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Review

Biomedical applications of polyhydroxyalkanoates, an overview of animal testing and *in vivo* responses

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Polyhydroxyalkanoates (PHAs) have been established as biodegradable polymers since the second half of the twentieth century. Altering monomer composition of PHAs allows the development of polymers with favorable mechanical properties, biocompatibility and desirable degradation rates, under specific physiological conditions. Hence, the medical applications of PHAs have been explored extensively in recent years. PHAs have been used to develop devices, including sutures, nerve repair devices, repair patches, slings, cardiovascular patches, orthopedic pins, adhesion barriers, stents, guided tissue repair/regeneration devices, articular cartilage repair devices, nerve guides, tendon repair devices, bone-marrow scaffolds, tissue engineered cardiovascular devices and wound dressings. So far, various tests on animal models have shown polymers, from the PHA family, to be compatible with a range of tissues. Often, pyrogenic contaminants copurified with PHAs limit their pharmacological application rather than the monomeric composition of the PHAs and thus the purity of the PHA material is critical. This review summarizes the animal testing, tissue response, *in vivo* molecular stability and challenges of using PHAs for medical applications. In future, PHAs may become the materials of choice for various medical applications.

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Polyhydroxyalkanoates (PHAs) represent a complex class of biopolymers consisting of various hydroxyalkanoic acids (FIGURE 1). They are synthesized by bacteria as storage compounds for energy and carbon, normally in the presence of excess carbon and with at least one nutrient essential for growth, such as nitrogen, phosphorus, sulphur or oxygen, present in limiting concentration [1].

PHAs exhibit a high degree of polymerization and molecular weight values up to several million Daltons [2]. They are biodegradable, insoluble in water, nontoxic, biocompatible, piezoelectric, thermoplastic and or elastomeric [1]. These features make them suitable for several applications in the packaging industry, medicine, pharmacy, agriculture, food industry, as raw materials for enantiomerically pure chemicals and in the production of paints [1]. PHAs can be obtained from renewable resources; hence, there has been a tremendous amount of commercial interest in these polymers.

Poly(3-hydroxybutyric acid) (P[3HB]), the most well characterized among all PHAs, was first discovered from the bacterium *Bacillus megaterium* in 1926 (BOX 1) [3].

R-(3HB) remained the only known constituent of bacterial polyhydroxyalkanoic acids (PHA) until constituents other than 3HB were reported in the 1960s and 1970s. Wallen and Rohwedder first reported the identification of hydroxyalkanoates (HA), such as 3-hydroxyvalerate (3HV) and 3-hydroxyhexanoate (3HHx) [4]. Later, Witholt and colleagues noted the presence of 3-hydroxyoctanoate (3HO) units [5] and small amounts of 3HHx units [6] in *Pseudomonas oleovorans* when grown on *n*-octane.

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The discovery of various monomeric components revealed a variety of potentially useful properties of the PHAs and increased interest in these polymers. At this stage, the first industrial production of a copolymer consisting of 3HB and 3HV, sold under the tradename of Biopol®, was reported by Imperial Chemical Industries (ICI) (BOX 1) [101]. These polymers were developed as renewable and biodegradable replacements for petroleum-derived plastics. As a result of this commercial interest, both P(3HB) and P(3HB-*co*-3HV) became widely available, which provided opportunities for their evaluation as medical biomaterials. While these efforts have resulted in several promising clinical trials (for a review please refer to [7,8]) and development efforts continue, products containing these materials have yet to be approved for *in vivo* medical use [7].

The research interests then moved to identify and characterize all the different potential HA units that could possibly be a constituent of this bacterial polyester. This resulted in the discovery of numerous HA constituents, including 4-hydroxyalkanoates [9] and 5-hydroxyalkanoates [10], by the end of the 1980s (BOX 1). To date, approximately 150 different constituents occurring in PHAs alone as homopolyesters or in combination as copolyesters are known [11].

After the late 1980s, development in research concerning bacterial PHA involved the cloning and characterization of genes involved in the biosynthesis of these reserve polyesters (BOX 1). This breakthrough provided a new means of tailoring the properties of PHA polymers for particular applications and represents a potentially important step in the development of technologies to produce designer biomaterials for medical use.

At present, the topic of interest involves the determination of tertiary and quaternary structures of PHA synthases, the key enzymes involved in PHA biosynthesis, their catalytic mechanisms, substrate specificity and the factors that determine the molecular weight of the PHA produced. PHAs can be divided into two subgroups based on the number of carbon atoms present in their monomeric units, that is, PHAs with hydroxyalkanoate monomers of less than six carbon atoms are termed short-chain-length (SCL) PHAs (PHA_{SCL}) and those with six to 14 carbon atoms are termed medium-chain-length (MCL) PHAs (PHA_{MCL}) [12].

The relatively higher cost of PHAs when compared with other biodegradable polymers has limited their use in bulk product manufacture. Nevertheless, PHAs are being explored for biomedical applications, such as wound management [13–15], nerve repair [16–20], drug-delivery systems [7,21–23], stents [24], soft-tissue repair [25–29,102], hard-tissue regeneration [30–39] and heart tissue engineering [7,8,40,41]. In the past 3 years, PHAs have become one of the leading classes of biomaterials under investigation for the development of tissue-engineered scaffolds, both for hard and soft tissues, including cardiovascular products. These polymers offer properties unavailable in existing synthetic absorbable polymers, such as polyglycolic acid (PGA) [7]. This review summarizes advances in the development of biomedical devices based on PHAs, covering the animal models investigated, tissue response, *in vivo* molecular stability and addressing the challenges of using PHAs for a range of medical applications.

