ISSN 1990-7508, Biochemistry (Moscow) Supplement Series B: Biomedical Chemistry, 2011, Vol. 5, No. 1, pp. 10–21. © Pleiades Publishing, Ltd., 2011.
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Poly(3-Hydroxybutyrate) and Poly(3-Hydroxybutyrate)-Based Biopolymer Systems

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Received September 24, 2009

Abstract—Biodegradable biopolymers attract much attention in biology and medicine due to its wide application. The present review considers a biodegradable and biocompatible polymer of bacterial origin, poly(3-hydroxybutyrate), which has wide perspectives in medicine and pharmaceutics. It highlights basic properties of biopolymer (biodegradability and biocompatibility) and also biopolymer systems: various materials, devices and compositions based on the biopolymer. Application of poly(3-hydroxybutyrate)-based biopolymer systems in medicine as surgical implants, in bioengineering as cell culture scaffolds, and in pharmacy as novel drug dosage forms and drug systems are also considered.

Keywords: biopolymer system, polyhydroxyalkanoates (PHA), poly(3-hydroxybutyrate) (PHB), biodegradation, biocompatibility.

DOI: 10.1134/S1990750811010045

INTRODUCTION

The past 2–3 decades are characterized by intensive development of biomedical materials based on bacterial poly(3-hydroxybutyrate) (PHB). PHB belongs to the class of polyhydroxyalkanoates (PHA), bacterial biodegradable polyesters. The other widely known biodegradable polymers are polymers of lactic and glycolic acids (polylactides and polyglycolides, respectively) and their copolymers, polycaprolactone, poly(orthoesters), polyanhydrides, poly(propylene fumarate), some polysaccharides (starch, chitosan, alginates), and proteins (collagen). Since some of these polymers may be synthesized chemically (e.g. widely used polymers of lactic and glycolic acids) it is rather incorrect to define them as the biopolymers. Biodegradable biopolymers attract much attention in biology and medicine due to their wide application. Only medical fields of application of these biopolymers include medical implants for surgery, tissue engineering, design of novel drug dosage forms in pharmaceutics, design of novel materials for dentistry and others. In addition biodegradable biopolymers are used in food packaging industry and agriculture [1–6]. Each biopolymer attracting attention of both science and technology "forms" a wide multidisciplinary network, which usually includes the following field: basic and technological problems of biopolymer biosynthesis; economical problems associated with large-scale biopolymer production; studies of mechanical, physical, physicochemical, chemical, and biological properties of the polymer of interest; technology of preparation of various materials and products made using this biopolymer; studies of physicochemical and biological properties of these materials and products, preclinical and clinical trials of these materials and products; a market analysis and perspectives of practical application of the developed products and many other problems. A wide spectrum of biopolymer application is formed due to development of various materials, products and composites based on these polymers, which we define here as the biopolymer systems. 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.

In contrast to polyglycolides, polylactides, and their copolymers (polyglycolactides) poly(3-hydroxybutyrate) is considered as a material demonstrating moderate resistance to biodegradation in the body. Periods of its use may significantly vary in dependence on chemical nature of a particular biopolymer, crystallinity, morphology, molecular mass, stereoisomer ratio, technology of sample preparation, etc. [7, 8]. Sometimes physicochemical factors influencing the biodegradation process do not provide a comprehensive picture and may even contradict to each other. Similar inconsistencies have been also observed during studies of PHB biocompatibility. Existence of inconsistent results may be attributed to different biotechnological procedures used for PHB preparation with

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various bacterial strains; this complicates comparisons of characteristics of the polymeric product and thus requires standardization in the future. In addition, PHB samples used in various biomedical studies may have different size and shape and they are produced using various technologies [9–15]. In this connection, understanding of mechanisms of PHB degradation and compatibility in the body requires certain summarization and intensification of subsequent studies.

Every year spheres of application of PHB-based biopolymer systems become more and more diverse. These include intensive design and studies of various medical implants, PHB-based biopolymer beads and scaffolds for tissues engineering. There is active development of novel drug dosage forms based on PHB micro- and nanoparticles with encapsulated drugs with various pharmacological spectra. New directions appear in this field. These include development of PHB-based biopolymer systems with encapsulated inorganic nanoparticles and also biopolymer systems of combined therapeutic action. PHB-based biopolymer systems may be used for therapy of a wide range of diseases, including oncologic, infectious, chronic inflammatory and cardiovascular diseases. Thus, development and study of PHB-based biopolymer systems represent an important and perspective direction in modern medicine and pharmacology.

1. BIODEGRADATION AND BIOCOMPATIBILITY OF PHB AND BHB-BASED MEDICAL DEVICES

During several decades PHB is intensively used for the development and production of a wide range of medical devices. The widest field of PHB application includes surgical implants used in hernioplasty, dentistry, cardiovascular surgery, orthopedic surgery, etc. The biopolymer is used for development of biodegradable sutures [13, 14, 16, 17], biodegradable screws and staples [18, 19], periodontal membranes in dentistry, surgical meshes with PHB-based coatings [18], wound coatings [20], surgical patches for defects of intestine, pericardium, or bone tissues [9, 10, 21–23] and some others. Figure 1 shows some of our biopolymer devices. Biodegradation and biocompatibility of PHB and PHB-based devices have been investigated in several studies [9-15, 18, 21-23].

Hydrolytic cleavage of PHB starts with penetration of water molecules into the polymer volume [24, 25]. Appearance of additional functional groups formed during hydrolysis increases hydrophilicity of the polymer matrix and therefore its swelling. Comparative analysis of the polymer film cross sections before and after hydrolysis have shown the internal layer is characterized by increased porosity and formation of pores with the diameter <0.5 μm. Molecular mass distribution remained unimodal, and the molecular mass decrease followed the first-order kinetics [7]. PHB hydrolysis occurs in a two-step mechanism. The first step is characterized by statistical cleavage of macromolecules accompanied by the decrease of molecular weight of the biopolymer sample; the decrease of a weight of the polymer sample begins when molecular mass reaches about 10 kDa and relative low molecular fragments of PHB begin to desorb into the solution [26].

