
- [24] Bonartsev, A. P., Myshkina, V. L., Nikolaeva, D. A., Furina, E. K., Makhina, T. A., Livshits, V. A., Boskhomdzhiyev, A. P., Ivanov, E. A., Iordanskii, A. L., & Bonartseva, G. A. (2007). Biosynthesis, biodegradation, and application of poly(3-hydroxybutyrate) and its copolymers - natural polyesters produced by diazotrophic bacteria. In: A. Méndez-Vilas (Ed.), *Communicating Current Research and Educational Topics and Trends in Applied Microbiology*, V. 1, Spain: Formatex, pp. 295–307.
- [25] Bonartsev, A. P. private communication.
- [26] Cha, Y., & Pitt, C. G. (1990). The biodegradability of polyester blends. *Biomaterials*, 11(2), 108–112.
- [27] Schliecker, G., Schmidt, C., Fuchs, S., Wombacher, R., & Kissel, T. (2003). Hydrolytic degradation of poly(lactide-co-glycolide) films: effect of oligomers on degradation rate and crystallinity. *Int. J. Pharm.*, 266(1–2), 39–49.
- [28] Scandola, M., Focarete, M. L., Adamus, G., Sikorska, W., Baranowska, I., Swierczek, S., Gnatowski, M., Kowalczyk, M., & Jedlinski, Z. (1997). Polymer blends of natural poly(3-hydroxybutyrate-co-hydroxyvalerate) and a synthetic atactic poly(3-hydroxybutyrate). Characterization and biodegradation studies. *Macromolecules*, 30, 2568–2574.
- [29] Doi, Y., Kanesawa, Y., Kawaguchi, Y., & Kunioka, M. (1989). Hydrolytic degradation of microbial poly(hydroxyalkanoates). *Makrom. Chem. Rapid. Commun.*, 10, 227–230.
- [30] Wang, Y.-W., Yang, F., Wu, Q., Cheng, Y. C., Yu, P. H., Chen, J., & Chen G.-Q. (2005). Evaluation of three-dimensional scaffolds made of blends of hydroxyapatite and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) for bone reconstruction. *Biomaterials*, 26(8), 899–904 (a).
- [31] Muhamad, I. I., Joon, L. K., Noor, M. A. M. (2006). Comparing the degradation of poly- β -(hydroxybutyrate), poly- β -(hydroxybutyrate-co-valerate)(PHBV) and PHBV/Cellulose triacetate blend. *Malaysian Polymer J.*, 1, 39–46.
- [32] Mergaert, J., Webb, A., Anderson, C., Wouters, A., & Swings, J. (1993). Microbial degradation of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) in soils. *Applied and Environmental Microbiology*, 59(10), 3233–3238.
- [33] Choi, G. G., Kim, H. W., & Rhee, Y. H. (2004). Enzymatic and non-enzymatic degradation of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolyesters produced by *Alcaligenes* sp. MT-16. *The Journal of Microbiology*, 42(4), 346–352.
- [34] Yang, X., Zhao, K., & Chen, G. Q. (2002). Effect of surface treatment on the biocompatibility of microbial polyhydroxyalkanoates. *Biomaterials*, 23(5), 1391–1397.
- [35] Zhao, K., Yang, X., Chen, G. Q., & Chen, J. C. (2002). Effect of lipase treatment on the biocompatibility of microbial polyhydroxyalkanoates. *J. material science: materials in medicine*, 13, 849–854.
- [36] Wang, H. T., Palmer, H., Linhardt, R. J., Flanagan, D. R., & Schmitt, E. (1990). Degradation of poly(ester) microspheres. *Biomaterials*, 11(9), 679–685.
- [37] Doyle, C., Tanner, E. T., & Bonfield, W. (1991). In vitro and in vivo evaluation of polyhydroxybutyrate and of polyhydroxybutyrate reinforced with hydroxyapatite. *Biomaterials*, 12, 841–847.
