Polyhydroxyalkanoates: Biodegradable Plastics and Their Applications

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Abstract

Human civilization has experienced several ages such as stone, bronze, iron, and steel, and now it is a plastic age. Due to multifaceted properties of plastic, it is one of the most widely available and overused item in the world today and became a necessary evil. When disposed, it does not decompose and pollutes the land or air nearby when burned in the open air. Plastic pollution is affecting the global economy. Polyhydroxyalkanoates (PHAs) have been drawing much attention as

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L. M. T. Martínez et al. (eds.), *Handbook of Ecomaterials*,
https://doi.org/10.1007/978-3-319-68255-6_84

biodegradable substitutes for conventional nondegradable plastics. PHAs are synthesized by numerous bacteria as carbon and energy storage capsules and are good candidates as biodegradable plastic material. Because of their versatility and wide range of properties, biodegradable PHAs are being used in various areas of modern benefits. With the currently increased interest level and the extensive research being carried out in this area, PHAs are potentially emerging as environmentally friendly materials of the next generation with a wide range of applicability. This chapter deals with the recent advances in applicability of PHAs.

List of Abbreviations

3HV	3-Hydroxyvaleric acid
3HA	Hydroxyalkanoic acid
3HB	3-hydroxybutyric acid
3HD	3-Hydroxydecanoic acid
3HO	3-Hydroxyoctanoate
4HB	4-Hydroxybutyric acid
GHB	Gamma-hydroxybutyric acid
HB	3-Hydroxybutyrate
HV	3-Hydroxyvalerate
P(3HB-3 HV-3HHx)/ P(3HB-co-3 HV-co-3HHx)	Poly-3hydroxybutyrate-co-3hydroxyvalerate-co-3hydroxyhexanoate
P(3HB-3 HV-DHB)	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-2,3-dihydroxybutyrate)
P(3HB-3HO)	Poly-3hydroxybutyrate-co-3hydroxyoctanoate
P(3HB-4HB-3 HV)	Poly-3hydroxybutyrate-co-4hydroxybutyrate-co-3hydroxyvalerate
P(3HB-*co*- 3 HV)/ P(3HB-3 HV)	Poly-3hydroxybutyrate-co-3hydroxyvalerate
P(3HB-co(4HB)	Poly(3-hydroxybutyrate-co-4-hydroxybutyrate)
P(3HDD-*co*-3H9D)	Poly-3-hydroxydodecanoate-3-hydroxy-9-decenoate
P(3HO)	Poly(3-hydroxyoctanoate)
PE	Polyethylene
PGA	Polyglycollic acid
PHA	Polyhydroxyalkanoate
PHB/P3HB	Poly (3- hydroxybutyrate)
PHBHHx/P(3HO-co-3HHx)	P(3-hydroxybutyrate-co-3-hydroxyhexanoate)
PHBV	Poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid)/ polyhydroxybutyrate-co-valerate
PLA	Polyvinylacetate
PP	Polypropylene
pTHPP	5,10,15,20-Tetrakis(4-hydroxy-phenyl)-21H, 23H–porphine

Introduction

Plastics are utilized in almost every aspect of human life, from automobiles to medicine. Plastics score as materials of everyone's choice due to their useful characteristics, such as chemical resistance, elasticity, durability, moldability, and cost effectiveness. What makes plastics undesirable is the difficulty in their disposal. Plastics being xenobiotic are recalcitrant to microbial degradation [35]. The longevity of conventional plastics is estimated to be hundreds to thousands of years, but it seems to be even longer in the deep sea [5]. Waste from conventional petrochemically produced plastics accumulates in nature leading, for instance, to pollution of 165 million tons of plastics in the world's oceans [48]. Besides, their manufacture leads to increased usage of petroleum ending up in the slow depletion of the fossil fuels and thereby increasing the cost of petrochemicals. The menace of pollution has necessitated the development of biodegradable substitutes. Bio-based polymers represent an appropriate answer to the conventional plastics because most of them are biodegradable and even renewable, while some of them offer very similar properties as conventional petroleum-based polymers. Numerous types of biodegradable polymers are available including polylactides, polyglycolic acids, polyhydroxyalkanoates (PHAs), aliphatic polyesters, and polysaccharides.

Among the various groups of biopolymers, PHAs are unique. The PHAs are produced and accumulated intracellularly by a large number of microorganisms as energy and carbon reserves under nutrient stress conditions. PHAs are synthesized entirely by a biological process that involves conversion of carbon sources directly into PHA through microbial fermentation [105]. Up to 90% of the cell dry weight PHAs can be accumulated intracellularly under conditions of nutrient stress [24, 64]. The molecular mass of PHA is in the range of 50,000–1,000,000 Da and varies with the microbial growth conditions, PHA producer, and downstream processes. The monomer units are all in D-(−) configuration owing to the stereospecificity of biosynthetic enzymes [24, 75, 97, 121]. The PHAs are nontoxic, biocompatible, biodegradable thermoplastics, and have a high degree of polymerization, are highly crystalline, optically active and isotactic (stereochemical regular in repeating units), peizoelectric, and insoluble in water. These features make them highly competitive with polypropylene, the petrochemical-derived plastic.

The first PHA, PHB was discovered in *Bacillus megaterium* by the French scientist Lemoigne in 1926 [56]. Since then over 150 different kinds of PHAs have been discovered [55, 70, 78]. Basically, PHAs comprise of (R)-3-hydroxyalkanoic acid monomers of C_3 to C_{14}. Their general structure is shown in Fig. 1. The identity of a typical monomer unit of a PHA is determined by the alkyl side chain, R, which can be saturated, unsaturated, straight, or branched, containing aliphatic or aromatic side groups or halogenated, epoxidized, and branched monomers [55]. The value of n (in Fig. 1) depends on the pendant group (R in Fig. 1) and the microorganisms in which the polymer is produced. It usually varies from 100 to 30,000 [55] (Table 1).

Based on the chain length of the fatty acid monomers, PHAs can be classified into three categories: short-chain-length (scl) PHAs with 3–5 carbon atoms, medium-

Fig. 1 General structure of PHA

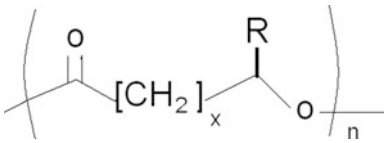

Table 1 The general structure of members of PHA family

SN	Type of alkyl group	PHA candidate	Popular name
1	R = H; x = 1	Poly(3-hydroxypropionate)	P3HP
2	R = CH_3	Poly(3-hydroxybutyrate)	P3HB/PHB
4	R = C_3H_7	Poly(3-hydroxyhexanoate)	P3HHx
5	R = C_5H_{11}	Poly(3-hydroxyoctanoate)	P3HO
6	R = C_7H_{15}	Poly(3-hydroxydecanoate)	P3HD
7	R = $C_{11}H_{23}$	Poly(3-hydroxytetradecanoate)	P3HTD
8	R = $C_{15}H_{31}$	Poly(3-hydroxyoctadecanoate)	P3HOD
9	R = H; x = 2	Poly(4-hydroxybutyrate)	P4HB
10	R = H; x = 2	Poly(5-hydroxyvalerate)	P5HV

chain-length (mcl) PHAs with 6 to 14 carbon atoms, and long-chain-length (lcl) PHAs with more than 14 carbon atoms [80] .

Properties of Polyhydroxyalkanoates

Physical Properties

After extraction from bacterial cell, PHA can appear in various forms according to its composition and process of purification. These polymers are characterized by their thermal properties, such as the glass transition temperature (*Tg*) of the amorphous phase and the melting temperature (*Tm*) of the crystalline phase. Most of PHAs behave as thermoplastics with melting temperatures between 50 °C and 180 °C. For example, P3HB has properties similar to polypropylene due to a similar melting point, tensile strength, and glass transition temperature.

Biocompatibility

Even though the PHAs are synthesized by biological cells, in order to use them in biological systems, their biocompatibility study is necessary. An ideal biocompatible material must not cause severe immune reactions when introduced to soft tissues or blood of a host organism [94]. Some studies have showed that PHA-based molecules are not toxic and are widely distributed in biological cells [13, 49]. Moreover, PHA molecules are able to pervade aqueous and hydrophobic environment of the cell membrane and, therefore, are present in cytoplasm and intracellular fluids as well as in membranes and lipoproteins [13, 49]. For example, P4HB and P3HB are

biocompatible and extremely well-tolerated in vivo, given that their hydrolysis yields 4HB and 3HB, respectively, which are common metabolites occurring naturally in the human body and suggests nontoxicity of implanted biopolymers [55, 73]. These are also found to be immunologically inert and resorbable in vivo [115]. In vitro tests have shown that P3HB was nontoxic in various human cell lines, including osteoblasts, fibroblasts, epithelial cells, and ovine chondrocytes [72, 90].

Biodegradability

As per ISO norms, a polymer is classified as biodegradable when "the breakdown by microorganisms in the presence of oxygen leads to carbon dioxide, water and mineral salts of any other elements present and new biomass" [92, 108]. The property that distinguishes PHA from petroleum-based plastics is their biodegradability that allows microorganisms to break down the polymer into simple components upon exposure to soil, compost, or marine sediment.

Biodegradation of PHAs under aerobic conditions results in carbon dioxide and water, whereas in anaerobic conditions the degradation products are carbon dioxide and methane. Studies have shown that 85% of PHAs were degraded in 7 weeks [35, 46]. The degradation rate depends on many factors, viz., first, the physical properties of the polymer itself, in particular to its surface area, its molecular weight, its monomeric composition, and its crystallinity and second, it depends on environmental conditions such as temperature, moisture level, pH, and available nutrients [91, 148].

Modifications of PHAs

PHAs are still far away from replacing petroleum-based polymers such as PP or PE in a competitive manner. Various PHA blends have been developed to improve their performance. The blending of PHAs will offer more scope to expand their range of applications. PHB is the simplest homopolymer of the PHA family, with high degree of crystallinity resulting in a stiff and brittle nature. The copolymers of PHB can be made more flexible and less crystalline by the introduction of long alkyl side chain such as HB and HV leading to the formation of P(3HB-co-4HB) and P(3HB-co-3 HV), respectively. The P(3HB)/PLA blend is one of the most studied blends, which exhibits mechanical properties that are intermediate between the individual components. Zhao et al. [145] reported the preparation of a P(3HB-*co*-3 HV)/PLA blend using a corotating twin-screw extruder and found better performance. Zembouai et al. [140] reported that the thermal stability of the P(3HB-*co*-3 HV)/PLA blends could be improved by increasing the amount of PLA. The T_m for the blend was found to be in between these two polymers. Ethyl cellulose (EtC) is also a biomaterial like P(3HB) that is approved by the FDA (Food and Drug Administration) and is widely used as a blood coagulant, in coatings for pharmaceutical tablets, and matrices for poorly soluble drugs. Zhang et al. [142] had investigated the

miscibility, thermal behavior, and morphological structure of P(3HB) with ethyl cellulose (EtC) blends.