Enzymatic destruction of PHB in vitro results in changes of molecular weight, crystallinity, and mechanical characteristics. For these parameters there was a direct correlation between a loss of polymer mass and the loss of its molecular weight. At the same time at high degradation degree there was an increase in the crystallinity degree; this confirms notions of PHB degradation preferentially occurring in amorphous regions [27]. This caused disappearance of the amorphous phase and the increase in the crystallinity degree and the resultant samples became more fragile and simultaneously more friable [28]. Here one should take into consideration the mode of sample preparation. Film samples obtained by solvent evaporation may include pores and microvoids of a rather large size (0.1–0.5 μm). Smaller sized lipase molecules used for nonspecific enzymatic cleavage of PHB could penetrate inside the polymer and perform polymer biodegradation not only on the surface but also in the polymer volume due to a branched system of pores in the sample volume [29]. Besides porosity two other factors may influence the process of enzymatic degradation: segmental polymer mobility, which is significantly higher in amorphous inter-crystal regions of PHB and polymer hydrophobicity, i.e. the degree of polymer conversion (hydrolysis), which results in hydrophilic group formation [7]. A gradual decrease of mechanistic characteristics (a 20% decrease in the Young's modulus and a 29% loss in breaking load) confirms changes in morphology and molecular mass of PHB during incubation of blood plasma [14].

PHB biodegradation in vivo in animal tissues were originally investigated by Miller et al. and Saito et al. 15–20 years ago [13, 15]. These studies provided principal characteristics of this process for the first time. The mechanism of PHB biodegradation includes combined effects of enzymatic and non-enzymatic degradation. However, this fact does not mean that such biodegradation represents simple combination addition of two these processes. Moreover, PHB biodegradation in vivo (resulted in the decrease in molecular weight and total mass of the sample) remains an object of studies that do not give clear interpretation. The major reason for discrepancies in the mechanism of hydrolytic destruction consist in the use of nonstandard and markedly differed technological conditions and also implantation conditions coupled with modeling of animal organism. The major proportion of biodegradation studies employed prototypes of medical devices of PHB with different sizes and geometry; in vivo experiments were performed on various

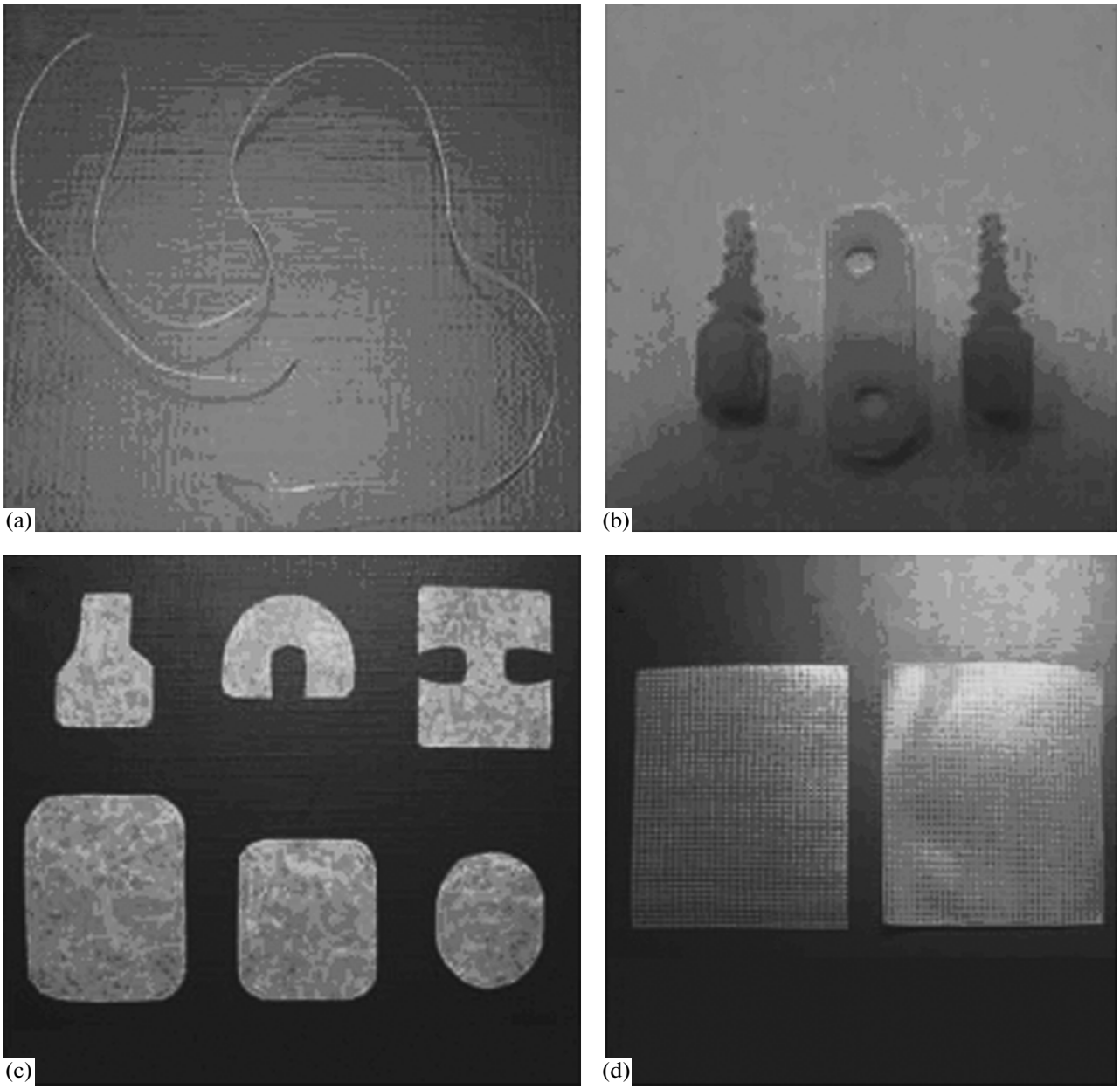

Fig. 1. PHB-based medical devices: (a) biodegradable surgical thread, (b) biodegradable screws and staples for fixation of bone fragments; (c) periodontal membranes; (d) polypropylene-based surgical meshes with PHB-based coating: on the left the mesh without drug, on the right the mesh with the coating with dipiridamol as the drug preventing platelet adhesion (adapted from [18]).

animals and implantation of PHB samples was performed using various modes and after different time intervals, etc. [3].

