Introduction Currently, the intensive development of biodegradable and biocompatible materials for medical implication provoke comprehensive interdisciplinary studies on biopolymer structures and functions. The well-known and applicable biodegradable polymers are polylactides (PLA), polyglycolides (PGA), and their copolymers, poly-ε-caprolactone, poly(orthoesters), poly-β-maleic acid, poly(propylene fumarate), polyalkylcyanoacrylates, polyorthoanhydrides, polyphosphazenes, poly(propylene fumarate), some natural polysaccharides (starch, chitosan, alginates, agarose, dextrane, chondroitin sulfate, hyaluronic acid), and proteins (collagen, silk fibroin, fibrin, gelatin, albumin). Since some of these polymers should be synthesized through chemical stages (e.g. via lactic and glycolic acids) it is not quite correct to define them as the biopolymers. Besides biomedicine applications, the biodegradable biopolymers attract much attention as perspective materials in wide areas of industry, nanotechnologies, farming and packaging owing to the relevant combination of biomedical, transport, and physical-chemical properties. It is worth to emphasis that only medical area of these biopolymers includes implants and prosthesis, tissue engineering scaffolds, novel drug dosage forms in pharmaceutics, novel materials for dentistry and others. Each potentially applicable biopolymer arranges a wide multidisciplinary network, which usually includes tasks of searching for efficacy ways of biosynthesis reactions; economical problems associated with large-scale production; academic studies of mechanical, physicochemical, biochemical properties of the polymer and material of interest; technology of preparation and using this biopolymer; preclinical and clinical trials of these materials and products; a market analysis and perspectives of application of the developed products and many other problems. Poly((R)-3-hydroxybutyrate) (PHB) is an illustrative example for the one of centers for formation of the above mentioned scientific-technological network and a basis for the development of various biopolymer systems [1-2]. In recent decades an intense development of biomedical application of bacterial PHB in producing of biodegradable polymer implants and controlled drug release systems [3-6] needs for comprehensive understanding of the PHB biodegradation process. Examination of PHB degradation process is also necessary for development of novel friendly environment polymer packaging [7-9]. 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 [6, 10]. Opposite to other biodegradable polymers (e.g. PGA and PLGA), PHB is considered to be moderately resistant to degradation in vitro as well as to biodegradation in biological media. The rates of degradation are influenced by the characteristics of the polymer, such as chemical composition, crystallinity, morphology and molecular weight [11, 12]. 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 sometimes even contradictory. The presence of conflicting data can be partially explained by the fact that biotechnologically produced PHB with standardized

properties is relatively rare and is not readily available due to a wide variety of its biosynthesis sources and different manufacturing processes. Above inconsistencies can be explained also 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 devices. Depending on applied biomedical purposes biodegradation of PHB was investigated under different geometry: films and plates with various thickness [13-16], cylinders [17-19], monofilament threads [20-22] and micro- and nanospheres [23, 24]. At these experiments PHB was used from various sources, with different molecular weight and crystallinity. Besides, different technologies of PHB devices manufacture affect such important characteristics as polymer porosity and surface structure [14, 15]. Reports regarding the complex theoretical research of mechanisms of hydrolysis, enzymatic degradation and biodegradation in vivo of PHB processes are relatively rare [13-15, 16, 25-27] that attaches 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 yet well clarified. PHB applications Medical implants and devices on the base of PHB and its biocompatibility The perspective area of PHB application is development of implanted medical devices for dental, cranio-maxillofacial, orthopaedic, cardiovascular, hernioplastic and skin surgery [3, 6]. A number of potential medical devices on the base of PHB: bioresorbable surgical sutures [6, 22-23, 28-30], biodegradable screws and plates for cartilage and bone fixation [6, 24, 31-32], biodegradable membranes for periodontal treatment [6, 31, 33-34], surgical meshes with PHB coating for hernioplastic surgery [6, 31], wound coverings [35], patches for repair of a bowel, pericardial and osseous defects [14-15, 36-40], nerve guidance channels and conduits [20-21], cardiovascular stents [41] etc. was developed (Fig. 1). Fig. 1- Medical devices on the base of 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) [31] 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 [13, 16, 31, 42]. On the one hand, it was shown that PHB elicited similar mild tissue response as PLA did [16], but on the other hand the use of implants consisting of polylactic acid, polyglicolic acid and their copolymers is not without a number of sequelae related with the chronic inflammatory reactions in tissue [42-47]. 