- [38] Coskun, S., Korkusuz, F., & Hasirci, V. (2005). Hydroxyapatite reinforced poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) based degradable composite bone plate. *J. Biomater. Sci. Polymer Edn.*, 16(12), 1485–1502.

- [39] Sudesh, K., Abe, H., & Doi, Y. (2000). Synthesis, structure and properties of polyhydroxyalkanoates: biological polyesters. *Prog. Polym. Sci.*, 25, 1503–1555.
- [40] Lobler, M., Sass, M., Kunze, C., Schmitz, K. P., & Hopt, U. T. (2002). Biomaterial patches sutured onto the rat stomach induce a set of genes encoding pancreatic enzymes. *Biomaterials*, 23, 577–583.
- [41] Tokiwa, Y., Suzuki, T., & Takeda, K. (1986). Hydrolysis of polyesters by *Rhizopus arrhizus* lipase. *Agric. Biol. Chem.*, 50, 1323–1325.
- [42] Hoshino, A., & Isono, Y. (2002). Degradation of aliphatic polyester films by commercially available lipases with special reference to rapid and complete degradation of poly(L-lactide) film by lipase PL derived from *Alcaligenes* sp. *Biodegradation*, 13, 141–147.
- [43] Jendrossek, D., Schirmer, A., & Schlegel, H. G. (1996). Biodegradation of polyhydroxyalkanoic acids. *Appl. Microbiol. Biotechnol.*, 46, 451–463.
- [44] Winkler, F. K., D'Arcy, A., & Hunziker, W. (1990). Structure of human pancreatic lipase. *Nature*, 343, 771–774.
- [45] Mergaert, J., Anderson, C., Wouters, A., Swings, J., & Kersters, K. (1992). Biodegradation of polyhydroxyalkanoates. *FEMS Microbiol. Rev.*, 9(2–4), 317–321.
- [46] Tokiwa, Y., & Calabia, B. P. (2004). Degradation of microbial polyesters. *Biotechnol. Lett.*, 26(15), 1181–1189.
- [47] Mokeeva, V., Chekunova, L., Myshkina, V., Nikolaeva, D., Gerasin, V., & Bonartseva, G. (2002). Biodestruction of poly(3-hydroxybutyrate) by microscopic fungi: tests of polymer on resistance to fungi and fungicidal properties. *Mikologia and Fitopatologia*, 36(5), 59–63.
- [48] Bonartseva, G. A., Myshkina, V. L., Nikolaeva, D. A., Kevbrina, M. V., Kallistova, A. Y., Gerasin, V. A., Iordanskii, A. L., & Nozhevnikova, A. N. (2003). Aerobic and anaerobic microbial degradation of poly-beta-hydroxybutyrate produced by *Azotobacter chroococcum*. *Appl Biochem Biotechnol.*, 109(1–3), 285–301.
- [49] Spyros, A., Kimmich, R., Briesse, B. H., & Jendrossek, D. (1997). ¹H NMR imaging study of enzymatic degradation in poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate). Evidence for preferential degradation of the amorphous phase by PHB depolymerase B from *pseudomonas lemoignei*. *Macromolecules*, 30(26), 8218–8225.
- [50] Hocking, P. J., Marchessault, R. H., Timmins, M. R., Lenz, R. W., & Fuller, R. C. (1996). Enzymatic degradation of single crystals of bacterial and synthetic poly(-hydroxybutyrate). *Macromolecules*, 29(7), 2472–2478.
- [51] Bonartseva, G. A., Myshkina, V. L., Nikolaeva, D. A., Rebrov, A. V., Gerasin, V. A., & Makhina, T. K. (2002). The biodegradation of poly-beta-hydroxybutyrate (PHB) by a model soil community: the effect of cultivation conditions on the degradation rate and the physicochemical characteristics of PHB. *Mikrobiologiya*, 71(2), 258–263, Russian.