Biodegradation time, a degree of sample mass loss and PHB molecular mass strongly varied. For example, according one study [6], during 6 months the films of 150–200 μm thick implanted subcutaneously in rabbits were characterized by a 6% sample mass loss and the decrease in biopolymer molecular mass by 60%, whereas other authors [18] reported that total biodegradation of films implanted subcutaneously in rats occurred within 3 months [3]. Similar situation was observed during comparison of biodegradation times of PHB-based devices. Studies on subcutaneous PHB monofilament implantation for 6 months gave different results in different laboratories: in one report there was a 30% decrease in sample mass loss [14], whereas the other study [13] did not reveal monofilament biodegradation at all [13].

Using PHB as a material for medical devices it is important to investigate tissues reactions to the administered implant in vivo. In most cases of PHB

application good biocompatibility was observed. For example, no acute inflammatory reaction, abscesses or tissue necroses were observed near the polymer. Moreover, cell reactions or cell mobilizations were not detected at a distant location from the implant [6, 9, 18, 30]. Comparison of tissue reactions to PHB or polylactides, polyglycolides, or their copolymers shows that, on one hand, such reaction is rather moderate within a short time interval [9]; on the other hand, polylactides, polyglycolides, or their copolymers may induce chronic inflammation compared with PHB [31].

Results of subcutaneous implantation of PHB films for one month suggest that such samples are surrounded by a rather well organized and homogenous capsule (80–100 μm thick). This vascularized capsule consisted of connective tissues cells (round, young fibroblasts), which were oriented parallel to the implanted surface. Moderate inflammatory reaction was determined by the presence of mononuclear macrophages and lymphocytes. Three months after the implantation the fibrous capsule thickened and reached 180–200 μm due to the increase in the volume of capsule forming cells. A significant decrease in the inflammatory cell concentration was observed 6 months after the implantation with simultaneous decrease in the capsule thickness up to 80–100 μm. At that stage the capsule mainly consisted of collagen fibers and the number of connective tissue cells significantly decreased. A small inflammatory exudate was observed in tissues adjacent or adherent to the implant within 3–6 months after PHB implantation [6, 9]. Moderate biocompatibility of PHB was demonstrated during subcutaneous administration of PHB polymeric films of different molecular mass (300, 450, 1000 kDa). In this case the tissue reaction did not differ from the reaction of the same tissues to a control glass plate [18].

During contacts of PHB implants with bones tissue reactions promoted a high defect-healing rate due to rapid formation of novel bone tissue so that osteogenic characteristics of PHB exceeded the same characteristics of such synthetic plastics as polyethylene. On the initial step of implantation the implant surrounding medium consists of combination of soft connective tissue containing active fibroblasts and tightly screwed osteon bundles of 100 μm in diameter. Giant cells usually detected on the early stages of implantation were not found in this case. These connective tissue formations demonstrate time-dependent orientation becoming parallel to the implant surface. Three months after the implantation the bone tissue was formed not only near the polymer surface but more intensively at a certain distance from the implant and later (6 months and later) the implant was totally inserted into the newly formed bone tissue. These results suggest possible successful use of PHB-based materials as effective implants for bone tissues [32].

Fifteen years ago Swedish cardiologists reported that long-term observation (3–24 months) on the regeneration and newly formed vascular wall tissues revealed that the PHB transannular patches inserted into the ventricular outflow tract or pulmonary artery did not cause any pathologies compared with the similar tissues of the natural artery. Biodegradable PHB patches covering defects of cardiac septum or atrial walls also promoted cell regeneration, which resulted in formation of basically native tissues; this promoted restoration of the cardiac elements. These results suggest possibility of the PHB use as the scaffold material for tissue regeneration and growth of vessels and organs as evidenced by a significant number of modern studies [21, 23].

Similarly to cardiovascular implants, the PHB patches for reconstructive surgery of the gastrointestinal tract did not cause serious inflammatory reactions and initiate fibrosis as evidenced by animal experiments performed for 6 months [7, 33].

Development of nerve conduits, the directing channels for nerve fiber growth, represents another interesting direction of PHB application. Such recently developed procedure is especially important for treatment of the spinal cord injury. Restoration of nerve tissue growing in the cylindrical space of the PHB implant was observed within one month. The histological analysis of the implanted conduits revealed that they contain numerous myelinized axons and Schwann cells. In this case inflammatory processes were not registered. Moreover, nerve endings and conduit walls were spanned with a capillary system; this means that growth and restoration of nerve conduction channels was accompanied by progressive angiogenesis [12].

First experiments on administration of PHB microspheres were performed in the beginning of 90th of the last century. Their biocompatibility was investigated in [30] during implantation of rat femoral muscle. The PHB microspheres of 450 kDa were surrounded by one or (rarely) two layers of spindle cells. One week after the implantation the presence of mononuclear cells and inflammatory cells was detected. After four weeks the number of inflammatory cells decreased and the layers started to flatten. After 8 weeks the inflammatory cells were not detected in the encapsulated microsphere environment. These implants exhibited minimal toxicity towards normal tissues [3]. A later study also demonstrated low toxicity of injected PHB-based microspheres. Intraperitoneal administration of high doses (320 and 800 mg/kg) of PHB microspheres (35 μm in diameter) did not cause death of laboratory mice and changes of their weight [34].