Market Status of PHA

As preliminary efforts, development and commercialization of PHB got a push initially in 1950s by W.R. Grace Co., a North-American Company. This process was not feasible owing to little production as well as lack of appropriate purification technique. In 1970, Imperial Chemical Industries (ICI, UK) started manufacturing PHA polymers under the trade name of BIOPOL® by *Cupriavidus necator* NCIB 11599 from diverse carbon substrates like butyrolactone 1,6-hexanediol, etc. Today, there are several companies, viz., Metabolix Inc. (USA), Shenzhen Ecomann Technology Co. Ltd. (China), Tianjin GreenBio Materials Co. Ltd. (China), Meredian Inc. (USA), and Biomer (Germany), which are involved in the PHA bioplastic business. Two companies, USA-based Metabolix Inc. and China-based Shenzhen Ecomann Technology, have shown maximum growth and developments (*Global Trends* & *Forecasts to 2018*) (Table 2).

Applications of Polyhydroxyalkanoates

PHAs resemble plastics, yet are biodegradable and biocompatible. This makes them an attractive agent in medical, packaging, and in many other applications [136, 148]. It is difficult to differentiate the applications of PHA as many of these are interlinked. In this chapter, we have divided the applications broadly into medical and other nonmedical commercial applications with subheadings.

Medical and Healthcare Applications

Cardiovascular Applications

Cardiovascular tissue engineering aims to provide new and better approaches for treating various diseases of the cardiovascular system. For therapeutic and regenerative purposes, PHAs pose solutions with multiple advantages over synthetic materials owing to their biocompatible nature.

Vascular grafting is carried out to repair or replace damaged, diseased, or malfunctioned blood vessels in the arterial or venous systems. Tim et al. [114] used a polymeric scaffold consisting of two components in a tubular conduit for tissue engineering the abdominal aorta in a lamb model. The inner layer was fabricated with a nonwoven PGA mesh and three layers of nonporous P(3HO-co-3HHx), with 10% of 3-hydroxyhexanoic acid, in the outer layer. The scaffolds were seeded with autologous cells and kept implanted for up to 5 months. The grafting results were promising since all the P(3HHx-co-3HO)/PGA grafts were found to

Table 2 Commercial producers of PHAs

Product	Producer	Commercial name	Country
Biomer P209; Biomer P226; Biomer P240	Biomer	Biomer	Germany
BIOCYCLE 1000; BIOCYCLE 18 BC-1; BIOCYCLE 189C-1; BIOCYCLE 189D-1	PHB industrial	Biocycle	Brazil
ENMAT Y1000; ENMAT Y1000P; ENMAT Y3000; ENMAT Y3000P	Tianan biological material Polyone	Ecogen	China
P(3HB)	Jiangsu Nantian group	Jiangsu Nantian	China
MINERV-PHA™	Bio-on	Minerv-PHA	Italy
Biogreen	Mitshubishi gas	Biogreen	Japan
Mirel P4001; Mirel P4010; Mirel P5001; Mirel P5004; Mirel M2100; Mirel M2200; Mirel M4100	Metabolix	Mirel	USA
Mvera™ B5011; Mvera™ B5010	Telles LLC	Metabolix	USA
Nodax™	P&G chemicals	Nodax	USA/ Japan
(PHB88/PHV12)	Goodfellow Cambridge ltd	Goodfellow	UK

allow unrestricted blood flow (except in one case), and no inflammatory responses were observed.

Stock et al. [107] used (3HO-co-3HHx) with 10% of 3-hydroxyhexanoic acid, as a component of an autologous cell seeded tissue engineered vascular graft in lambs. During the course of study, proteoglycans/glycosaminoglycans (component of extracellular matrix) increased and reached a maximum value at day 112 in the tissue engineered conduit. Qu et al. [85] also investigated the role of P(3HB-co-3HHx) for vascular grafting.

Tissue engineered heart valves are deemed to have the potential to overcome shortcomings of prosthetic valves and homograft valves that are currently being used for valve replacements. One of the early studies using an elastomeric P(3HO) for the fabrication of a tri-leaflet heart valve scaffold was carried out by Sodian et al. [104]. Vascular cells were harvested from ovine carotid arteries, expanded in vitro, and seeded onto the heart valve scaffold. When this artificial tri-leaflet scaffold was incorporated in the test animal model, all animals survived the procedure and the valves showed minimal regurgitation. Stock et al. [106] also evaluated the feasibility of creating tri-leaflet, valved, pulmonary conduits from autologous ovine vascular cells and P(3HO) in lambs. Histological examination showed the development of thrombus formation on all three leaflets after 4 weeks. Wu et al. [131] developed hybrid valves, which were fabricated from decellularized porcine aortic valves coated with P(3HB-co-3HHx). The in vitro test to study the biomechanical performances of the hybrid valve revealed that coating with P(3HB-co-3HHx) increased

the tensile strength of the valve. For the in vivo study, hybrid valve conduits were implanted in the pulmonary region in sheep without a cardiopulmonary bypass. Recently, Harrington et al. [40] disclosed a stent that included sparse comb PHA systems. The stent included a stent body, scaffold, or substrate made partially or completely of polymer material including PHA.

Tissue Engineering

PHAs are star biomaterials to be used in tissue engineering applications because of their biodegradability, biocompatibility, and low cytotoxicity. In a study carried out by Lomas et al. [61] the suitability of P(3HB-co-3HHx) and collagen hybrid constructs was investigated with a view to creating a future tissue-engineered product. Here, human embryonic stem cells (hESCs), spontaneously differentiated hESCs (SDhESCs), and mesenchymal stem cells (hMSCs) were tested. It was shown that hMSCs and SDhESCs demonstrated good long-term viability with an overall lack of stimulus independent differentiation.

Unmodified/raw mcl-PHAs, without any copolymerization, were tested as a novel scaffold for tissue engineering applications using MSCs. The data revealed that this biomaterial supports cell attachment [71]. Ceftiofur-loaded P(3HB-co-3 HV) microparticles (PHBV-CEF) have been formulated and successfully developed as a drug delivery system to treat infectious diseases. The in vitro release experiment showed a sustained release profile of ceftiofur for 7 days, and the antimicrobial test demonstrated that ceftiofur kept its antibiotic activity after the encapsulation process, and the cell proliferation assay showed a very low cytotoxicity of PHBV-CEF on Hep-G2 cells with an IC50 $>$ 10 mg/mL [117]. A model anticancer drug, ellipticine, was successfully encapsulated in P(3HB-co-3 HV) to increase the cytotoxic effect of ellipticine by increasing its bioavailability [66]. Dinjaski et al. [30] reported that a naturally functionalized bacterial polyhydroxyalkanoates (PHACOS) inhibited the growth of methicillin-resistant *Staphylococcus aureus*. The authors observed that the more antimicrobial thioester ligands linked to the polyester back bone, the more was the effective biocide activity of PHACOS. Furthermore, Pramual et al. [83] suggested that pTHPP-loaded PHAs nanoparticles exhibited high photocytotoxicity against HT-29 cancer cells and suggested an attractive biopolymerin photodynamic therapy, as an alternative to conventional cancer treatments.

Bone is a complex, living, constantly changing tissue, which consists of cancellous and cortical bone. This architecture allows the skeleton to perform its essential mechanical functions. Chemically, bone is composed of an inorganic mineral phase of hydroxyapatite (HA) (60% by weight) and an organic phase, which is mainly type I collagen. There have been a variety of studies on scaffold preparation to address the problem of bone loss. In an investigation by Wang et al. [126], rabbit bone marrow cells were inoculated on 3D scaffolds of PLA, P(3HB) and P(3HB-co-3HHx), to evaluate their in vitro biocompatibilities. The results showed that the cells on the P (3HB-co-3HHx) scaffolds were able to maintain typical osteoblast phenotypes: round cell shape, high alkaline phosphatase activity, strong calcium deposition, and fibrillar collagen synthesis. Lu et al. [62] evaluated the effect of HA and

orientation of fibers on cell proliferation and differentiation in vitro. MSCs were seeded on scaffolds made from blends of P(3HB-co-HV) and HA. The results confirmed that the MSCs attached and proliferated more favorably on random-oriented P(3HB-co-3 HV) nanofibrous meshes without HA. Recently, the adhesive strength of bone-implant interface and in vivo degradation of P3HB composites was investigated by Meischel et al. [67]. Their results showed that P3HB composites with ZrO_2 and a high percentage of Herafill® (30%) (a composite made of calcium sulfate, calcium carbonate, and glycerol tripalmitate) showed the highest values of bone accumulation around the implant and no significant degradation of the implants was found after 36 weeks in vivo. However, improvement of the mechanical properties of the studied P3HB composites is necessary in order to obtain an appropriate load-bearing material.

Cartilage Repair

Cartilage is the slippery tissue that covers the ends of bones in a joint. Several investigations have been carried out on three-dimensional polymer scaffold systems consisting of a blend of P(3HB) and P(3HB-co-3HHx) for their use as a matrix for cartilage tissue engineering [27, 28, 146]. Among the first of such studies carried out was by Deng et al. [27], who showed that chondrocytes proliferated better on the blend P(3HB)/P(3HB-co-3HHx) scaffolds than on the simple P(3HB) scaffold, and the blended scaffold was able to promote both extracellular matrix formation on the surface and regeneration of chondrocytes on the inside. In another study [28], rabbit articular cartilage (RAC) was seeded on blend P(3HB)/P(3HB-co-3HHx) scaffolds (weight ratios 1:0, 2:1, 1:1, 1:2, 0:1) to investigate the production of the extracellular matrix of articular cartilage chondrocytes in vitro. Biochemical analysis and RT-PCR confirmed that the blend polymer of P(3HB)/P(3HB-co-3HHx) was capable of initiating a redifferentiation process, which allowed chondrocytes to express and produce type II collagen to a higher extent than the P(3HB)-only scaffold (the control). Zhao et al. [146] have studied RAC on a 3D scaffold matrix made of P(3HB-co-3HHx)/P(3HB) consisting of 60 wt% P(3HB-co-3HHx). EDX analysis of the extracellular matrix on the scaffolds demonstrated a high level of calcium and phosphorus elements in a Ca/P molar ratio of 1.66, which is approximately equal to that of natural hydroxyapatite, which has a Ca/P ratio of 1.67. This suggests that P(3HB-co-3HHx)/P(3HB) scaffolds can be effectively used for regeneration of cartilage [146]. Matrices of collagen-containing calcium phosphate (CaP-Gelfix) and matrices of PHBV were produced to create a cartilage via tissue engineering [50]. Macroscopic examination showed that PHBV (with or without chondrocytes) maintained its integrity for 21 days, while CaP-Gelfix got deformed and degraded within 15 days. PHBV was found to have better healing response than CaP-Gelfix. Using electrospinning of blends of P(3HB)/P(3HO), Ching et al., [17] produced polymer scaffolds and optimized their structure, stiffness, degradation rates, and biocompatibility. Scaffolds with ratio of 1:0.25 of P(3HB)/P(3HO) exhibit randomly oriented fibers that closely mimic the collagen fibrillar meshwork of native cartilage and match the stiffness of native articular cartilage too.