- [52] Kramp, B., Bernd, H. E., Schumacher, W. A., Blynnow, M., Schmidt, W., Kunze, C., Behrend, D., & Schmitz, K. P. (2002). Poly-beta-hydroxybutyric acid (PHB) films and plates in defect covering of the osseous skull in a rabbit model. *Laryngorhinootologie*, 81(5): 351–356 [Article in German].
- [53] Malm, T., Bowald, S., Karacagil, S., Bylock, A., & Busch, C. (1992). A new biodegradable patch for closure of atrial septal defect. An experimental study. *Scand. J. Thorac. Cardiovasc. Surg.*, 26(1), 9–14 (a).
- [54] Malm, T., Bowald, S., Bylock, A., Saldeen, T., & Busch, C. (1992). Regeneration of pericardial tissue on absorbable polymer patches implanted into the pericardial sac. An immunohistochemical, ultrastructural and biochemical study in the sheep. *Scandinavian Journal of Thoracic and Cardiovascular Surgery*, 26(1), 15–21 (b).
- [55] Malm, T., Bowald, S., Bylock, A., & Busch, C. (1992). Prevention of postoperative pericardial adhesions by closure of the pericardium with absorbable polymer patches. An experimental study. *The Journal of Thoracic and Cardiovascular Surgery*, 104, 600–607 (c).
- [56] Malm, T., Bowald, S., Bylock, A., Busch, C., & Saldeen, T. (1994). Enlargement of the right ventricular outflow tract and the pulmonary artery with a new biodegradable patch in transannular position. *European Surgical Research*, 26, 298–308.

- [57] Duvernoy, O., Malm, T., Ramström, J., & Bowald, S. (1995, Oct.). A biodegradable patch used as a pericardial substitute after cardiac surgery: 6- and 24-month evaluation with CT. *Thorac. Cardiovasc. Surg.*, 43(5), 271–274.
- [58] Kawaguchi, T., Tsugane, A., Higashide, K., Endoh, H., Hasegawa, T., Kanno, H., Seki, T., Juni, K., Fukushima, S., & Nakano, M. (1992). Control of drug release with a combination of prodrug and polymer matrix: antitumor activity and release profiles of 2',3'-Diacyl-5-fluoro-2'-deoxyuridine from poly(3-hydroxybutyrate) microspheres. *Journal of Pharmaceutical Sciences*, 87(6), 508–512.
- [59] Baptist, J. N. (1965). Assignor to W.R. Grace Et Co., New York. Patent US No. 3 225 766.
- [60] Holmes, P. (1988). Biologically produced (R)-3-hydroxy-alkanoate polymers and copolymers. In: D. C. Bassett (Ed.), *Developments in Crystalline Polymers*, Vol. 2, Elsevier: London, pp. 1–65.
- [61] Saad, B., Ciardelli, G., Matter, S., Welti, M., Uhlschmid, G. K., Neuenschwander, P., & Suterl, U. W. (1996). Characterization of the cell response of cultured macrophages and fibroblasts to particles of short-chain poly[(R)-3-hydroxybutyric acid]. *Journal of Biomedical Materials Research*, 30, 429–439.
- [62] Fedorov, M., Vikhoreva, G., Kildeeva, N., Maslikova, A., Bonartseva, G., & Galbraikh, L. (2005). Modeling of surface modification process of surgical suture. *Chimicheskie volokna*, (6), 22–28 [Article in Russian].
- [63] Rebrov, A. V., Dubinskii, V. A., Nekrasov, Y. P., Bonartseva, G. A., Shtamm, M., & Antipov, E. M. (2002). Structure phenomena at elastic deformation of highly oriented polyhydroxybutyrate. *Vysokomol. Soedin. (Russian)*, 44, 347–351 [Article in Russian].
- [64] Kil'deeva, N. R., Vikhoreva, G. A., Gal'braikh, L. S., Mironov, A. V., Bonartseva, G. A., Perminov, P. A., & Romashova, A. N. (2006). Preparation of biodegradable porous films for use as wound coverings. *Prikl. Biokhim. Mikrobiol.*, 42(6), 716–720 [Article in Russian].