Two facts have been noted considering biochemical signs of tissue reaction of animal tissues on implantation of PHB patches to the pericardium regions. First, the reaction of thrombomodulin, a multifunctional

protein exhibiting anticoagulant properties, was positive in both colonies of mesothelial cells and endothelial cells. The level of prostacyclin production responsible for cytoprotective effect in pericardium and prevention of pericardial adhesion was similar to that of native pericardium. This may be considered as the evidence for biocompatibility of PHB patches, because the inflammatory symptoms have not been observed macroscopically. Simultaneously, the level of the inflammatory process associated with proinflammatory cytokine mRNA concentrations insignificantly changed after implantation regardless of some increase in interleukin-1β and interleukin-6 [21]. Regeneration of the normal filament structure was observed using immunohistochemical methods [22], which demonstrated formation of the intermediate filament cytokeratin of epithelial and mesodermal cells. In addition, the layers of these cells also contain heparin-sulfate, a proteoglycan, which is a known marker for basal membranes [22].

PHB represents a perspective material for cell engineering owing to their high biocompatibility. This is confirmed by examples of cell cultures demonstrating satisfactory level of cell adhesion, proliferation and viability during contacts with PHB-based films and polymer cell scaffolds. These include mouse and human fibroblasts [6, 35, 36], mesenchymal stem cells [37], rabbit bone osteoblasts [38], human osteosarcoma cells [39], rabbit joint chondrocytes [40], and rabbit smooth muscle cells [41]. For PHB films it was also demonstrated that fibroblasts, endothelial cells, and isolated hepatocytes cultured on the surface of PHB films exhibit relatively high level of cell adhesion and growth [42].

High viability and proliferation of macrophages and fibroblasts was observed during their cultivation in the presence of low molecular weight PHB [43]. We also demonstrated that PHB biopolymer microspheres did not influence viability of human MCF-7 cells [34]. However, at cell concentrations from 1×10^3 to 2×10^5 cells per cm^2 the cell growth was relatively low [35]. Some impairment in the interaction between the PHB matrix and cytoskeleton of cultivated cells was demonstrated in [44]. At the same time such polymer characteristics as chemical composition, surface morphology, surface energy and polymer hydrophobicity significantly influence cell viability and growth [45]. Summarizing existing information we may conclude that this polymer, PHB, may be used as a cell scaffold under in vivo conditions for tissue engineering [35, 40].

It was demonstrated that morphology of the PHB surface significantly influenced cell adhesion and growth [46, 47] and various cells exhibited different "preferences" towards the structure surface. For example, preferred development of osteoblasts was observed on rough surfaces with suitable sizes of hollows and pores [46]; fibroblast and epithelial cells preferred smooth surfaces [47]. Such cell sensitivity to pore sizes and surface roughness is obviously associated with vital activity of cells, with need of gas exchange and also exchange of various substances (i.e. realization of some diffusion and hydrodynamic conditions required for nutrient supply) and features of protein adsorption [38, 40]. Sevastianov et al. reported that PHB films contacted with blood did not influence the cell component of the hemostasis system but could activate the coagulation system and complement reaction (i.e. act at the molecular level) [48].

Such multi-aspect assessment of PHB biocompatibility is determined by several reasons. First, PHB is a natural biopolymer involved into functional activity of pro- and eukaryotes [49]. Being a natural product, this biopolymer has a strict stereo-regular structure, which consists of residues of D-3-hydroxybutyric acid [50]. A low-molecular-weight PHB (up to 150 hydroxybutyrate monomer units) forming complexes with other biological molecules is an integral component of pro- and eukaryotes [49]. PHB complexes were found in various mammalian organs and tissues including human sources (blood, kidneys, vessels, nerves, lipoprotein particles, platelets, etc.). Concentrations of PHB complexes vary from 3–4 μg/g in nervous tissues and the brain to 12 μg/g in blood plasma [51, 52]. In human plasma concentration of the biopolymer varies widely from 0.60 to 18.2 mg/l (an average value of 3.5 mg/l) [52]. An intermediate of PHB biodegradation, D-3-hydroxybutyrate, has also been found in blood and animal tissues at the concentrations 0.3–1.3 mM [53]. Phosphate complexes of PHB can form ion channels in erythrocyte plasma membrane and hepatocyte mitochondrial membranes [54, 55]. The ability of these objects to form hydrophobic channels and to perform metabolite transfer through hydrophobic membrane regions determines their specific physiological niche in cell metabolism [49]. However, mechanism of their synthesis of eukaryotes is not well investigated. It is reasonable to suggest that PHB complexes represent one of symbiotic products originated due to interaction between animals and their intestinal microorganisms. This may be illustrated by the following example: *E. coli* cells can synthesize a low-molecular-weight PHB to perform certain functions in the bacterial cells [56].

As it has been demonstrated above PHB is characterized by a low rate of degradation in the body compared with degradation of polylactides and polyglycolides or their copolymers. A decreased biodegradation rate results in a decreased concentration of degradation products near the implant (in the case of PHB 3-hydroxybutyrate dominates) [9]. During rapid hydrolysis of polylactides and their copolymers hydrolytic products are not unitized in the body and this is accompanied by a sharp decrease of environmental pH near the implant. Chronic tissue irritation caused by the pH decrease is a serious problem associated with application of polyglycolactide-based polymer

implants [57]. Chronic inflammation as a response to polylactide and polyglycolide destruction may be accompanied by an immune response to release of non-stereo-regular water soluble oligomers formed due to degradation of these polymers [58].