Ophthalmological Applications

The eye is a complex and highly evolved organ. Common eye disorders may arise from anatomical changes to the cornea and lens. Retinal pigment epithelial cells (RPE) constitute a simple layer of cuboidal cells that are strategically situated behind the photoreceptor (PR) cells [8]. RPE degeneration causes retinal disorders. Tezcaner et al. [112] reported the growth of retinal pigment epithelium cells on PHBV8 film chosen as a temporary substrate. The cells were also grown to confluency as an organized monolayer suggesting PHBV8 film as a potential temporary substrate for subretinal transplantation to replace diseased or damaged retinal pigment epithelium. PHBHHx scaffolds have been evaluated for use in eyelid reconstruction during experiments on animals [147]. Although the scaffold performed satisfactorily, it produced some inflammation that took about 2 weeks to clear.

Nerve Regeneration

PHAs have also gained interest as biomaterial for nerve regeneration [69, 139]. PHAs like P(3HO) and copolymers, for example P(3HB-co-3HHx), have also been used as biopolymer scaffolds for the regeneration of nerve axons [79, 130]. Yang et al. [134] showed that fetal mouse cerebral cortex cells were able to grow well when seeded on P(3HB-co-3HHx) films. Xu et al. [132] carried out further studies on the behavior of neural stem cells on PHA nanofiber scaffolds to study its potential for repairing central nervous system (CNS) injuries. Three different PHAs, P(3HB), P (3HB-co(4HB), and P(3HB-co-3HHx), were fabricated into 3D nanofiber matrices via a novel phase separation process to mimic natural extracellular matrix. Cell culture studies with neural stem cells showed that all the three PHA materials were able to support NSC growth, synaptic outgrowth, and synaptogenesis as opposed to the 2D films. In another study [122, 124], the *ter* polymer of P(3HB-co-3 HV-co-3HHx) was used as a matrix material for the differentiation of human bone marrow MSC (hBMSC) in nerve cells. The polymer was fabricated into a porous 3D scaffold using thermally induced phase separation. The P(3HB-co-3 HV-co-3HHx) scaffold was found to provide the most suitable environment for hBMSC proliferation and differentiation into nerve cells.

Skin Defect Repair

The skin is the largest organ of the human body, representing approximately one tenth of the body mass, and serves several important functions, including physical barrier to the external environment, thermal regulation, and retention of normal hydration, and therefore is critical to our survival. The development of novel materials for effective regeneration of injured skin is one of the serious concerns in reconstructive medicine [101]. PHAs are instrumental in skin defect repair in many ways. In the studies carried out by Tang et al. [110], copolymers of poly(3HB-co-5 mol%-3HHx), poly(3HB-co-7 mol%-4HB), and poly(3HB-co-97 mol%-4HB) were used to fabricate nanostructured fibrous scaffolds. Tensile strength and Young's modulus of these scaffolds were comparable to those of human skin. Histological evaluation showed that subcutaneous implantation of the electrospun PHA scaffolds was well-tolerated in vivo. Li et al. [60] developed nanofibrous matrices using

blends of P(3HB)/P(3HB-co-3HHx) and P(3HB)/P(3HB-co-4HB) resembling collagen fibers. The human keratinocyte cell line, HaCat, was seeded on these scaffolds and their response compared with HaCat seeded on solvent cast films of the same composition. It was seen that cell behavior including morphology, adhesion ability, and viability on the nanofibrous matrices were better than those on the ordinary solid matrices. 2D films fabricated using the homopolymer of P(3HO) were also studied as matrix material for skin tissue engineering. The films possess a smooth surface with moderate hydrophilicity. When seeded with HaCaT cells, these P(3HO) films were found to support cell adhesion, proliferation, and maturation of the seeded HaCaT cells. In addition to its biocompatibility**,** the flexible and elastomeric nature of the film also makes it suitable for applications in difficult contours of the body [86]. Zonari et al. [149] produced thin nanoporous membranes and 3D porous scaffolds of PHBV that were combined in bilayer structures to recreate the epidermal and dermal layers, respectively. Results confirmed suitability of the PHBV structures to support cell adhesion and proliferation with human keratinocytes (hKC) and dermal fibroblasts (hDFb). Recently, Shishatskaya et al. [101] reported the efficacy of nonwoven membranes of P(3HB/4HB) carrying the culture of allogenic fibroblasts against model skin defects in Wistar rats. The morphological, histological, and molecular studies revealed the presence of fibroblasts on dressing materials which facilitated wound healing, vascularization, and regeneration. The wounds under the P(3HB/4HB) membrane carrying cells healed 1.4 times faster than the wounds under the cell-free membrane and 3.5 times faster than the wounds healing under eschar (control). The complete wound healing process was achieved at day 14. Nonwoven membranes developed from P(3HB/4HB) helped in reducing inflammation, enhancing angiogenic properties of skin, and facilitating better wound healing process.

Drug Delivery Systems

Due to various limitations of conventional drug therapy, administered either intravenously or via an extravascular route (oral, nasal, sublingual, or rectal), controlled drug delivery of pharmacologically active agents to the specific site of action at the therapeutically optimal rate and dose regimen is a major attraction in designing drug delivery systems [102]. Since 1990s, PHAs became star candidates for use as carriers for prolonged release of therapeutics due to their biodegradability, biocompatibility, and their degradation by surface erosion. Many drug delivery studies have been carried out using PHAs [4, 44, 57, 82, 102]. Kawaguchi et al. [47] reported the preparation of microspheres of PHB containing the antitumor drug 2′,3′-diacyl-5-fluoro-2′-deoxyuridine. The PHB microspheres showed low toxicity and good compatibility in mice and rats. Studies have been carried out on the application of PHA and dendrimer matrix for efficient transdermal drug delivery system (TDDS) by Wang et al. [127]. They used tamsulosin as the model drug to the dendrimer. The dendrimer-containing PHA matrix achieved the clinically required amount of tamsulosin permeating through the skin model. In an interesting study, P(3HB-co-3HHx) was studied as a matrix for the control release of triamcinolone acetonide for possible treatment of cystoid macular edema and acute posterior segment inflammation associated with uveitis. Results showed that 90% of the drug was released

within the first 24 h, then at a slower rate. It was also reported that drug release profile can be adjusted by changing the drug/polymer ratio and the particle size distribution [6]. Shishatskaya et al. [100] incorporated rubomycin in absorbable PHB matrix in the form of microparticles. Rubomycin deposited in polymeric microparticles exhibited pronounced antitumor activity in mice, inhibited the proliferative activity of Ehrlich ascitic carcinoma, and improved survival of mice with tumors. A sustained release system of P13 K inhibitor (TGX221) based on PHA nanoparticle was developed and used to block the proliferation of cancer cell lines [63]. It was found that TGX221 gradually released from PHA-based nanoparticles, and the growth of cancer cell lines was significantly slower in cells treated with TGX221 nanoparticles. Shah et al. [98] determined the efficacy of cisplatin-loaded self-assembled amphiphilic copolymer nanoparticles against tumor. The in vitro release profile of cisplatin from P(3 HV-co-4HB)-b-mPEG showed a sustained release of the drug.

As Cell Anchor

PHAs have also been used as cell-seeded biomaterials with the aim of promoting new tissue formation or regeneration. Cheng et al. [16] have shown that 3HB (0.02 g $ml^{-}1$) promoted cell proliferation in cultured L929 cells plated at high cell density (1×105 cells/well) but not at lower cell densities. Although 3HB did not affect cell cycle progression, it significantly inhibited cell death. 3HB treatment prevented necrosis, reducing cell membrane permeability after 4 h following serum withdrawal from the medium, and for all subsequent time points. In an interesting study [51], random or aligned PHB electrospun nanofibers that generate bone and spinal axon scaffolds were combined with MSCs. The adhesion and proliferation of cells on these membranes were examined. It was observed that the MSCs maintained their characteristic properties on the membranes, adhered to the membranes, and preserved their viability.

Wound Management

In current surgery practices, a need is seen for absorbable fibers and surgical meshes with a prolonged strength retention that can be used as a suture material. Over the past 20 years, PHA and its composites have been used to develop devices including sutures, suture fasteners, meniscus repair devices, rivets, tacks, staples, screws, interference screws, bone plates and plating systems, surgical mesh, repair patches, slings, cardiovascular patches, orthopedic pins, bone filling augmentation material, adhesion barriers [23], stents, guided tissue repair/regeneration devices, articular cartilage repair devices [125, 128], nerve guides [7], tendon repair devices, atrial septal defect repair devices, pericardial patches, bulking and filling agents, vein valves, bone marrow scaffolds, meniscus regeneration devices, ligament and tendon grafts, ocular cell implants, spinal fusion cages, skin substitutes, dural substitutes, bone graft substitutes, bone dowels, wound dressings, and hemostats [13]. Martin et al. [65] claimed to develop absorbable polyester fibers, braids, and surgical meshes with prolonged strength retention derived from copolymers or homopolymers of P4HB. These devices are believed to provide a wider range of in vivo

strength retention, antiadhesion properties, reduced risks of infection, or other postoperative problems resulting from absorption and eventual elimination of the device and competitive cost. The devices may be suitable for use in pediatric applications where their absorption should not hinder growth and wound healing with long-term mechanical stability. The devices may additionally be combined with autologous, allogenic, and/or xenogenic tissues to provide implants with improved mechanical, biological, and handling properties. Some remarkable applications are listed in Table 3.

Commercial Nonmedical Applications

In addition to their biomedical applications, PHAs can potentially replace petrochemicals-based plastics in several other areas, prime among which are discussed in this section.

Packaging Applications

Packaging is an important aspect to maintain the quality of food products during storage, transportation, and consumption. Also, packaging is a powerful marketing tool, which brings to the consumer all the information needed to make a choice of the brand. Biodegradable packaging materials can be degraded into carbon dioxide and water by microorganisms via metabolism under natural conditions of temperature, humidity, and oxygen [33, 93]. As the only biodegradable polymer that comes entirely from nature, PHA, has been processed into bottles, bags, and films using injection-moulding, blowing, and pressing particularly due to its excellent biodegradability and oxygen permeability resistance characteristics [19, 34]. The potential of PHAs for truly biodegradable packaging was recognized in the 1980s with the commercial release of Biopol®, thermoplastic resins of P(3HB) with various copolymer loadings of (3 HV), by Imperial Chemical Industries (ICI, now Zeneca). PHAs were initially used to make articles of daily use such as shampoo bottles [41], moisture barriers in sanitary products [54], and packaging materials by Wella (Germany) [129]. PHA were also developed as packaging films mainly for uses as shopping bags, containers and paper coatings, disposable items such as razors, utensils, diapers, hygiene products for women, cosmetic containers and cups, as well as medical surgical garments, upholstery, carpet, packaging, compostable bags and lids, or tubs for thermoformed articles by Proctor & Gamble, Biomers, Metabolix, and several other companies [18, 68].