- [65] Solheim, E., Sudmann, B., Bang, G., & Sudmann, E. (2000). Biocompatibility and effect on osteogenesis of poly(ortho ester) compared to poly(DL-lactic acid). *J. Biomed. Mater. Res.*, 49(2), 257–263.
- [66] Bostman, O., & Pihlajamaki, H. (2000). Clinical biocompatibility of biodegradable orthopaedic implants for internal fixation: a review. *Biomaterials*, 21(24), 2615–2621.
- [67] Lickorish, D., Chan, J., Song, J., & Davies, J. E. (2004). An in-vivo model to interrogate the transition from acute to chronic inflammation. *Eur. Cell. Mater.*, 8, 12–19.
- [68] Khouw, I. M., van Wachem, P. B., de Leij, L. F., & van Luyn, M. J. (1998). Inhibition of the tissue reaction to a biodegradable biomaterial by monoclonal antibodies to IFN-gamma. *J. Biomed. Mater. Res.*, 41, 202–210.
- [69] Su, S. H., Nguyen, K. T., Satasiya, P., Greilich, P. E., Tang, L., & Eberhart, R. C. (2005). Curcumin impregnation improves the mechanical properties and reduces the inflammatory response associated with poly(L-lactic acid) fiber. *J. Biomater. Sci. Polym. Ed.*, 16(3), 353–370.
- [70] Lobler, M., Sass, M., Schmitz, K. P., & Hopt, U. T. (2003). Biomaterial implants induce the inflammation marker CRP at the site of implantation. *J. Biomed. Mater. Res.*, 61, 165–167.
- [71] Novikov, L. N., Novikova, L. N., Mosahebi, A., Wiberg, M., Terenghi, G., & Kellerth, J. O. (2002). A novel biodegradable implant for neuronal rescue and regeneration after spinal cord injury. *Biomaterials*, 23, 3369–3376.
- [72] Cao, W., Wang, A., Jing, D., Gong, Y., Zhao, N., & Zhang, X. (2005). Novel biodegradable films and scaffolds of chitosan blended with poly(3-hydroxybutyrate). *J. Biomater. Sci. Polymer Edn.*, 16(11), 1379–1394.
- [73] Wang, Y.-W., Yang, F., Wu, Q., Cheng, Y. C., Yu, P. H., Chen, J., & Chen, G.-Q. (2005). Effect of composition of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) on growth of fibroblast and osteoblast. *Biomaterials*, 26(7), 755–761 (b).
- [74] Ostwald, J., Dommerich, S., Nischan, C., & Kramp, B. (2003). In vitro culture of cells from respiratory mucosa on foils of collagen, poly-L-lactide (PLLA) and poly-3-hydroxy-butyrat (PHB). *Laryngorhinootologie*, 82(10), 693–699 [Article in German].

- [75] Wollenweber, M., Domaschke, H., Hanke, T., Boxberger, S., Schmack, G., Gliesche, K., Scharnweber, D., & Worch, H. (2006 Feb.). Mimicked bioartificial matrix containing chondroitin sulphate on a textile scaffold of poly(3-hydroxybutyrate) alters the differentiation of adult human mesenchymal stem cells. *Tissue Eng.*, 12(2), 345–359.
- [76] Wang, Y.-W., Wu, Q., & Chen, G. Q. (2004). Attachment, proliferation and differentiation of osteoblasts on random biopolyester poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) scaffolds. *Biomaterials*, 25(4), 669–675.
- [77] Nebe, B., Forster, C., Pommerenke, H., Fulda, G., Behrend, D., Bernewski, U., Schmitz, K. P., & Rychly, J. (2001). Structural alterations of adhesion mediating components in cells cultured on poly-beta-hydroxy butyric acid. *Biomaterials*, 22(17), 2425–2434.
- [78] Qu, X.-H., Wu, Q., & Chen, G.-Q. (2006). In vitro study on hemocompatibility and cytocompatibility of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate). *J. Biomater. Sci. Polymer Edn.*, 17(10), 1107–1121 (a).