2. PHB-BASED BIOPOLYMER SYSTEMS WITH ENCAPSULATED DRUGS

Use of biopolymers for encapsulation of various drugs opens wide perspectives for design of novel biopolymer systems in medicine. Development of injection systems for controlled release, in which drugs are encapsulated in microparticles (microspheres or microcapsules) obtained using biodegradable polymers, represents the foremost frontiers of modern pharmacology. Immobilization of biologically active components in a biopolymer matrix or reservoir followed by their subsequent release (desorption) from microparticles allows to create requested local concentrations of these drugs near the target organ; using this approach it is possible to maintain the requested concentrations for a long time. Prolonged target delivery of biologically active substances realized during their release from microparticles avoids some characteristic disadvantages typical for traditional methods of drug administration (peroral, injection, inhalation, etc. modes of drug administration), which include increased toxicity, drug instability and discontinuous mode of drug administration. Almost all these disadvantages may be overcome by creating microparticles with prolonged drug release; this is important for therapy of chronic diseases and also in the postoperative period [59]. Microparticles also have some limitations. For example, they should be rather homogeneous in size and the size of the largest fraction should not exceed 125 μm in diameter for local (subcutaneous or intramuscular) injections and 1 μm for systemic (intravenous) administration. Production of microparticles containing drug substances should employ reproducible, large-scale and sparing technology with high grade encapsulation [60].

In accordance with these requirements and due to good biocompatibility and controlled biodegradation capacity PHB represents a perspective material for production of polymer dosing systems in the forms of micro- and nanoparticles. A wide range of drugs is used as an active pharmacological component introduced into PHB and its derivatives. First, it includes a class of model compounds used for investigation of controlled release: 2,7-dichlorofluorescein [61], FITC-dextran [62], methyl red [63], 7-(hydroxyethyl)theophylline [64], coumarin [65]. Second, these are drugs used in therapeutic practice: antibiotics and antibacterial compounds (rifampycin [18, 66], tetracycline [67], cefoperazone and gentamicin [68], sulperazone and duocide [69], sulbactam [70], levofloxacine, metronidazole [18]), antitumor drugs (5-fluorouracil [71], 2',3'-diacyl-5-fluoro-2'-deoxyuridine [30], paclitaxel, dexamethasone, chlorambucil, doxorubicin, etoposide [34, 65]), anti-inflammatory agents (indomethacin [72]), analgetics (tramadol [73]), vasodilatators and antithrombotic drugs (dipiridamol [18, 72, 74]). Besides studies of kinetic profiles most of these systems were tested for biocompatibility and pharmacological activity [18, 30, 34, 66, 69, 70, 73]. It should be noted that PHB homologues (poly-3-hydroxyalkanoates) are used as a biodegradable matrix (microspheres) more frequently than the PHB homopolymer [30, 63, 64, 66, 72, 73]. A photo shows general view and morphology of the PHB biopolymer microspheres with the encapsulated drug, paclitaxel (Fig. 2).

Originally, PHB was used as a delivery system for controlled release in [64], which reported rapid release of encapsulated 7-(hydroxyethyl)theophylline from the PHB tablets with molecular weight of 2000 kDa; this was accompanied by the total mass loss of the subcutaneously implanted sample. Authors also made a preliminary conclusion that PHB with molecular mass exceeding 100 kDa is not suitable for long-acting dosage forms [64].

Pouton and Akhtar analyzed controlled release of low molecular drugs from a PHB matrix characterized by increased porosity and high water permeability [75]. Retention and desorption of a model compound (methyl red) from the PHB matrix formed by crystallization of a polymer melt depended crystallization kinetics and crystal phase morphology [63]. Surprisingly, a total crystallization degree did not influence the release rate. It is well known that crystal regions of PHB are impermeable for drug molecules and therefore these regions represent obstacles to drug diffusion transport. Taking into consideration high porosity this result may by explained as a consequence of drug substance movement in the aqueous phase filling the formed pore system and therefore in this situation we should observe a high rate of release and crystallites do not prevent movement of the drug substance. Indeed, the high rate of release (so-called the "burst" effect) on the initial step of drug release followed by the subsequent phase of a linear release do not contradict our suggestion.

More detailed analysis of chemical structure of a drug substance and molecular mass of the polymer on controlled release has been demonstrated in [30]. Microspheres (of 100–300 μm in diameter) prepared using PHB with various molecular masses (65, 135, and 450 kDa) were loaded with a prodrug, 5-fluoro-2'-deoxyuridine (FDU). In dependence of nature of a substituted radical in the ester group hydrophilicity (solubility in water) changed from 70 mg/ml for FDU to 0.1 mg/ml for butyryl-FDU. Thus, this study demonstrated that the rate of drug delivery may be regulated by changing hydrophilicity/hydrophobicity balance in the order propionyl-FDU > butyryl-FDU > pentanoyl-FDU. In addition, molecular mass of PHB