PHB fibers with high tensile strength were prepared by stretching the fibers after isothermal crystallization near the glass-transition temperature [109]. However, the tensile strength still restricts further application of PHAs as a packaging material. In order to solve this problem, PVA was grafted on PIP (poly-cis-1,4-isoprene) and mixed with PHB, had better tensile properties and impact strength than PHB/PIP blends, which were immiscible [137]. On the other hand, Zhang et al. [141] reported improved mechanical properties for blends of PHB/PLA compared with the

Table 3 Medical applications of PHAs

SN	PHA type	Sub area	Remarks	References
1.	P(3HO-co-3HHx)/PGA acid	Cardiovascular	Blood vessels repair	Tim et al. [114]
2.	(3HO-co-3HHx)		Vascular graft	Stock et al. [107]; Qu et al. [85]
3.	P(3HO)		Tri-leaflet heart valve scaffold	Sodian et al. [104]; stock et al. [106]
4.	P(3HB-co-3HHx)		Hybrid valves	Wu et al. [131]
5.	P(3HB-co-3 HV)	Cancer therapy	Ellipticine microparticles	Masood et al. [66]
6.	pTHPP-loaded PHAs		Biopolymerin photodynamic therapy	Pramual et al. [83]
7.	PHB		Rubomycin microparticles	Shishatskaya et al. [100]
8.	P(3HB-co-3 HV)	Drug delivery	Ceftiofur-loaded microparticles	Vilos et al. [117]
9.	Functionalized PHAs		Inhibited the growth of methicillin-resistant *Staphylococcus aureus*	Dinjaski et al. [30]
10.	P(3HB)		Extended release tablet containing ibuprofen	Jirage et al. [45]
11.	PHB		2′,3′-Diacyl-5-fluoro-2′-deoxyuridine microparticles	Kawaguchi et al. [47]
12.	PHB		Controlled delivery of tetracycline	Panith et al. [76]
13.	P(3HB-co-3HHx)	Orthopedic	To maintain osteoblast phenotypes	Wang et al. [126]
14.	P3HB		Bone accumulation	Meischel et al. [67]
15.	P(3HB) and P(3HB-co-3HHx)	Cartilage engineering	Regeneration of chondrocytes	Zhao et al. [146]
16.	PHB	As cell anchor	MSCs	[51]
17.	P4HB	Wound management	Absorbable polyester fibers, braids, and surgical meshes	Martin et al. [65]

(*continued*)

Table 3 (continued)

SN	PHA type	Sub area	Remarks	References
18.	P(3HB-*co*-4HB)		Wound dressing	Vigneswari et al. [116]
19.	P(3HB)/P(3HO)		Scaffolds	Ching et al. [17]
20.	PHBV with ZnO nanocrystals		Antibacterial wound dressings	Abdalkarim et al. [1]
21.	PHBV8	Ophthalmological	Growth of retinal pigment epithelium cells	Tezcaner et al. [112]
22.	PHBHHx		For use in eyelid reconstruction	Zhou et al. [147]
23.	P(3HB-co-3HHx)	Nerve regeneration	Fetal mouse cerebral cortex cells were able to grow	Yang et al. [134]
24.	P(3HB), P(3HB-co(4HB), and P(3HB-co-3HHx)		Development of natural extracellular matrix	Xu et al. [132]
25.	P(3HB-co-3 HV-co-3HHx)		Scaffold for hBMSC proliferation and differentiation into nerve cells	Wang et al. [122, 124]
26.	Poly (3HB-co-5 mol%-3HHx), poly(3HB-co-7 mol%-4HB), poly(3HB-co-97 mol%-4HB)	Skin regeneration	To fabricate nanostructured fibrous scaffolds	Tang et al. [110]
27.	P(3HB)/P(3HB-co-3HHx) and P(3HB)/P (3HB-co-4HB)		To seed human keratinocyte cell line	Li et al. [60]
28.	PHBV		To recreate the epidermal and dermal layers	Zonari et al. [149]
29.	P(3HB/4HB)		Culture of allogenic fibroblasts	Shishatskaya et al. [101]
30.	P(3HDD-*co*-3H9D)	Controllable protein adsorption	Comb-like temperature-responsive graft polymer	Yao et al. [135]
31.	GHB	Alcohol withdrawal therapy		Addolorato et al. [2]
32.	PHAs	Immunodiagnostics	PHA chip for the immobilization of recombinant proteins	Park et al. [77]

common PHB. Combined with synthetic plastics or starch, PHAs make excellent packaging films [113]. Fabra et al. [32] created an innovative way to develop renewable microbial-based biopolyester multilayer structures with enhanced barrier performance, which is of significant interest for food packaging applications. These multilayer structures were based on PHBV with a valerate content of 12% (PHBV12) containing a high barrier interlayer of zein electrospun nanofibers. The incorporation of HV in PHB, resulting in PHBV, increased impact strength, elongation modulus, tensile strength, and decreased Young's modulus, making the film more flexible and more resistant [99]. PHBV degraded between five and 6 weeks in a microbiologically active environment, resulting in water and carbon dioxide in aerobic conditions. In an anaerobic environment, degradation was faster, producing methane [103]. Diez-Pascual and Diez-Vicente [29] dispersed ZnO into scl-PHA to obtain a composite film with antibacterial properties, which could be applied in the food packaging industry. Without the use of a coupling agent, nano-ZnO achieved a uniform dispersion in a PHBHV matrix, and also proved effective in the nucleation of PHBHV. With the increased addition of nano-ZnO, the thermal property, rigidity, strength, and tenacity of PHBHV film increased significantly. Kovalcik et al. [52] prepared PHBHV/Kraft lignin films using a thermo-hydraulic press for packaging of various perishable goods. A reduction of permeability was observed for oxygen by 77% and for carbon dioxide by 91%, in 1 wt % lignin blended PHBHV. Chea et al. [12] investigated the sorption behavior, mechanical properties, and barrier properties of PHBHV film under realistic conditions of storage and food contact. It was found that PHBHV film was very stable in all the food liquid simulants tested (viz., water, acetic acid 3% (*w*/*v*), ethanol 20% (*v*/*v*), isooctane, and olive oil). Biodegradable and optically transparent films were prepared by blending PLA with P3HB using the melt processing method. P3HB and acetyl tributyl citrate (ATBC) were used to improve the crystallinity of PLA and the processability as well as the flexibility of the film. In addition, the thermal stability of PLA/P3HB film could be improved by the addition of CNC. The interaction between ATBC and CNC improved the dispersion of CNC and further increased the intermolecular interaction between PLA and PHBHV chains [3].

As Fiber Material

PHA fibers can be obtained using melt spinning, gel spinning, or electrospinning methods. PHBHV fibers with a strength of 1.85 cN/dtex and an elongation of 47.9% could be obtained after stretching through a high-speed nozzle [14]. After the formation of PHA fibers, a transition from visco-elasticity to brittleness could be observed with the increase of storage time, which restricted their further application. To solve this problem, Li et al. [58] blended PLA with PHBHV and obtained PLA/PHBHV fibers via the conventional melt-spinning and hot-drawing processes. The tensile strength of PLA/PHBHV (70/30) fibers obtained under a speed of 2500 m/min and a draw ratio of 1.6 was above 2.0 cN/dtex, which pointed to their potential for application in the textile industry. Hufenus et al. [42] combined PHBHV with PLA in a core/sheath configuration and

introduced a new spin pack concept. To further improve the mechanical properties of PHA fibers, drawing and heat-setting processes were conducted after the formation of P3HB and PHBHV fibers [43]. PHA fibers can be prepared using the gel-spinning method, incorporating the processes of extrusion, hot drawing, and annealing [37]. As distinct from melt- and gel-spinning, the electro-spinning method is often used to prepare PHA nanofibers with special morphology [31], such as self-bundling yarn [123], coral-like structure [133], and beaded structure [138], which are of potential benefit in the areas of tissue engineering, filtration, and sensors. Electrospun scl-PHA fibers also have porous surface structure and microstructure, which provides the possibility for hydrophilic or hydrophobic control and for the adhesion regeneration of cells. Interestingly, the surface structure of nanofibers can be further adjusted to control the interfacial parameters using plasma treatment technology [138].

As Biofuels

The application of PHA as a source of biofuel is promising if low-cost PHA is used in this process. It can be obtained from activated sludge or food industry waste [36, 88]. The role of PHA-based materials as biofuels was first proposed by Zhang et al. [144]. Various PHAs like PHB and mcl PHA can be converted to (R)-3-hydroxybutyrate methyl ester (3HBME) and medium chain-length hydroxyalkanoate methyl ester (3HAME) by acid catalyzed hydrolysis. It was found that 3HBME and 3HAME had combustion heat values of 20 kJ g^{-1} and 30 kJ g^{-1}, respectively. Addition of 10% 3HBME or 3HAME to ethanol, which has a combustion heat of 27 kJ g^{-1}, enhanced the combustion heat of ethanol to 30 kJ g^{-1} and 35 kJ g^{-1}, respectively. The production cost of PHA-based biofuels was also roughly estimated to be around $1200 per ton. In their subsequent work, they blended 3HBME with 97% gasoline in volume ratios of 5%, 8.5%, 10%, 15%, and 20% and found that 3HBME had similar or better properties as a fuel additive compared with ethanol in terms of oxygen content, dynamic viscosity, flash point, and boiling point [122, 124].

As a Precursor of Carbon Material

As a kind of polymer with plenty of alkyls and hydroxy fatty acids, PHA can introduce an additional oxygen element into a carbon precursor. Chen et al. [15] synthesized a spindle-like hierarchical carbon structure of submicron dimension by the pyrolysis of a PHA/ferrocene/chloroform precursor. The electrochemical performance of the obtained carbon material showed a specific capacitance of 188 F/g with excellent stability over 10,000 cycles. In addition, PHA can also be used to improve the melt spinnability of Kraft lignin and prepare continuously spooled lignin/PHA fibers, which could be converted to low-cost carbon fibers.