- [79] Pompe, T., Keller, K., Mothes, G., Nitschke, M., Teese, M., Zimmermann, R., & Werner, C. (2007). Surface modification of poly(hydroxybutyrate) films to control cell-matrix adhesion. *Biomaterials*, 28(1), 28–37.
- [80] Deng, Y., Lin, X. S., Zheng, Z., Deng, J. G., Chen, J. C., Ma, H., & Chen, G.-Q. (2003). Poly(hydroxybutyrate-co-hydroxyhexanoate) promoted production of extracellular matrix of articular cartilage chondrocytes in vitro. *Biomaterials*, 24(23), 4273–4281.
- [81] Zheng, Z., Bei, F.-F., Tian, H.-L., & Chen, G.-Q. (2005). Effects of crystallization of poly-hydroxyalkanoate blend on surface physicochemical properties and interactions with rabbit articular cartilage chondrocytes. *Biomaterials*, 26, 3537–3548.
- [82] Qu, X.-H., Wu, Q., Liang, J., Zou, B., & Chen, G.-Q. (2006 May). Effect of 3-hydroxyhexanoate content in poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) on in vitro growth and differentiation of smooth muscle cells. *Biomaterials*, 27(15), 2944–2950 (b).
- [83] Shishatskaya, E. I., & Volova, T. G. (2004). A comparative investigation of biodegradable polyhydroxyalkanoate films as matrices for in vitro cell cultures. *J. Mater. Sci.-Mater. M.*, 15, 915–923.
- [84] Fischer, D., Li, Y., Ahlemeyer, B., Kriglstein, J., & Kissel, T. (2003). In vitro cytotoxicity testing of polycations: influence of polymer structure on cell viability and hemolysis. *Biomaterials*, 24(7), 1121–1131.
- [85] Nitschke, M., Schmack, G., Janke, A., Simon, F., Pleul, D., & Werner, C. (2002). Low pressure plasma treatment of poly(3-hydroxybutyrate): toward tailored polymer surfaces for tissue engineering scaffolds. *J. Biomed. Mater. Res.*, 59(4), 632–638.
- [86] Chancel-Lesrat, D. J., Pellen-Mussi, P., Auroy, P., & Bonnaure-Mallet, M. (1999). Evaluation of the in vitro biocompatibility of various elastomers. *Biomaterials*, 20, 291–299.
- [87] Boyan, B. D., Hummert, T. W., Dean, D. D., & Schwartz, Z. (1996). Role of material surfaces in regulating bone and cartilage cell response. *Biomaterials*, 17, 137–146.
- [88] Bowers, K. T., Keller, J. C., Randolph, B. A., Wick, D. G., Michaels, C. M. (1992). Optimization of surface micromorphology for enhanced osteoblasts responses in vitro. *Int. J. Oral. Max. Impl.*, 7, 302–310.
- [89] Cochran, D., Simpson, J., Weber, H., & Buser, D. (1994). Attachment and growth of periodontal cells on smooth and rough titanium. *Int. J. Oral. Max. Impl.*, 9, 289–297.
- [90] Sevastianov, V. I., Perova, N. V., Shishatskaya, E. I., Kalacheva, G. S., & Volova, T. G. (2003). Production of purified polyhydroxyalkanoates (PHAs) for applications in contact with blood. *J. Biomater. Sci. Polym. Ed.*, 14, 1029–1042.
- [91] Seebach, D., Brunner, A., Burger, H. M., Schneider, J., & Reusch R. N. (1994). Isolation and 1H-NMR spectroscopic identification of poly(3-hydroxybutanoate) from prokaryotic and eukaryotic organisms. Determination of the absolute configuration (R) of the monomeric unit 3-hydroxybutanoic acid from *Escherichia coli* and spinach. *Eur. J. Biochem.*, 224(2), 317–328.