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 [13, 16]. The biocompatibility of PHB has been demonstrated in vivo under subcutaneous implantation of PHB films. Tissue reaction to films from PHB of different molecular weight (300, 450, 1000 kDa) implanted subcutaneously was relatively mild and didn't change from tissue reaction to control glass plate [18, 31]. 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 [48]. 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 transannular 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 [39]. 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 [65]. 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 [38]. The regeneration of normal filament structure of restored tissues was observed by immunohistochemical methods after PHB devices implantation [37]. The immunohistochemical demonstration of cytokeratine, an intermediate filament, which is constituent of epithelial and mesodermal cells, agreed with observations on intact mesothelium. Heparin sulfate proteoglycan, a marker of basement membrane, was also identified [37]. However, in spite of good tissue reaction to implantation of cardiovascular PHB patches, PHB endovascular stents in the rabbit iliac arteria caused intensive inflammatory vascular reactions [41]. 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 [14, 49]. 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-guality nerve regeneration in PHB guidance channels [21, 50]. Biocompatibility of PHB was evaluated by implanting microspheres from PHB (Mw = 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 [42]. 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 [51-52, 38, 49]. 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 [52, 38]. 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-1β 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 [51, 49]. 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 [15, 34, 53-58], human mesenchymal stem cells [59], rabbit bone marrow cells (osteoblasts) [55, 60-61], human osteogenic sarcoma cells [62], human epithelial cells [56, 62], human endothelial cells [63-64], rabbit articular cartilage chondrocytes [65-66] and rabbit smooth muscle cells [67], human neurons (schwann cells) [68] 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. 2) [69]. A series of 2D and 3D PHB scaffolds was developed by various methods: polymer surface modification [64], blending [60, 34, 54, 56, 59, 66, 70], electrospinning [71-73], salt leaching [60, 61, 74-75], microspheres fusion [76], forming on porous mold [77], laser cutting [78]. Fig. 2 - 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 [55] It was shown also that cultured cells produced collagen II and glycosaminoglycan, the specific structural

biopolymers formed the extracellular matrix [62, 65, 66]. 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 [79]. However it was shown that cell growth on the PHB films was relatively poor: the viable cell number ranged from 1×103 to 2×105 [53, 55, 66]. An impaired interaction between PHB matrix and cytoskeleton of cultured cells was also demonstrated [62]. It was reported that a number of polymer properties including chemical composition, surface morphology, surface chemistry, surface energy and hydrophobicity play important roles in regulating cell viability and growth [80]. The investigation showed that this biomaterial can be used to make scaffolds for in vitro proliferating cells [53, 55, 65]. 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 [53, 55, 65, 81]. 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 [40, 113]. Lipase treatment increases the viable cell number on the PHB film from 100 to 200 times compared to the untreated PHB film. NaOH treatment on PHB film also indicated an increase of 25 times on the viable cell number compared with the untreated PHB film [53]. 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 hydrophylicity (but with no change of microstructure) of polymer surface [56]. 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 [82]. But a microstructure of PHB film surface can be also responsible for cell adhesion and cell growth [83-85]. 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 [53]. Different cells prefer different surface. For example, osteoblasts preferred rougher surfaces with appropriate size of pores [83-84] while fibroblast prefer smoother surface, yet epithelial cells only attached to the smoothest surface [85]. This appropriate roughness affects cell attachment as it provides the right space for osteoblast growth, or supplies solid anchors for filapodia. 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. Moreover, the semicrystalline

surface PHB ultrastructure can be connected with protein adsorption and cell adhesion [57, 58, 86]. The appropriate surface properties may also promote cell attachment and proliferation by providing more spaces for better gas/nutrients exchange or more serum protein adsorption [36, 94, 98]. 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 [87]. 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-hydrohybutyric acid [88-90]. Low molecular weight PHB (up to 150 resides of 3-hydrohybutyric acid), complexed to other macromolecules (cPHB), was found to be a ubiquitous constituent of both prokaryotic and eukaryotic organisms of nearly all phyla [91-95]. Complexed cPHB was found in a wide variety of tissues and organs of mammals (including human): blood, kidney, vessels, nerves, vessels, eye, brain, as well as in organelles, membrane proteins, lipoproteins, and plaques. cPHB concentration ranged from 3-4 μg/g wet tissue weight in nerves and brain to 12 μg/g in blood plasma [96-97]. In humans, total plasma cPHB ranged from 0.60 to 18.2 mg/l, with a mean of 3.5 mg/l. [97]. It was shown that cPHB is a functional part of ion channels of erythrocyte plasma membrane and hepatocyte mitochondria membrane [98-99]. 