For Paper Finishing

PHAs are water insoluble, hydrophobic in nature, possess excellent film-forming properties, provide a barrier against oxygen, and offer good UV resistance with low water vapor permeability (WVP) compared to other biopolymers, which make then

ideal candidates for paper finishing. Cyras et al. [20] coated PHB over paper by solvent casting using chloroform. When the PHB concentration exceeds 10 wt %, they observed reduction in moisture and water absorption, WVP and surface roughness, and increased surface hydrophobicity along with improvement in the surface tensile properties over the paper. When PHB was coated over paperboard by compression molding, similar results were obtained in terms of improved barrier and mechanical properties, which were further enhanced when the paperboard was acetylated to have better adhesion between the PHB coating and the paperboard [21]. PHAs have also been used for surface coating of paper and as sizing agents [9] to improve mechanical properties of papers resulting in improvement of water resistance and tensile strength. The degradation of P(3HB-*co*-4HB) coated brown Kraft paper and its components was investigated in a constructed soil environment by Dagnon et al. [22]. Soil burial tests were carried out over 8 weeks, and a substantially higher weight loss was observed *vis-a-vis* the pure biopolymer under the same conditions, which was attributed to a higher microbial population gathered over the coated papers than on the pure biopolymer.

Agricultural Applications

Various agriculture practices require the usage of large quantities of plastics. In order to overcome the serious drawback of using huge quantities of plastics, researches have recently used PHAs for various applications in agricultural industries.

The use of PHAs as a carrier for targeted and controlled delivery of pesticides (alpha-hexachlorocyclohexane and lindane) to soil was investigated by Voĭnova et al. [118]. Pesticides embedded in PHA carrier were released gradually and slowly, without bursts, as the polymer got degraded by the soil microflora. The microbial soil component actively responded to the addition of the polymer as an additional nutrient substrate; the latter was degraded and then utilized. PHAs have been used as controlled release agents for herbicides in agriculture. Controlled release potentially reduces the impact of the herbicides on nontarget species and reduces the need for repeated applications [38]. Micro- and nanoparticles of PHB and PHBV were used in a controlled release formulation of the herbicide ametryn [38]. PHB has been successfully tested for removing lipid-soluble organic pollutants from water by adsorption [143]. P(3HB/3 HV) was used to develop sustained-release formulations of the herbicide Zellek Super in the form of films and microgranules by Prudnikova et al. [84]. They found that PHAs can be used effectively to construct environmentally friendly sustained-release PHA-herbicide systems that can be placed into the soil together with seeds. Boyandin et al. [10] studied the formulations of herbicide metribuzin embedded in matrices of P3HB and its composites with PEG and PCL, and wood powder prepared in the form of pressed pellets containing 75% polymeric basis (pure P3HB, or its composite with a second component with a ratio of 7:3) and 25% metribuzin. The most active release of metribuzin (about 60% of the embedded herbicide over 35 days) was detected for the P3HB/PEG carrier compared to the P3HB, P3HB/wood, and P3HB/PCL forms (30–40%). The study shows that herbicide release can be controlled by the matrix formulation.

PHAs appear to be potentially useful in controlling bacterial pathogens in certain aquaculture applications also [25, 26, 74]. For example, administering 1000 mg L^{-1} of PHB particles of an average diameter of 30 μm, or addition of inactivated cells (10^7 cells L^{-1}) of PHB-containing *Brachymonas* bacteria (equivalent to 10 mg L^{-1} PHB) to the culture water of brine shrimp (*Artemia nauplii*) larvae, conferred a complete protection from a virulent strain of the intestinal pathogen *Vibrio campbellii* [26]. Other similar reports have claimed an inhibitory effect of PHB on certain gut microflora of the giant freshwater larvae of prawn (*Macrobrachium rosenbergii*) [74]. Addition of PHB (5 g L^{-1}) in the feed significantly increased the survival rate of the prawn larvae and improved their development. The total bacterial counts and *Vibrio* spp. counts were significantly reduced in PHB-fed larvae compared to the control larvae.

Formulations of the fertilizer urea loaded in the P3HB in the form of films, pellets, and coated granules were constructed and investigated by Volova et al. [119]. Nitrogen release into soil occurred as the polymer was degraded, and it was dependent on the geometry of the carrier and the amount of nitrogen loaded in it, showing that nitrogen release can last for 30 days or longer and that release rates can be controlled by varying the fabrication technique. P3HB/urea formulations were shown to have a favorable effect on the soil microbial community also. The use of such slow-release formulations can decrease the amounts of chemicals in the environment and prevent their adverse effects on the biosphere.

Stabilization of Nanoparticles

Silver nanoparticles have attracted much attention because of their antibacterial properties [59], but slurries of such particles tend to be unstable. Prolonged stability of such slurries has been reported after incorporating PHAs in them [81].

Other Applications

PHAs are applicable in numerous other areas of modern usage, which exceed beyond the theme limit of this chapter. Some remarkable applications include development of artificial esophagus [13], as raw materials for the production of latex paints [95], denitrification [39, 53], in restoration of seagrass habitats in marine environment [96], in diagnosis and endotoxin removal [78], to produce strong fibers for fisheries industry [11], and in conductive nanocomposite electrodes [111]. Some of these applications are listed in Table 4.

Perspectives

In recent years, PHAs have gained much attention both in research and industry for a wide range of applications. Without any doubts, they are valuable biomaterials with versatile properties. Nevertheless, limitations still persist such as the elevated cost of commercial PHA and limited market availability. A lot of progress has been made recently through the formulation of PHA with tailored additives and blends leading to greatly improved mechanical profiles, as well as suitable processability via extrusion or injection moulding. Using current knowledge and progress in genetic

Table 4 Nonmedical applications of PHAs

SN	PHA type	Subarea	Remarks	References
1.	PHB	Packaging	Packaging films	Tharanathan [113]
2.	PHAs		Shampoo bottles	Hocking and Marchessault [41]
3.	PHAs		Moisture barriers in sanitary products	Lauzier et al. [54]
4.	(PHBV12)		Food packaging	Fabra et al. [32]
5.	ZnO doped PHBHV		Food packaging with antibacterial properties	Diez-Pascual and Diez-Vicente [29]
6.	PHBHV/ Kraft lignin films		Packaging of perishable goods	Kovalcik et al. [52]
7.	P (HB–HV)		Water-proof films on the back of diaper sheets	Reddy et al. [89]
8.	PLA/ PHBHV	Fiber material	–	Li et al. [58]; Hufenus et al. [42]
9.	PHB and mcl PHA	Biofuels	Converted to (R)-3-hydroxybutyrate methyl ester (3HBME) and medium chain-length hydroxyalkanoate methyl ester (3HAME)	Zhang et al. [144]; [122, 124]
10.	PHAs	Precursor of carbon material	Synthesized a spindle-like hierarchical carbon structure	Chen et al. [15]
11.	PHB	Paper finishing	Solvent casting using chloroform	Cyras et al. [20]
12.	PHAs		Surface coating of paper and as sizing agents	Bourbonnais and Marchessault [9]
13.	(P(3HB-*co*-4HB))		Coating of brown Kraft paper	Dagnon et al. [22]
14.	PHAs	Agricultural applications	Carrier for pesticides (alpha-hexachlorocyclohexane and lindane)	Voĭnova et al. [118]
15.	PHB and PHBV		Controlled release formulation of the herbicide ametryn	Grillo et al. [38]
16.	PHB		Removing lipid-soluble organic pollutants from water by adsorption	Zhang et al. [143]
17.	P(3HB/ 3 HV)		Sustained-release formulations of the herbicide Zellek super	Prudnikova et al. [84]
18.	PHB		Inhibitory effect on certain gut microflora of the giant freshwater prawn (*Macrobrachium rosenbergii*) larvae	Nhan et al. [74]

(*continued*)

Table 4 (continued)

SN	PHA type	Subarea	Remarks	References
19.	PHB		Protection from a virulent strain of the intestinal pathogen *Vibrio campbellii*	Defoirdt et al. [26]
20.	P3HB		Controlled release fertilizers	Volova et al. [119]
21.	P3HB		Controlled release fertilizers	Boyandin et al. [10]
22.	PHAs	Stabilization of nanoparticles	Silver nanoparticles	Li et al. [59]; Phukon et al. [81]
23.	PHAs	Immobilization	Of alkaline polygalacturonate lyase	Ran et al. [87]
24.	PHAs		Active bacteriophages	Wang et al. [120]

engineering and synthetic biology it seems to be possible to construct an ideal PHA producer, which can biosynthesize new biopolymers and could be used cost-effectively for industrial production. Research and developmental studies are going on to improve the yield of PHA, for instance, by genetic modification of the bacteria or the use of waste for their growth. Further improvements are still needed to allow a broader market introduction of PHAs. The use of waste feedstock for the culture of the microorganisms accumulating PHAs is also needed as a vehicle to lead to their greater economic viability and sustainability. Further investigations and efforts must be undertaken by researchers to reduce the production costs of these PHAs and increase the industrial sustainability and commercialization of PHAs. It is quite possible that further improvements could produce even more flexible grades of PHAs or even transparent ones through the control of the crystallization process.

References

1. Abdalkarim SYH, Yu HY, Wang D, Ya J (2017) Electrospun poly(3-hydroxybutyrate-co-3-hydroxy-valerate)/cellulose reinforced nanofibrous membranes with ZnO nanocrystals for antibacterial wound dressings. Cellulose 24:2925–2938. https://doi.org/10.1007/s10570-017-1303-0
2. Addolorato G, Balducci G, Capristo E, Attilia ML, Taggi F, Gasbarrini G. Ceccanti M(1999) Gamma-hydroxybutyric acid (GHB) in the treatment of alcohol withdrawal syndrome: a randomized comparative study versus benzodiazepine. Alcohol Clin Exp Res 23:596–604
3. Arrieta MP, Fortunati E, Dominici F, Lopez J, Kenny JM (2015) Bionanocomposite films based on plasticized PLA-PHB/cellulose nanocrystal blends. Carbohydr Polym 121:265–275
4. Bansal SS, Goel M, Aqil F, Vadhanam MV, Gupta RC (2011) Advanced drug-delivery systems of curcumin for cancer chemoprevention. Cancer Prev Res 4:1158
5. Barnes DKA, Galgani F, Thompson RC, Barlaz M (2009) Accumulation and fragmentation of plastic debris in global environments. Philos Trans R Soc Lond Ser B Biol Sci 364:1985–1998
6. Bayram C, Denbas EB (2008) Preparation and characterization of triamcinolone acetonide-loaded poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHx) microspheres. J Bioactive Comp Polym 23:334–347
7. Bian YZ, Wang Y, Guli S, Chen GQ, Wu Q (2009) Evaluation of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) conduits for peripheral nerve regeneration. Biomaterials 30:217–225