- [92] Reusch, R. N. (1989). Poly- β -hydroxybutyrate/calcium polyphosphate complexes in eukaryotic membranes. *Proc. Soc. Exp. Biol. Med.*, 191, 377–381.

- [93] Reusch, R. N. (1992). Biological complexes of poly- β -hydroxybutyrate. *FEMS Microbiol. Rev.*, 103, 119–130.
- [94] Reusch, R. N. (1995). Low molecular weight complexed poly(3-hydroxybutyrate): a dynamic and versatile molecule in vivo. *Can. J. Microbiol.*, 41(Suppl. 1), 50–54.
- [95] Müller, H. M., & Seebach, D. (1994). Polyhydroxyalkanoates: a fifth class of physiologically important organic biopolymers? *Angew. Chemie.*, 32, 477–502.
- [96] Huang, R., & Reusch, R. N. (1996). Poly(3-hydroxybutyrate) is associated with specific proteins in the cytoplasm and membranes of *Escherichia coli*. *J. Biol. Chem.*, 271, 22196–22201.
- [97] Reusch, R. N., Bryant, E. M., & Henry, D. N. (2003). Increased poly-(R)-3-hydroxybutyrate concentrations in streptozotocin (STZ) diabetic rats. *Acta Diabetol.*, 40(2), 91–94.
- [98] Reusch, R. N., Sparrow, A. W., & Gardiner, J. (1992). Transport of poly- β -hydroxybutyrate in human plasma. *Biochim. Biophys. Acta*, (1123), 33–40.
- [99] Reusch, R. N., Huang, R., & Kosk-Kosicka, D. (1997). Novel components and enzymatic activities of the human erythrocyte plasma membrane calcium pump. *FEBS Lett.*, 412(3), 592–596.
- [100] Pavlov, E., Zakharian, E., Bladen, C., Diao, C. T. M., Grimbly, C., Reusch, R. N., & French, R. J. (2005). A large, voltage-dependent channel, isolated from mitochondria by water-free chloroform extraction. *Biophysical Journal*, 88, 2614–2625.
- [101] Theodorou, M. C., Panagiotidis, C. A., Panagiotidis, C. H., Pantazaki, A. A., & Kyriakidis, D. A. (2006). Involvement of the AtoS-AtoC signal transduction system in poly-(R)-3-hydroxybutyrate biosynthesis in *Escherichia coli*. *Biochim. Biophys. Acta*, 1760(6), 896–906.
- [102] Wiggam, M. I., O'Kane, M. J., Harper, R., Atkinson, A. B., Hadden, D. R., Trimble, E. R., & Bell, P. M. (1997). Treatment of diabetic ketoacidosis using normalization of blood 3-hydroxy-butyrate concentration as the endpoint of emergency management. *Diabetes Care*, 20, 1347–1352.
- [103] Larsen, T., & Nielsen, N. I. (2005). Fluorometric determination of beta-hydroxybutyrate in milk and blood plasma. *J. Dairy Sci.*, 88(6), 2004–2009.
- [104] Agrawal, C. M., & Athanasiou, K. A. (1997). Technique to control pH in vicinity of biodegrading PLA-PGA implants. *J. Biomed. Mater. Res.*, 38(2), 105–114.
- [105] Ignatius, A. A., & Claes, L. E. (1996). In vitro biocompatibility of bioresorbable polymers: poly(l, dl-lactide) and poly(l-lactide-co-glycolide). 17(8), 831–839.
- [106] Rihova, B. (1996). Biocompatibility of biomaterials: hemocompatibility, immunocompatibility and biocompatibility of solid polymeric materials and soluble targetable polymeric carriers. *Adv. Drug. Delivery Rev.*, 21, 157–176.
- [107] Ceonzo, K., Gaynor, A., Shaffer, L., Kojima, K., Vacanti, C. A., & Stahl, G. L. (2006). Polyglycolic acid-induced inflammation: role of hydrolysis and resulting complement activation. *Tissue Eng.*, 12(2), 301–308.