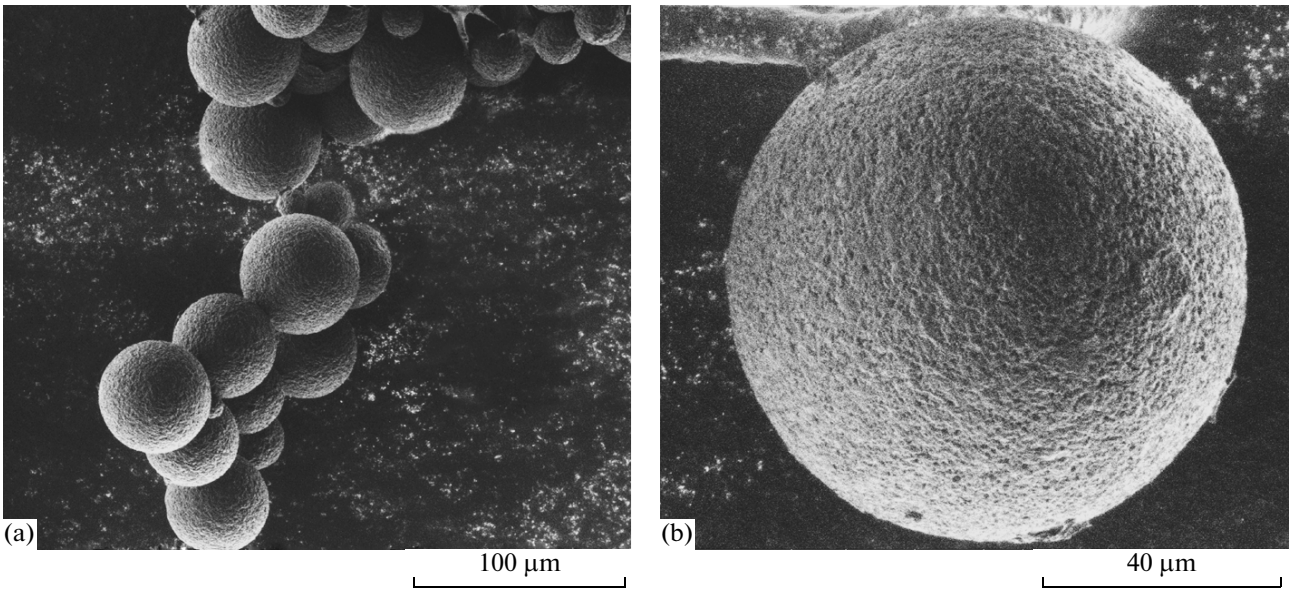

Fig. 2. PHB-based microspheres with encapsulated paclitaxel (scanning microscopy): (a) general view, (b) individual microsphere (adapted from [34]).

also influenced controlled release. In the case of FDU derivatives, drug release from microspheres obtained using low-molecular weight PHB (65 kDa) was significantly higher than that from microspheres prepared using high molecular weight PHB (135 and 450 kDa). The importance of this study also consists in demonstration of an accelerating effect of increased drug concentration on the rate of its release [30].

Pharmacological activity of drugs in combination with PHB biocompatibility has been investigated in several studies [18, 30, 66, 73]. These studies demonstrated that implanted films of this biopolymer containing dipiridamol or indomethacin induced a rather moderate response of surrounding animal tissues. Inflammatory process was observed during a limited time interval and toxicity was minimal [18]. In the case of microspheres loaded with the analgesics (tramadol) toxicity was not registered at all. Use of analgesic loaded microspheres is very promising as the analgesic effect of the drug encapsulated into microparticles was observed during 21 h, whereas an equivalent dose of injected tramadol maintained the analgesic effect only during 5 h [73]. Intraperitoneal administration of PHB microspheres (30 μm in diameter) with the encapsulated antitumor drug, paclitaxel (10% w/w) at the dose of 320 mg of microspheres per kg or 32 mg of drug substance per kg did not cause death of laboratory mice. Intraperitoneal administration of the same dose of paclitaxel as the traditional drug dosage form Taxol® (32 mg of drug substance per kg) caused death of 100% of animals. Effectiveness of PHB biopolymer microspheres with encapsulated antitumor drugs (paclitaxel, etoposide, dexamethasone, chlorambucil, and dipiridamol) was demonstrated in experiments with human breast cancer cell line MCF-7. Using the MTT method it was demonstrated that cell cultivation with PHB microspheres with all encapsulated drugs (10%, w/w) for 48 h at the microsphere concentration of 3 mg/ml (0.3 mg of drug substance per ml) resulted in significant inhibition of MCF-7 cells proliferation. The cell survival represented 4% (microspheres with chlorambucil), 34% (microspheres with paclitaxel), 55% (microspheres with etoposide), and 60% (microspheres with either dexamethasone or dipiridamol) of control [34].

Use of PHB microspheres as an embolization system followed by subsequent histopathological studies was reported in [66]. Comparison of renal angiograms obtained before and after embolization and similar histopathological observations revealed high efficiency of PHB microspheres as the chemoembolization agent, i.e. as a matrix that can cause embolization of blood vessels and desorb a drug substance to the embolizing region simultaneously.

Development of biodegradable polymer-based biopolymer systems for prolonged release of biologically active proteins and nucleic acids is a relatively novel and actively developing field of pharmacology. On the basis of PHB matrices and microcapsules systems with encapsulated bovine serum albumin (BSA) and horseradish peroxidase (HRP) have been created. These systems demonstrate prolonged release of BSA and HRP for 7–10 days [76]. Unfortunately, these are the only examples of such PHB based system found in the literature. However, other systems based on PHB copolymers do exist [77].

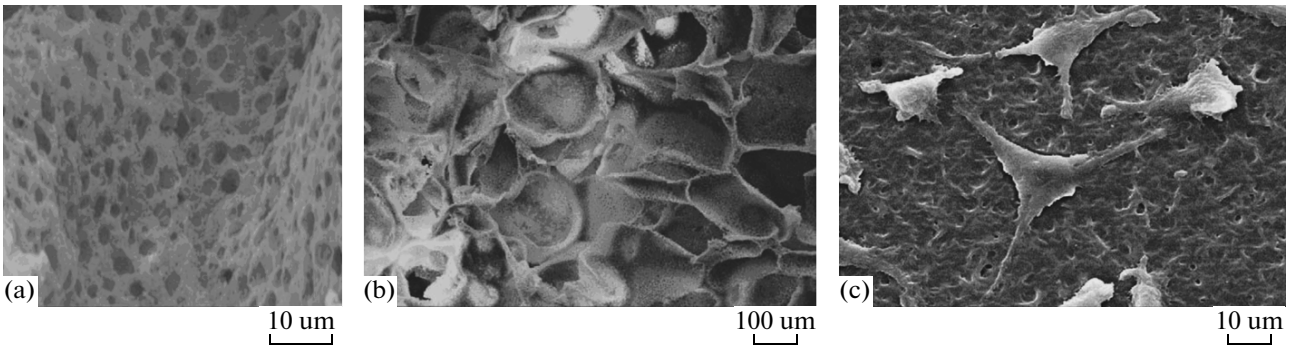

Fig. 3. PHB blendes used as 3D-scaffolds for tissue engineering; (a) 3D-scaffold based on the PHB blend with hydroxyapatite; (b) 3D-scaffold based on the PHB blend with its copolymer, 3-hydroxyhexanoate, (c) mouse L929 fibroblasts cultivated on the 3D-scaffold based on the PHB blend with its copolymer, 3-hydroxyhexanoate (adapted from [38, 82]).