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 [93]. 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 [95, 100]. 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 contains in all animal tissues [101-102]. 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 prevent from increase of 3-hydroxybutyric acid concentration in surrounding tissues [13,16], 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 [103-104]. 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 released during degradation of synthetic polymers [104-106]. Novel drug dosage forms on the base of PHB An improvement of medical materials on the base of biopolymers by encapsulating different drugs opens up the wide prospects in applications of new devices with pharmacological activity. The design of injection

systems for sustained drug delivery in the forms of microspheres or microcapsules prepared on the base of biodegradable polymers is extremely challenging in the modern pharmacology. The fixation of pharmacologically active component 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). 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 [1, 3, 6, 107]. 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 profile. The manufacturing method should produce the microspheres which are reproducible, scalable, and benign to some often delicate drugs, with a high encapsulation efficiency [108-109]. PHB as biodegradable and biocompatible is a promising material for producing of polymer systems for controlled drug release. A number of drugs with various pharmacological activities were used for development of polymer controlled release systems on the base of PHB and its copolymers: model drugs (2,7-dichlorofluorescein [110], dextran-FITC [111], methyl red [90, 112, 113], 7-hydroxethyltheophylline [114-115]), antibiotics and antibacterial drugs (rifampicin [116-117], tetracycline [118], cefoperazone and gentamicin [119], sulperazone and duocid [120-123], sulbactam and cefoperazone [124], fusidic acid [125], nitrofural [126]), anticancer drugs (5-fluorouracil [130], 2',3'-diacyl-5-fluoro-2'-deoxyuridine [42], paclitaxel [131-132], rubomycin [133], chlorambucil and etoposide [134]), anti-inflammatory drug (indomethacin [135], flurbiprofen [136], ibuprofen [137]), analgesics (tramadol [138], vasodilator and antithrombotic drugs (dipyridamole [139-140], nitric oxide donor [141-142], nimodipine [143], felodipine [144]), proteins (hepatocyte growth factor [145], mycobacterial protein for vaccine development [146], bone morphogenetic protein 7 [147]). Various methods for manufacture of drug-loaded PHB matrices and microspheres were used: films solvent casting [112-115], emulsification and solvent evaporation [116-135], spray drying [148], layer-by-layer self-assembly [136], supercritical antisolvent precipitation [149]. The biocompatibility and pharmacological activity of some of these systems was studied [42,116,122-124, 131-132, 138, 142]. But only a few drugs were used for production of drug controlled release systems on the base of PHB homopolymer: 7-hydroxethyltheophylline, methyl red, 2',3'-diacyl-5-fluoro-2'-deoxyuridine, rifampicin, tramadol, indomethacin, dipyridamole and paclitaxel [42, 122-124, 131-142]. The latest trend in PHB drug delivery systems study is PHB nanoparticles development loaded with different drugs

[150-151]. The first drug sustained delivery system on the base of PHB was developed by Korsatko W. et al., who observed a rapid release of encapsulated drug, 7-hydroxethyltheophylline, from tablets of PHB (Mw = 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 [114]. Pouton C.W. and Akhtar S. describing the release of low molecular drugs from PHB matrices reported that the latter have the tendencies to enhance water penetration and pore formation [152]. The entrapment and release of the model drug, methyl red (MR), from melt-crystallized PHB 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. MR released from PHB films for 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 [112-113]. 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 (65000, 135000, and 450000) were loaded with prodrugs of 5-fluoro-2'deoxyuridine (FdUR) 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 FdUR to 0.1 mg/ml for butyryl- FdUR). The release rates from the spheres depended on both the lipophilicity of the prodrug and the molecular weight of the polymer. Regardless of the polymer, the relative release rates were propionyle- FdUR > butyryl- FdUR > pentanoyl- FdUR. The release of butyryl- FdUR and pentanoyl- FdUR from the spheres consisting of low-molecular-weight polymer (Mw = 65000) was faster than that from the spheres of higher molecular weight (Mw = 135000 or 450000). 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 FdUR continued for more than 5 days [42]. 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. The drug overloading of up to 0,41 g rifampicin/g PHB were achieved. Drug release was 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 [117]. 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 with time.