- [108] Chasin, M., & Langer, R. (Eds.). (1990). *Biodegradable Polymers as Drug Delivery Systems*, Marcel Dekker: New York.
- [109] Johnson, O. L., & Tracy, M. A. (1999). Peptide and protein drug delivery. In: E. Mathiowitz (Ed.), *Encyclopedia of Controlled Drug Delivery*, Vol. 2, John Wiley and Sons: Hoboken, NJ, pp. 816–832.
- [110] Jain, R. A. (2000). The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. *Biomaterials*, 21, 2475–2490.
- [111] Gursel, I., & Hasirci, V. (1995). Properties and drug release behaviour of poly(3-hydroxybutyric acid) and various poly(3-hydroxybutyrate-hydroxyvalerate) copolymer microcapsules. *J. Microencapsul.*, 12(2), 185–193.
- [112] Li, J., Li, X., Ni, X., Wang, X., Li, H., & Leong, K. W. (2006). Self-assembled supramolecular hydrogels formed by biodegradable PEO-PHB-PEO triblock copolymers and α -cyclodextrin for controlled drug delivery. *Biomaterials*, 27(), 4132–4140.
- [113] Akhtar, S., Pouton, C. W., & Notarianni, L. J. (1992). Crystallization behaviour and drug release from bacterial polyhydroxyalkanoates. *Polymer*, 33(1), 117–126.

- [114] Akhtar, S., Pouton, C. W., & Notarianni, L. J. (1991). The influence of crystalline morphology and copolymer composition on drug release from solution cast and melting processed P(HB-HV) copolymer matrices. *J. Controlled Release*, 17, 225–234.
- [115] Korsatko, W., Wabnegg, B., Tillian, H. M., Braunegg, G., & Lafferty, R. M. (1983). Poly-D-hydroxybutyric acid-a biologically degradable vehicle to regard release of a drug. *Pharm. Ind.*, 45, 1004–1007.
- [116] Korsatko, W., Wabnegg, B., Tillian, H. M., Egger, G., Pfragner, R., & Walser, V. (1984). Poly D(-)-3-hydroxybutyric acid (poly-HBA)-a biodegradable former for long-term medication dosage. 3. Studies on compatibility of poly-HBA implantation tablets in tissue culture and animals. *Pharm. Ind.*, 46, 952–954.
- [117] Kassab, A. C., Piskin, E., Bilgic, S., Denkbaz, E. B., & Xu, K. (1999). Embolization with polyhydroxybutyrate (PHB) micromerspheres: in vivo studies. *J. Bioact. Compat. Polym.*, 14, 291–303.
- [118] Kassab, A. C., Xu, K., Denkbaz, E. B., Dou, Y., Zhao, S., & Piskin, E. (1997). Rifampicin carrying polyhydroxybutyrate microspheres as a potential chemoembolization agent. *J. Biomater. Sci. Polym. Ed.*, 8, 947–961.
- [119] Sendil, D., Gursel, I., Wise, D. L., & Hasirci, V. (1999). Antibiotic release from biodegradable PHBV microparticles. *J. Control. Release*, 59, 207–17.
- [120] Gursel, I., Yagmurlu, F., Korkusuz, F., & Hasirci, V. (2002). In vitro antibiotic release from poly(3-hydroxybutyrate-co-3-hydroxyvalerate) rods. *J. Microencapsul.*, 19, 153–164.
- [121] Turesin, F., Gursel, I., & Hasirci, V. (2001). Biodegradable polyhydroxyalkanoate implants for osteomyelitis therapy: in vitro antibiotic release. *J. Biomater. Sci. Polym. Ed.*, 12, 195–207.
- [122] Turesin, F., Gumusyazici, Z., Kok, F. M., Gursel, I., Alaeddinoglu, N. G., & Hasirci, V. (2000). Biosynthesis of polyhydroxybutyrate and its copolymers and their use in controlled drug release. *Turk. J. Med. Sci.*, 30, 535–541.