4. BIOPOLYMER SYSTEMS BASED ON PHB BLENDS WITH OTHER BIOPOLYMERS AND MATERIALS

Active construction of PHB blends with other biopolymers started after assessment of their capacities and perspectives of medical applications. PHB blends were mainly constructed in order to increase its mechanical properties (preferentially for increased plasticity), polymer hydrophilicity and the rate of its biodegradation.

A blend of bacterial high-molecular weight (>100 kDa) PHB with chemically synthesized amorphous low-molecular weight (10 kDa) atactic PHB (atPHB) is the simplest system. Smith et al. prepared and investigated in vitro and in vivo the PHB composites with atPHB and their medical devices. The resultant composites demonstrated high elasticity and high biodegradation rates. For example, in bacterial PHB the elastic modulus and the elongation at break were 3350 MPa and 1.5%, respectively, whereas a composite prepared using equal parts of PHB and PHB was characterized by the elastic modulus and the elongation at break of 660 MPa and 10.9%. A study of the rate of hydrolytic destruction of PHB revealed that the loss of sample mass and molecular weight of bacterial PHB were < 1% and 64%, respectively, whereas in the case of the blend these parameters were 12 and 93%, respectively. Authors explained these differences by a significant decrease of total crystallinity in the composite (from 70% in PHB to 40% in the composite) and by the increase in the number of amorphous regions [9, 10].

Most of studies were focused on the development and investigation of PHB blends with polylactides and polyglycolides (most widely used biodegradable polymers) [78, 79]. Creation of these composites was carried out using atPHB. For example, a resultant polylactide-based blend with atPHB had lower crystallinity and the highest biodegradation rate [78]. Blends of bacterial PHB and polylactide also demonstrated improved mechanical properties [80].

Biosynthesis of various PHB copolymers, exhibiting altered mechanical, adsorption, and biological properties resulted in the creation of blends of various PHB copolymers with PHB homopolymers. These include the PHB composite with a PHB copolymer with 3-hydroxyhexanoate and the PHB composite with a PHB copolymer with 4-hydroxybutyrate. It was demonstrated that scaffolds for tissue engineering prepared using nanometer fibers (50–500 nm thick) were characterized by improved mechanical characteristics, higher rates of biodegradation and improved biocompatibility than polylactide-based polymeric scaffolds [81]. Chinese researchers demonstrated better adhesion, extracellular matrix formation and growth of cell cultures (fibroblasts, chondrocytes, osteoblasts) on polymer beads and 3D-scaffolds prepared using PHB composites with its copolymer, 3-hydroxyhexanoate, as compared with PHB (Fig. 3) [35, 38, 82].

The creation of a PHB composite with a hydrophilic polymer, polyvinyl alcohol, allowed to regulate permeability of this polymer material by varying polyvinyl alcohol content in the composite from 0 to 20% (w/w) [83].

Besides atPHB, PHB copolymers with polylactides other polymers were also used for creation of PHB blends with: poly(*p*-dioxanone) [84], poly(ε-caprolactone) [79], polyvinyl alcohol, polyethylene, polyamide [83], cellulose acetate butyrate [85], collagen and even soy nutrition proteins [86]. Thus, creation of PHB blends significantly extends application of PHB-based polymer systems.

5. BIOPOLYMER SYSTEMS BASED ON PHB NANOCOMPOSITES WITH INORGANIC NANOPARTICLES

Design of nanocomposite polymers with inorganic nanoparticles is a new perspective direction in polymer science and technology. Administration of inorganic nanoparticles into a polymeric matrix provides requested changes in physicochemical and biological features of biopolymers: mechanic properties, thermal