From 2 to 7 days the drug release was with zero-order rate. The entire amount of tramadol was released after 7 days [138]. The kinetics of different drug release from PHB micro- and nanoparticles loaded with dipyridamole, indomethacin and paclitaxel was studied [131, 132, 135, 139, 140]. It was found that the release occurs via two mechanisms, diffusion and degradation, operating simultaneously. Vasodilator and antithrombotic drug, 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. [135]. The biodegradable microspheres on the base of PHB designed for controlled release of dipyridamole and paclitaxel were kinetically studied. The profiles of release from the microspheres with different diameters present the progression of nonlinear and linear stages. Diffusion kinetic equation describing both linear (PHB hydrolysis) and nonlinear (diffusion) stages of the dipyridamole and paclitaxel 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 and paclitaxel 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 PHB microspheres is responsible for the linear stage of dipyridamole and paclitaxel release profiles (Fig. 3) [24, 131, 132, 139, 140].
Fig. 3 - Kinetics profiles of DPD release from PHB microspheres in vitro (phosphate buffer, 37oC). A: General view of kinetic curves for the microspheres with different diameters: 4(1), 19(2), 63(3), and 92(4) μm. The lines show the second stage of release following the zero-order equation. B: Details of the curves for the microspheres with the smaller diameters: 4(1), 19(2) The biocompatibility and pharmacological activity of advanced drug delivery systems on the base of PHB was studied [42, 116, 138-140]. It was shown that implanted PHB microspheres loaded with paclitaxel caused the mild tissue reaction. The inflammation accompanying implantation of PHB matrices is temporary and additionally toxicity relative to normal tissues is minimal [140]. No signs of toxicity were observed after administration of PHB microspheres loaded with analgesic, tramadol, [138]. A single intraperitoneal injection of PHB microspheres containing anticancer prodrugs, butyryl- FdUR and pentanoyl- FdUR, resulted in high antitumor effects against P388 leukemia in mice over a period of five days [42]. 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 [116]. 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 [138]. The observed data indicate the wide prospects in applications of drug-loaded medical devices and microspheres on the base of PHB as implantable and injectable therapeutic systems in medicine for treatment of various diseases: cancer, cardio-vascular diseases, tuberculosis, osteomyelitis, arthritis etc [6]. Conclusions The natural poly-3-hydroxybutyrate (PHB) is unique biodegradable thermoplastics of considerable commercial importance. With this review, we have attempted to systematically evaluate the impact of physicochemical factors on the hydrolysis and the biodegradation of natural PHB both in vitro and in vivo. Clearly, the degradation behavior observed is very dependent upon both physicochemical conditions. Geometry and structural and microbial properties. If these conditions of (bio)degradation are known, the systems on the base PHB can be designed in such biomedicine areas as medical devices (Section 3.1), tissue scaffolds in bioengineering (Section 3.2) and development of novel biodegradable therapeutic systems for drug delivery.
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