- [123] Gursel, I., Korkusuz, F., Turesin, F., Alaeddinoglu, N. G., & Hasirci, V. (2001). In vivo application of biodegradable controlled antibiotic release systems for the treatment of implant-related osteomyelitis. *Biomaterials*, 22(1), 73–80.
- [124] Korkusuz, F., Korkusuz, P., Eksioglu, F., Gursel, I., & Hasirci, V. (2001). In vivo response to biodegradable controlled antibiotic release systems. *J. Biomed. Mater. Res.*, 55(2), 217–228.
- [125] Yagmurlu, M. F., Korkusuz, F., Gursel, I., Korkusuz, P., Ors, U., & Hasirci, V. (1999). Sulbactam-cefoperazone polyhydroxybutyrate-co-hydroxyvalerate (PHBV) local antibiotic delivery system: In vivo effectiveness and biocompatibility in the treatment of implantrelated experimental osteomyelitis. *J. Biomed. Mater. Res.*, 46, 494–503.
- [126] Khang, G., Kim, S. W., Cho, J. C., Rhee, J. M., Yoon, S. C., & Lee, H. B. (2001). Preparation and characterization of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) microspheres for the sustained release of 5-fluorouracil. *Biomed. Mater. Eng.*, 11, 89–103.
- [127] Bonartsev, A. P., Bonartseva, G. A., Makhina, T. K., Mashkina, V. L., Luchinina, E. S., Livshits, V. A., Boskhomdzhiyev, A. P., Markin, V. S., & Iordanskii, A. L. (2006). New poly-(3-hydroxybutyrate)-based systems for controlled release of dipyrindamole and indomethacin. *Prikl. Biokhim. Mikrobiol.*, 42(6), 710–715.
- [128] Salman, M. A., Sahin, A., Onur, M. A., Oge, K., Kassab, A., & Aypar, U. (2003). Tramadol encapsulated into polyhydroxybutyrate microspheres: in vitro release and epidural analgesic effect in rats. *Acta Anaesthesiol. Scand.*, 47, 1006–1012.
- [129] Bonartsev, A. P., Livshits, V. A., Makhina, T. A., Myshkina, V. L., Bonartseva, G. A., & Iordanskii, A. L. (2007). Controlled release profiles of dipyrindamole from biodegradable microspheres on the base of poly(3-hydroxybutyrate). *Express Polymer Letters*, 1(12), 797–803 (b).
- [130] Bonartsev, A. P., Postnikov, A. B., Myshkina, V. L., Artemieva, M. M., & Medvedeva, N. A. (2005). A new system of nitric oxide donor prolonged delivery on basis of controlled-release polymer, polyhydroxybutyrate. *American Journal of Hypertension*, 18(5A), A.
- [131] Bonartsev, A. P., Postnikov, A. B., Mahina, T. K., Myshkina, V. L., Voinova, V. V., Boskhomdzhiyev, A. P., Livshits, V. A., Bonartseva, G. A., & Iorganskii, A. L. (2007). A

- new in vivo model of prolonged local nitric oxide action on arteries on basis of biocompatible polymer. *The Journal of Clinical Hypertension*, 9(5, Suppl. A.), A152 (c).
- [132] Pouton, C. W., & Akhtar, S. (1996). Biosynthetic polyhydroxyalkanoates and their potential in drug delivery. *Adv. Drug Deliver. Rev.*, 18, 133–162.
- [133] Kots, A. Y., Grafov, M. A., Khropov, Y. V., Betin, V. L., Belushkina, N. N., Busygina, O. G., Yazykova, M. Y., Ovchinnikov, I. V., Kulikov, A. S., Makhova, N. N., Medvedeva, N. A., Bulargina, T. V., & Severina, I. S. (2000). Vasorelaxant and antiplatelet activity of 4,7-dimethyl-1,2, 5-oxadiazolo[3,4-d]pyridazine 1,5,6-trioxide: role of soluble guanylate cyclase, nitric oxide and thiols. *Br. J. Pharmacol.*, 129(6), 1163–1177.