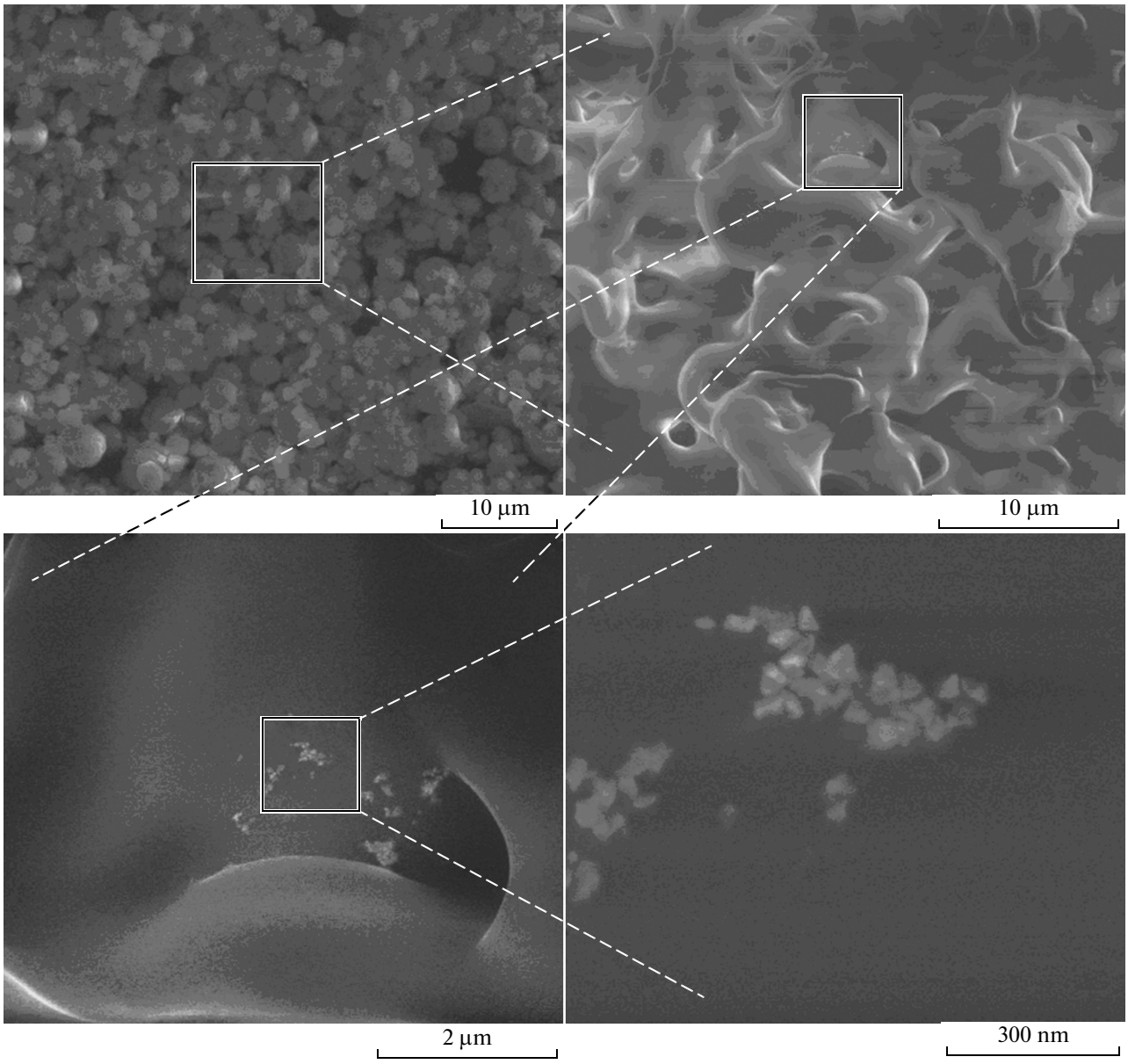

Fig. 4. Gold nanoparticles in the microsphere PHB polymer matrix loaded with paclitaxel (scanning electron microscopy; a series of photos with sequentially increased magnification; at high magnification biopolymer microspheres melted and gold nanoparticles appeared in the polymer matrix) (adapted from [94]).

properties, chemical stability, permeability for liquids and gases, surface properties, biocompatibility and biological activity. In addition, nanoparticles may impart principally new properties to the polymer material (e.g. magnetic properties or electric conductance). The following nanoparticles have been used for creation of nanocomposites with polymers: clay nanoparticles (montmorillonit), hydroxyapatite, silicon oxide, gold and silver nanoparticles, metal oxide (titan oxide, iron oxide, aluminum oxide, etc.) nanoparticles, carbon nanotubes, and fullerenes. The examples of such systems include polylactide and polyglycolide nanocomposites with gold nanoparticles [87] and iron oxide nanoparticles [88]. Biopolymer systems with inorganic nanoparticles are not limited by biopolymer nanocomposites with nanoparticles. It is possible to develop more complex systems, for example, based on biopolymer microcapsules with encapsulated inorganic nanoparticles [89]. In recent years PHB has been used for creation of biopolymer systems based on PHB nanocomposites with inorganic nanoparticles [5].

PHB with encapsulated hydroxyapatite nanoparticles, montmorillonit, and bioactive glass was one of the first PHB based nanocomposites. Incorporation of nanosized hydroxyapatite into the biopolymer

improved its mechanical properties, increased water absorption and hydrophilicity, and also increase the rate of biodegradation. PHB with encapsulated hydroxyapatite nanoparticles also acquired biological activity and promoted acceleration of regenerative processes during implantation and bone replacement [90]. Insertion of montmorillonit nanoplates also significantly improved mechanical properties of PHB but decreased the rate of material biodegradation [91]. Misra et al. compared biocompatibility of a PHB nanocomposite with bioactive glass nanoparticles and a traditional PHB composite with the bioactive glass. It should be noted that the bioactive glass is a mineral that consists of calcium and sodium phosphates with silicon oxide; it may represent particles of micrometer and nanometer sizes. These researchers demonstrated that the nanocomposite exhibited better mechanical properties, better water absorption, higher absorption capacity for protein and MG-63 cells; this suggests better biocompatibility and higher biological activity compared with the traditional composite [92]. In this connection it should be noted that clay and hydroxyapatite microparticles have been already used for creation of composites with PHB (Fig. 3a) [93].

Unfortunately, the use of nanomaterials for creation of nanocomposites with PHB is mainly limited by montmorillonit and hydroxyapatite. In this connection it should be noted that such nanoparticles as iron oxide or gold nanoparticles could acquire completely new properties to PHB-based materials. We have developed PHB-based biopolymer systems with gold, iron oxide, titan oxide nanoparticles, and also fullerene nanoparticles. The biopolymer system based on PHB microspheres with encapsulated antitumor drug paclitaxel and gold nanoparticles is one of the best our systems. On a series of photos with sequentially increased magnification (Fig. 4) one can see gold nanoparticles encapsulated in the polymer matrix of the PHB microspheres [94].

Perspectives of the use of these systems are associated with inorganic nanoparticles, which may perform new functions regardless their binding at the polymer matrix (or PHB microcapsules) or release during PHB biodegradation. For example, gold nanoparticles, may represent a diagnostic agent for the biopolymer system or during their release they may be used for photothermal therapy of tumors during electromagnetic radiation. Released titan oxide nanoparticles may be used for photodynamic therapy of tumors also during electromagnetic radiation. Iron oxide nanoparticles may be used for targeted delivery of micro- and nanoparticles of the biopolymer system and identification of these particles in various tissues and organs and during their release for thermal therapy of tumors treated with variable electromagnetic field of high frequency. During encapsulation of various drug substances with inorganic nanoparticles into the biopolymer a real possibility of combination of various methods of therapeutic and other functional effects in a single biopolymer system appears. This includes chemotherapy, physicochemical modes of therapy (photothermal, photodynamic, and magnitothermal), and also targeted delivery and diagnostics.

ACKNOWLEDGMENTS

This work was supported by the Russian Federal Agency for Science and Innovations of Russian Federation (State contract no. 02.512.12.2004 of June 10, 2008) and also by the Ministry of Education and Science of Russian Federation (State contact nos. P2266 of November 13, 2009, P2429 of November 19, 2009, and P548 of May 17, 2010).

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