8. Bok D, Hall MO (1971) The role of the pigment epithelium in the etiology of inherited retinal dystrophy in the rat. J Cell Biol 49:664–682
9. Bourbonnais R, Marchessault RH (2010) Application of polyhydroxyalkanoate granules for sizing of paper. Biomacromolecules 11:989–993. https://doi.org/10.1021/bm9014667
10. Boyandin AN, Zhila NO, Kiselev EG, Volova TG (2016) Constructing slow-release formulations of Metribuzin based on degradable poly(3-hydroxybutyrate). J Agric Food Chem 64:5625–5632. https://doi.org/10.1021/acs.jafc.5b05896
11. Bugnicourt E, Cinelli P, Lazzeri A, Alvarez V (2014) Polyhydroxyalkanoate(PHA): review of synthesis, characteristics, processing and potentialapplications in packaging. Express Polym Lett 8:791–808
12. Chea V, Angellier-Coussy H, Peyron S, Kemmer D, Gontard N (2016) Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) films for food packaging: physical-chemical and structural stability under food contact conditions. J Appl Polym Sci 133:41850
13. Chen GQ, Wu Q (2005) The application of polyhydroxyalkanoates as tissue engineering materials. Biomaterials 26:6565–6578
14. Chen L, Wang B, Chen YM, Zhang Y, Zhu MF (2007) The formation of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) fibers. J Donghua Univ 33:425–430
15. Chen Q, Xiao SJ, Zhang RJ, Guo FM, Wang KL, Zhu HW (2016) Spindle-like hierarchical carbon structure grown from polyhydroxyalkanoate/ ferrocene/chloroform precursor. Carbon 103:346–351
16. Cheng S, Chen GQ, Leski M, Zou B, Wang Y, Wu Q (2006) The effect of D,L-b-hydroxybutyric acid on cell death and proliferation in L929 cells. Biomaterials 27:3758–3765
17. Ching KY, Andriotis OG, Li S, Basnett P, Su B, Roy I, Tare RS, Sengers BG, Stolz M (2016) Nanofibrous poly(3-hydroxybutyrate)/poly(3-hydroxyoctanoate) scaffolds provide a functional microenvironment for cartilage repair. J Biomater Appl 31:77–91. https://doi.org/10.1177/0885328216639749
18. Clarinval AM, Halleux J (2005) Classification of biodegradable polymers. In: Smith R (ed) Biodegradable polymers for industrial applications. CRC, Boca Raton, pp 3–56
19. Cretois R, Follain N, Dargent E, Soulestin J, Bourbigot S, Marais S, Lebrun L (2014) Microstructure and barrier properties of PHBV/organoclays bionanocomposites. J Membr Sci 467:56–66
20. Cyras VP, Commisso MS, Mauri AN, Vázquez A (2007) Biodegradable double-layer films based on biological resources: polyhydroxybutyrate and cellulose. J Appl Polym Sci 106:749–756
21. Cyras VP, Soledad CM, Analía V (2009) Biocomposites based on renewable resource: acetylated and non acetylated cellulose cardboard coated with polyhydroxybutyrate. Polymer 50: 6274–6280
22. Dagnon KL, Thellen C, Ratto JA, D'Souza NA (2010) Physical and thermal analysis of the degradation of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) coated paper in a constructed soil medium. J Polym Environ 18:510–522
23. Dai ZW, Zou XH, Chen GQ (2009) Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) as an injectable implant system for prevention of post-surgical tissue adhesion. Biomaterials 30:3075–3083
24. Dawes EA, Senior PJ (1973) The role and regulation of energy reserve polymers in microorganisms. Adv Microb Physiol 10:135–266
25. De Schryver P, Dierckens K, Bahn Thi QQ, Amalia R, Marzorati M, Bossier P, Boon N, Verstraete W (2011) Convergent dynamics of the juvenile European sea bass gut microbiota induced by polyhydroxybutyrate. Environ Microbiol 13(4):1042–1051
26. Defoirdt T, Sorgeloos P, Bossier P (2011) Alternatives to antibiotics for the control of bacterial disease in aquaculture. Curr Opin Microbiol 14(3):251–258
27. Deng Y, Zhao K, Zhang XF, Hu P, Chen GQ (2002) Study on the three-dimensional proliferation of rabbit articular cartilage-derived chondrocytes on polyhydroxyalkanoate scaffolds. Biomaterials 23:4049–4056. https://doi.org/10.1016/S0142-9612(02)00136-9
28. Deng Y, Linb XS, Zhenga Z, Deng JG, Chena JC, Mab H, Chen GQ (2003) Poly (hydroxybutyrate-co-hydroxyhexanoate) promoted production of extracellular matrix of articular cartilage chondrocytes *in vitro*. Biomaterials 24:4273–4281

29. Diez-Pascual AM, Diez-Vicente AL (2014) ZnO-reinforced poly(3-hydroxybutyrate-co-3-hydroxyvalerate) bionanocomposites with antimicrobial function for food packaging. ACS Appl Mater Interfaces 6:9822–9834
30. Dinjaski N, Fernández-Gutiérrez M, Selvam S, Parra-Ruiz F, Lehman SM, SanRomán J, García E, García JL, García AJ, Prieto MA (2014) PHACOS, a functionalized bacterial polyester with bactericidal activity against methicillin-resistant *Staphylococcus aureus*. Biomaterials 35:14–24
31. El-hadi AM, Al-Jabri FY (2016) Influence of electrospinning parameters on fiber diameter and mechanical properties of poly(3-hydroxybutyrate) (PHB) and polyanilines (PANI) blends. Polymer 8:97
32. Fabra MJ, Lopez-Rubio A, Lagaron JM (2013) High barrier polyhydroxyalcanoate food packaging film by means of nanostructured electrospun interlayers of zein. Food Hydrocoll 32:106–114
33. Fabra MJ, Lopez-Rubio A, Lagaron JM (2014a) On the use of different hydrocolloids as electrospun adhesive interlayers to enhance the barrier properties of Polyhydroxyalkanoates of interest in fully renewable food packaging concepts. Food Hydrocoll 39:77–84
34. Fabra MJ, Sanchez G, Lopez-Rubio A, Lagaron JM (2014b) Microbiological and ageing performance of polyhydroxyalkanoate-based multilayer structures of interest in food packaging. Lwt-Food Sci Technol 59:760–767
35. Flechter A (1993) plastics from bacteria and for bacteria: PHA as natural biodegradable polyesters. Springer, New York, pp 77–93
36. Gao X, Chen JC, Wu Q, Chen GQ (2011) Polyhydroxyalkanoates as a source of chemicals, polymers, and biofuels. Curr Opin Biotechnol 22:768–774
37. Gordeyev SA, Nekrasov YP, Shilton SJ (2001) Processing of gel-spun poly(_-hydroxybutyrate) fibers. J Appl Polym Sci 81:2260–2264
38. Grillo R, Pereira AES, de Melo NFS, Porto RM, Feitosa LO, Tonello PS, Filho NLD, Rosa AH, Lima R, Fraceto LF (2011) Controlled release system for ametryn using polymer microspheres: preparation, characterization and release kinetics in water. J Hazard Mater 186(2–3):1645–1651. https://doi.org/10.1016/j.jhazmat.2010.12.044
39. Gutierrez-Wing MT, Malone RF, Rusch KA (2014) Development of a model for PHA-based denitrification in a packed bed reactor. Aquac Eng 60:41–47
40. Harrington JE, Kossuth MB, Oberhauser J (2015) Branched polyhydroxyalkanoate systems for bioresorbable vascular scaffold applications. US 20150305899 A1
41. Hocking PJ, Marchessault RH (1994) Biopolyesters. In: GJL G (ed) Chemistry, technology of biodegradable polymers. Blackie Academic & Professional, London, pp 48–96
42. Hufenus R, Reifler FA, Maniura-Weber K, Spierings A, Zinn M (2012) Biodegradable bicomponent fibers from renewable sources: melt-spinning of poly(lactic acid) and poly (3-hydroxybutyrate)-co-(3-hydroxyvalerate). Macromol Mater Eng 297:75–84
43. Hufenus R, Reifler FA, Fernandez-Ronco MP, Heuberger M (2015) Molecular orientation in melt-spun poly(3-hydroxybutyrate) fibers: effect of additives, drawing and stress-annealing. Eur Polym J 71:12–26
44. Jain R, Tiwari A (2015) Role of polyhydroxyalkanoates in cancer and other drug delivery systems. J Can Res Ther 11:494–495
45. Jirage A, Shaikh K, Kate V, Payghan S (2015) Optimization of ibuprofen carrying poly-(3-hydroxybutyrate) extended release tablet by central composite design. Asian J Biomed Pharm Sci 6(59):7–30
46. Johnstone B (1990) A throw away answer. Far Eastern Econ Rev 147(6):62–63
47. 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. J Pharm Sci 81:508–512
48. Knight GD (2012) Plastic pollution, Raintree (ed). Heinemann, Library
49. Koller M, Atlic A, Dias M, Reiterer A, Braunegg G (2010) Microbial PHA production from waste raw materials. In: Steinbüchel A (series ed) Guo G, Chen Q (eds) Plastics from bacteria:

natural functions and applications. Microbiology monographs 14th edn. Springer, Berlin, Heidelberg, pp 85–119
50. Köse GT, Korkusuz F, Ozkul A, Soysal Y, Ozdemir T, Yildiz C, Hasirci V (2005) Tissue engineered cartilage on collagen and PHBV matrices. Biomaterials 26(25):5187–5197
51. Kose S, Aerts Kaya F, Denkbas EB, Korkusuz P, Cetinkaya FD (2016) Evaluation of biocompatibility of random or aligned electrospun polyhydroxybutyrate scaffolds combined with human mesenchymal stem cells. Turk J Biol 40:410–419
52. Kovalcik A, Machovsky M, Kozakova Z, Koller M (2015) Designing packaging materials with viscoelastic and gas barrier properties by optimized processing of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) with lignin. React Funct Polym 94:25–34
53. Krasnits E, Beliavsky M, Tarre S, Green M (2013) PHA based denitrification: municipal wastewater vs. acetate. Bioresour Technol 132:28–37
54. Lauzier CA, Monasterios CJ, Saracovan I, Marchessault RH, Ramsay BA (1993) Film formation and paper coating with poly(b-hydroxyalkanoate), abiodegradable latex. TAPPI J 76:71–77
55. Lee SY (1996) Bacterial polyhydroxyalkanoates. Biotechnol Bioeng 49(1):1–14
56. Lemoigne M (1926) Produits de deshydration et de polymerisation de l'acide b-oxybutyric. Bull Soc Chem Biol 8:770–782
57. Li HY, Chang J (2005) Preparation, characterization and in vitro release of gentamicin from PHBV /wollastonite composite microspheres. J Control Release 107:463–473
58. Li L, Huang W, Wang B, Wei W, Gu Q, Chen P (2015) Properties and structure of polylactide/poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PLA/PHBV) blend fibers. Polymer 68:183–194
59. Li WR, Xie XB, Shi QS, Zeng HY, YS OU-Y, Chen YB (2010) Antibacterial activity and mechanism of silver nanoparticles on *Escherichia coli*. Appl Microbiol Biotechnol 85(4): 1115–1122
60. Li XT, Zhang Y, Chen GQ (2008) Nanofibrous polyhydroxyalkanoate matrices as cell growth supporting materials. Biomaterials 29:3720–3728
61. Lomas AJ, Webb WR, Han J, Chen GQ, Sun X, Zhang Z, Haj AJ, Forsyth NR (2013) Poly (3-hydroxybutyrate-co-3-hydroxyhexanoate)/collagen hybridscaffolds for tissue engineering applications. Tissue Eng Part C Methods 19:577–585
62. Lu LX, Zhang XF, Wang YY, Ortiz L, Mao X, Jiang ZL, Xiao ZD, Huang NP (2013) Effects of hydroxyapatite-containing composite nanofibers onosteogenesis of mesenchymal stem cells in vitro and bone regeneration invivo. ACS Appl Mater Interfaces 5:319–330
63. Lu XY, Ciraolo E, Stefenia R, Chen GQ, Zhang Y, Hirsch E (2011) Sustained release of PI3K inhibitor from PHA nanoparticles and in vitro growth inhibition of cancer cell lines. Appl Microbiol Biotechnol 89:1423–1433
64. Madison LL, Huisman GW (1999) Metabolicengineering of poly(3-hydroxyalkanoates): from DNA to plastic. Microbiol Mol Biol Rev 63(1):21–53
65. Martin DP, Rizk S, Ahuja A, Williams SF (2016) Method of making a medical textile from polyhydroxyalkanoate fibers. US 9333066 B2. May 10
66. Masood F, Chen P, Yasin T, Fatima N, Hasan F, Hameed A (2013) Encapsulation of Ellipticine in poly-(3-hydroxybutyrate-co-3-hydroxyvalerate)based nanoparticles and its *in vitro* application. Mater Sci Eng C Mater Biol Appl 33:1054–1060
67. Meischel M, Eichler J, Martinelli E, Karr U, Weigel J, Schmoeller G, Tschegg EK, Fischerauer S, Weinberg AM, Stanzl-Tschegg SE (2016) Adhesive strength of bone-implant interfaces and in-vivo degradation of PHB composites for load-bearing applications. J Mech Behav Biomed Mater 53:104–118
68. Mikova G, Chodak I (2006) Properties and modification of poly(3-hydroxybutanoate). Chem List 100:1075–1083
69. Mosahebi A, Fuller P, Wiberg M, Terenghi G (2002) Effect of allogeneic Schwann cell transplantation on peripheral nerve regeneration. Exp Neurol 173:213–223
70. Mozejko-Ciesielska J, Kiewisz R (2016) Bacterial polyhydroxyalkanoates: still fabulous? Microbiol Res 192:271–282

71. Naveen SV, Tan IKP, Goh YS, Raghavendran HRB, Murali MR, Kamarul T (2015) Unmodified medium chain length polyhydroxyalkanoate (uMCL-PHA) asa thin film for tissue engineering application – characterization and *in vitro* biocompatibility. Mater Lett 141:55–58
72. Nebe B, Forster C, Pommerenke H, Fulda G, Behrend D, Bernewski U, Schmitz KP, Rychly J (2001) Structural alterations of adhesion mediating components in cells cultured on poly-β-hydroxybutyric acid. Biomaterials 22(17):2425–2434
73. Nelson T, Kaufman E, Kline J, Sokoloff L (1981) The extraneural distribution of γ-hydroxybutyrate. J Neurochem 37(5):1345–1348
74. Nhan DT, Wille M, De Schryver P, Defoirdt T, Bossier P, Sorgeloos P (2010) The effect of poly-β-hydroxybutyrate on larviculture of the giant freshwater prawn *Macrobrachium rosenbergii*. Aquaculture 302(1–2):76–81
75. Oeding V, Schlegel HG (1973) Beta-ketothiolase from *Hydrogenomonas eutropha HI6* and its significance in the regulation of poly beta-hydroxybutyrate metabolism. Biochem J 134: 239–248
76. Panith N, Assavanig A, Lertsiri S, Bergkvist M, Surarit R, Niamsiri N (2016) Development of tunable biodegradable polyhydroxyalkanoates microspheres for controlled delivery of tetracycline for treating periodontal disease. J Appl Polym Sci 133:44128
77. Park TJ, Park JP, Lee SJ, Hong HJ, Lee SY (2006) Polyhydroxyalkanoate chip for the specific immobilization of recombinant proteins and its applications in immunodiagnostics. Biotechnol Bioprocess Eng 11:173
78. Parlane NA, Gupta SK, Rubio-Reyes P, Chen S, Gonzalez-Miro M, Wedlock DN, Rehm BHA (2016) Self-assembled protein-coated polyhydroxyalkanoate beads: properties and biomedical applications. ACS Biomat Sci Eng. https://doi.org/10.1021/acsbiomaterials.6b00355
79. Philip S, Keshavarz T, Roy I (2007) Polyhydroxyalkanoates: biodegradable polymers with a range of applications. J Chem Technol Biotechnol 82:233–247
80. Phithakrotchanakoon C, Champreda V, Aiba S, Pootanakit K, Tanapongpipat S (2015) Production of polyhydroxyalkanoates from crude glycerol using recombinant *Escherichia coli*. J Polym Environ 23:38–44
81. Phukon P, Saikia JP, Konwar BK (2011) Enhancing the stability of colloidal silver nanoparticles using polyhydroxyalkanoates (PHA) from *Bacillus circulans* (MTCC 8167) isolated from crude oil contaminated soil. Colloids Surf B Biointerfaces 86(2):314–318. https://doi.org/10.1016/j.colsurfb.2011.04.014
82. Pouton CW, Akhtar S (1996) Biosynthetic polyhydroxyalkanoates and their potential in drug delivery. Adv Drug Deliv Rev 18:133–162
83. Pramual S, Assavanig A, Bergkvist M, Batt CA, Sunintaboon P, Lirdprapamongkol K, Svasti J, Niamsiri N (2016) Development andcharacterization of bio-derived polyhydroxyalkanoate nanoparticles as adelivery system for hydrophobic photodynamic therapy agents. J Mater Sci Mater Med 27:40. https://doi.org/10.1007/s10856-015-5655-4
84. Prudnikova SV, Boyandin AN, Kalacheva GS, Sinskey AJ (2013) Degradable polyhydroxyalkanoates as herbicide carriers. J Polymer Env 21:675–682
85. Qu XH, Wu Q, Liang J, Qu X, Wang SG, Chen GQ (2005) Enhanced vascular-related cellular affinity on surface modified copolyesters of 3- hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx). Biomaterials 26:6991–7001
86. Rai R (2010) Biosynthesis of polyhydroxyalkanoates and its medical applications, Ph.D thesis in School of Life Sciences, University of Westminster, London, p 291
87. Ran GQ, Tan D, Dai WE, Zhu XL, Zhao JP, Ma Q, Lu XY (2017) Immobilization of alkaline polygalacturonate lyase from *Bacillus subtilis* on the surface of bacterial polyhydroxyalkanoate nano-granules. Appl Microbiol Biotechnol 101:3247–3258
88. Ravindran R, Jaiswal AK (2016) Exploitation of food industry waste for high-value products. Trends Biotechnol 34:58–69
89. Reddy CSK, Ghai R, Rashmi KVC (2003) Polyhydroxyalkanoates: an overview. Bioresour Technol 87:137–146

90. Rivard CH, Chaput C, Rhalmi S, Selmani A (1996) Bioabsorbable synthetic polyesters and tissue regeneration: a study on the three-dimensional proliferation of ovine chondrocytes and osteoblasts. Ann Chir 50(8):651–658
91. Samantaray S, Bhati R, Mallick N (2014) Cyanobacterial polyhydroxyalkanoates: an alternative source for plastics. In: Cyanobacteria. Wiley, Chichester, pp 227–244. https://doi.org/10.1002/9781118402238.ch14
92. Sawada H (1998) ISO standard activities in standardization of biodegradability of plastics-development of test methods and definitions. Polym Degrad Stab 59(1–3):365–370
93. Scarfato P, Di Maio L, Incarnato L (2015) Recent advances and migration issues in biodegradable polymers from renewable sources for food packaging. J Appl Polym Sci 132:42597. https://doi.org/10.1002/app.42597
94. Schmalz G, Galler KM (2017) Biocompatibility of biomaterials - Lessons learned and considerations for the design of novel materials. Dent Mater 33:382–393. https://doi.org/10.1016/j.dental.2017.01.011
95. Scholz C (2000) Poly(β-hydroxyalkanoates) as potential biomedical materials: an overview. ACS Ser 764:328–334
96. Seggiani M, Cinelli P, Mallegni N, Balestri E, Puccini M, Vitolo S, Lardicci C, Lazzeri A (2017) New bio-composites based on polyhydroxyalkanoates and *Posidonia oceanica* fibres for applications in a marine environment. Dent Mater 10(4):326. https://doi.org/10.3390/ma10040326
97. Senior PJ, Beech GA, Ritchie GAF, Dawes EA (1972) The role of oxygen limitation in the formation of poly-b-hydroxybutyrate during batch and continuous culture of *Azobacter beijerinckii*. Biochem J 128:1193–1201
98. Shah M, Ullah N, Choi MH, Kim MO, Yoon SC (2012) Amorphous amphiphilic P(3HV-co-4HB)-b-mPEG block copolymer synthesized from bacterial copolyester via melt transesterification: nanoparticle preparation, cisplatin-loading for cancer therapy and in vitro evaluation. Eur J Pharm Biopharm 80(3):518–527
99. Shen Z, Simon GP, Cheng YB (2002) Comparison solution and melt intercalation of polymer-clay nanocomposites. Polymer 43:4251–4260
100. Shishatskaya EI, Goreva AV, Voinova ON, Inzhevatkin EV, Khlebopros RG, Volova TG (2008) Evaluation of antitumor activity of rubomycin deposited in absorbable polymeric microparticles. Bull Exp Biol Med 145:358–361
101. Shishatskaya EI, Nikolaeva ED, Vinogradova ON, Volova TG (2016) Experimental wound dressings of degradable PHA for skin defect repair. J Mater Sci Mater Med 27(11):165. https://doi.org/10.1007/s10856-016-5776-4
102. Shrivastav A, Kim HY, Kim YR (2013). Advances in the applications of polyhydroxyalkanoate nanoparticles for novel drug delivery system. Biomed Res Int 12 p. https://doi.org/10.1155/2013/581684
103. Siracusa V, Rocculi P, Romani S, Dalla Rosa M (2008) Biodegradable polymers for food packaging: a review. Trends Food Sci Technol 19(12):634–643
104. Sodian R, Hoerstrup SP, Sperling JS, Daebritz S, Martin DP, Moran AM, Kim BS, Schoen FJ, Vacanti JP, Mayer JE (2000) Early in vivo experience with tissue-engineered trileaflet heart valves. Circulation 102:22–29
105. Steinbüchel A (1991) Polyhydroxyalkanoic acids. In: Byrom D (ed) Biomaterials: novel materials from biological sources. Stockton Press, New York, pp 124–213
106. Stock UA, Nagashima M, Khalil PN, Nollert GD, Herden T, Sperling JS, Moran A, Lien J, Martin DP, Schoen FJ, Vacanti JP, Mayer JE (2000) Tissue engineered valved conduits in the pulmonary circulation. J Thorac Cardiovasc Surg 119:732–740
107. Stock UA, Wiederschain D, Kelly S, Shum-Tim D, Khalil PN, Vacanti JP, Mayer JE Jr, Moses MA (2001) Dynamics of extra-cellular matrix production and turnover in tissue engineered cardiovascular structures. J Cell Biochem 81:220–228
108. Sun J, Dai ZW, Chen GQ (2007) Oligomers of polyhydroxyalkanoates stimulated calcium ion channels in mammalian cells. Biomaterials 28:3896–3903

109. Tanaka T, Yabe T, Teramachi S, Iwata T (2007) Mechanical properties and enzymatic degradation of poly[(*R*)-3-hydroxybutyrate] fibers stretched after isothermal crystallization near T-g. Polym Degrad Stab 92:1016–1024
110. Tang H, Ishii D, Mahara A, Murakami S, Yamaoka T, Sudesh K, Samian R, Fujita M, Maeda M, Iwata T (2008) Scaffolds from electrospun polyhydroxyalkanoate copolymers: fabrication, characterization, bio absorption and tissue response. Biomaterials 29:1307–1317
111. Tematio C, Bassas-Galia M, Fosso N, Gaillard V, Mathieu M, Zinn M, Staderini EM, Schintke S (2017) Design and characterization of conductive biopolymer nanocomposite electrodes for medical applications. Mater Sci Forum 879:1921–1926
112. Tezcaner A, Bugra K, Hasirci V (2003) Retinal pigment epithelium cell culture on surface modified poly(hydroxybutyrate-co-hydroxyvalerate) thin films. Biomaterials 24(25):4573–4583
113. Tharanathan RN (2003) Biodegradable films and composite coatings: past, present and future. Trends Food Sci Technol 14:71–78
114. Tim DS, Stock U, Hrkach J, Shinoka T, Lien B, Moses MA, Stamp A, Taylor G, Moran AM, Landis W, Langer R, Vacanti JP, Mayer JE (1999) Tissue engineered of autologous aorta using a new biodegradable polymer. Ann Thorac Surg 68:2298–2305
115. Ueda H, Tabata Y (2003) Polyhydroxyalkanonate derivatives in current clinical applications and trials. Adv Drug Deliv Rev 55:501–518
116. Vigneswari S, Murugaiyah V, Kaur G, Abdul Khalil HPS, Amirul AA (2016) Biomacromolecule immobilization: grafting of fish-scale collagen peptides onto aminolyzed P(3HB-*co*-4HB) scaffolds as a potential wound dressing. Biomed Mater 11(5):055009. https://doi.org/10.1088/1748-6041/11/5/055009
117. Vilos C, Constandil L, Herrera N, Solar P, Escobar-Fica J, Velasquez LA (2012) Ceftiofur-loaded PHBV microparticles: a potential formulation for a long-actingantibiotic to treat animal infections. Electron J Biotechnol 15:1–13
118. Voĭnova ON, Kalacheva GS, Grodnitskaia ID, Volova TG (2009) Microbial polymers as a degradable carrier for pesticide delivery. [Article in Russian]. Prikl Biokhim Mikrobiol 45(4):427–431
119. Volova TG, Prudnikova SV, Boyandin AN (2016) Biodegradable poly-3-hydroxybutyrate as a fertiliser carrier. J Sci Food Agric 96(12):4183–4193. https://doi.org/10.1002/jsfa.7621
120. Wang C, Sauvageau D, Elias A (2016) Immobilization of active bacteriophages on polyhydroxyalkanoate surfaces. ACS Appl Mater Interfaces 8(2):1128–1138. https://doi.org/10.1021/acsami.5b08664
121. Wang JG, Bakken LR (1998) Screening of soil bacteria for poly beta-hydroxybutyric acid production and its role in the survival of starvation. Microb Ecol 35:94–101
122. Wang L, Wang ZH, Shen YS, You ML, Xiao JF, Chen GQ (2010a) Differentiation of human bone marrow mesenchymal stem cells grown in terpolyesters of 3-hydroxyalkanoates scaffolds into nerve cells. Biomaterials 31:1691–1698
123. Wang S, Li Y, Xiang H, Zhou Z, Chang T, Zhu M (2015) Low cost carbon fibers from biorenewable lignin/poly(lactic acid) (PLA) blends. Compos Sci Technol 119:20–25
124. Wang SY, Wang Z, Liu MM, Xu Y, Zhang XJ, Chen GQ (2010b) Properties of a new gasoline oxygenate blend component: 3-hydroxybutyrate methyl ester produced from bacterial poly-3-hydroxybutyrate. Biomass Bioenergy 34:1216–1222
125. Wang Y, Bian YZ, Wu Q, Chen GQ (2008a) Evaluation of three-dimensional scaffolds prepared from poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) for growth of allogeneic chondrocytes for cartilage repair in rabbits. Biomaterials 29:2858–2868
126. Wang YW, Wu Q, Chen GQ (2004) Attachment, proliferation and differentiation of osteoblasts on random biopolyester poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) scaffolds. Biomaterials 25(4):669–675
127. Wang Z, Itoh Y, Hosaka Y, Kobayashi I, Nakano Y, Maeda I, Umeda F, Yamakawa J, Kawase M, Yagi K (2003) Novel transdermal drug delivery system with Polyhydroxyalkanoate and starburst Polyamidoamine Dendrimer. J Biosci Bioeng 95:541–543

128. Wang ZH, Wu HN, Chen J, Zhang J, Chen GQ (2008b) A novel self-cleaving phasin tag for purification of recombinant proteins based on hydrophobic nanoparticles. Lab Chip 8:1957–1962
129. Weiner RM (1997) Biopolymers from marine prokaryotes. Trends Biotechnol 15:390–394
130. Witholt B, Kessler B (1999) Perspectives of medium chain length poly(hydroxyalkanoates), a versatile set of bacterial bioplastics. Curr Opin Biotechnol 10:279–285
131. Wu S, Liu YL, Cui B, Qu XH, Chen GQ (2007) Study on decellularized porcine aortic valve/poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) hybrid heart valve in sheep model. Artif Organs 31:689–697
132. Xu XY, Li XT, Peng SW, Xiao JF, Liu C, Fang G, Chen KC, Chen GQ (2010) The behaviour of neural stem cells on polyhydroxyalkanoate nanofiber scaffolds. Biomaterials 31:3967–3975
133. Yang DZ, Zhang JF, Xue J, Nie J, Zhang ZP (2013) Electrospinning of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) nanofibers with feature surface microstructure. J Appl Polym Sci 127:2867–2874
134. Yang F, Li X, Li G, Zhao N, Zhang X (2002) Study on chitosan and PHBHHx used as nerve regeneration conduit material. J Biomed Eng 19:25–29
135. Yao H, Wei D, Che X, Cai L, Tao L, Liu L, Wu L, Chen GQ (2016) Comb-like temperature-responsive polyhydroxyalkanoate-*graft*-poly(2-dimethylamino-ethylmethacrylate) for controllable protein adsorption. Polym Chem 7:5957–5965. https://doi.org/10.1039/C6PY01235C
136. Yao YC, Zhan XY, Zhang J, Zou XH, Wang ZH, Xiong YC, Chen J, Chen GQ (2008) A specific drug targeting system based on polyhydroxyalkanoate granule binding protein PhaP fused with targeted cell ligands. Biomaterials 29(36):4823–4830
137. Yoon JS, Lee WS, Jin HJ, Chin IJ, Kim MN, Go JH (1999) Toughening of poly(3-hydroxybutyrate) with poly(cis-1,4-isoprene). Eur Polym J 35(5):781–788
138. Yoon YI, Moon HS, Lyoo WS, Lee TS, Park WH (2008) Superhydrophobicity of PHBV fibrous surface with bead-on-string structure. J Colloid Interface Sci 320:91–95
139. Young RC, Wiberg M, Terenghi G (2002) Poly-3-hydroxybutyrate (PHB): a resorbable conduit for long-gap repair in peripheral nerves. Br J Plast Surg 55:235–240
140. Zembouai I, Kaci M, Bruzaud S, Benhamida A, Corre YM, Grohens Y (2013) A study of morphological, thermal, rheological and barrier properties of Poly (3-hydroxybutyrate-Co-3-Hydroxyvalerate)/polylactide blends prepared by melt mixing. Polym Test 32:842–851
141. Zhang L, Xiong C, Deng X (1996) Miscibility, crystallization and morphology of poly(β-hydroxybutyrate)/poly(d,l-lactide) blends. Polymer 37(2):235–241
142. Zhang L, Deng X, Huang Z (1997) Miscibility, thermal behaviour and morphological structure of poly(3-hydroxybutyrate) and ethyl cellulose binary blends. Polymer 38:5379–5387
143. Zhang X, Wei C, He Q, Ren Y (2010) Enrichment of chlorobenzene and o-nitrochlorobenzene on biomimetic adsorbent prepared by poly-3-hydroxybutyrate (PHB). J Hazard Mater 177 (1–3):508–515
144. Zhang XJ, Luo RC, Wang Z, Deng Y, Chen GQ (2009) Applications of (*R*)-3-hydroxyalkanoate methyl esters derived from microbial polyhydroxyalkanoates as novel biofuel. Biomacromolecules 10:707–711. https://doi.org/10.1021/bm801424e
145. Zhao H, Cui Z, Sun X, Turng HL, Peng X (2013) Morphology and properties of injection molded solid and microcellular polylactic acid/polyhydroxybutyrate-Valerate (PLA/PHBV) blends. Ind Eng Chem Res 52:2569–2581
146. Zhao K, Deng Y, Chen JC, Chen GQ (2003) Polyhydroxyalkanoate (PHA) scaffolds with good mechanical properties and biocompatibility. Biomaterials 24:1041–1054
147. Zhou J, Peng SW, Wang YY, Zheng SB, Wang Y, Chen GQ (2010) The use of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) scaffolds for tarsal repair in eyelid reconstruction in the rat. Biomaterials 31(29):7512–7518
148. Zinn M, Witholt B, Egli T (2001) Occurrence, synthesis and medical application of bacterial polyhydroxyalkanoate. Adv Drug Deliv Rev 53(1):5–21
149. Zonari A, Cerqueira MT, Novikoff S, Goes AM, Marques AP, Correlo VM, Reis RL (2014) Poly(hydroxybutyrate-co-hydroxyvalerate) bilayer skin tissue engineering constructs with improved epidermal rearrangement. Macromol Biosci 14(7):977–990. https://doi.org/10.1002/mabi.201400005