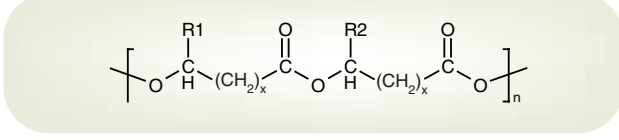

Figure 1. The general structure of polyhydroxyalkanoates (R_1,R_2=H,C_1-C_{13}; x = 1-4; n = 100-30000).

Box 1. The development of polyhydroxyalkanoate science and technology from the first half of the 20th century.

1920-1960:
- Identification of P(3HB)
- Determination of the functions of P(3HB) in bacterial cells

1960-1980:
- Determination of the properties of native P(3HB) granules
- Production of PHAs containing 3-, 4-, 5-hydroxyalkanoate units

1980-2000:
- Industrial production of P(3HB-*co*-3HV) or Biopol® by ICI, UK
- Identification of 3HHx and 3HO in PHAs
- Identification of 4HB in PHAs
- Cloning of genes involved in PHA biosynthesis
- Transfer of Biopol production from ICI, UK, to Zeneca Ltd, UK
- PHA production in recombinant bacteria and plants
- Transfer of Biopol production from Zeneca to Monsanto Co., MO, USA

2000- to date:
- Transfer of Biopol™ production from Monsanto to Metabolix Inc., MA, USA
- Large-scale manufacture of PHAs via transgenic approaches by Metabolix Inc.
- Medical products based on PHA polymers, such as P(4HB), by Tepha Inc., USA
- Development and large scale production of P(3HB-*co*-3HHx) or NODAX™ by Proctor & Gamble Co., USA and Kaneka Corp., Japan

3HHX: 3-hydroxyhexanoate; 3HO: 3-hydroxyoctanoate; ICI: Imperial Chemical Industries; P(3HB): Poly(3-hydroxybutyric acid); P(3HB-co-3HV): Poly(3-hydroxybutanate-co-3-hydroxyvalerate; PHA: Polyhydroxyalkanoates.

Properties of PHAs

Physical & chemical properties

Owing to the variable composition of PHAs, implants made of them can have different physiochemical properties and a tailored degradation rate in biological media, retaining their

mechanical strength for a given short or extended period of time. The disparity in the properties of various PHAs arises because of their chemical composition, either from the length of the pendant groups that extend from the polymer backbones or from the distance between the ester linkages in the polymer backbones [42]. For example, the homopolymer, P(3HB), is a relatively stiff, rigid material that has tensile strength comparable with that of polypropylene, whereas the homopolymer P(4HB) is a highly ductile, flexible polymer with an extension to break of approximately 1000%, compared with P(3HB), which has an extension to break of less than 10%. The introduction of a comonomer into this polymer backbone, significantly increases the flexibility and toughness of the polymer (extension to break and impact strength) and this is accompanied by a reduction in polymer stiffness (Young's modulus), as demonstrated in TABLE 1.

Although the relatively high brittleness of crystalline P(3HB) is a disadvantage for its medical applications, reports have demonstrated that the mechanical properties of P(3HB) films can be improved by the addition of plasticizers [43] or blending with other degradable polymers [8,27]. The rate of bioresorption of PHA polymers *in vivo* varies considerably and depends primarily on their chemical composition. Other factors, such as surface area (as in the case of P[4HB]), physical shape and form, crystallinity and molecular weight, are also found to be very important (for a detailed review, please refer to [7]).

In vivo *responses to PHAs*

Although useful information can be derived from *in vitro* studies, results of *in vitro* studies are not always good indicators of *in vivo* behavior. Hence, in this review, we will focus mainly on the *in vivo* responses to PHAs. Some of the earliest investigations of the *in vivo* tissue responses to PHA polymers were made by WR Grace and Co., in the mid-1960s [103]. In these early studies, strips of P(3HB) films were implanted subcutaneously and intramuscularly in rabbits and removed after 8 weeks. Examination of the implant sites revealed granulomatous foreign body reactions but these did not affect the underlying area. Since these early investigations, many groups have described the *in vivo* tissue responses of P(3HB) and P(3HB-co-3HV) in both biocompatibility and application-directed studies (for a detailed review, please refer to [7]).

However, most of the previously reported *in vivo* studies used industrial rather than medical grades of PHA polymers and hence need to be interpreted carefully depending upon the purity of the PHAs. The studies on the biodegradation of PHA polymers *in vivo* also demonstrated notable differences depending on their chemical composition, surface area, physical shape and form, crystallinity and molecular weight and hence have been a major topic in many reviews recently [7,8]. For example, the *in vivo* tissue reaction and biodegradation studies of P(3HB) and P(3HB-*co*-3HHx) in rabbits revealed that the degradation rate is higher for P(3HB-*co*-3HHx) compared with P(3HB) [44]. The *in vivo* degradation of P(3HB-*co*-3HHx) was found to occur in amorphous regions leading to more porous structures for further hydrolysis [44]. However, in the case of P(3HB), *in vivo* hydrolysis was found to start from a random chain scission both in amorphous and crystalline regions of the polymer matrix [44].

So far, only a few PHAs, such as P(3HB), P(3HB-*co*-3HV), (P4HB), P(3HB-*co*-4HB), P(3HB-*co*-3HHx) and P(3HHx-*co*-3HO), have undergone animal testing and *in vivo* tests for tissue response and have been found to be biocompatible in various host systems (TABLE 2). So far, the very limited human data on products containing these materials have hindered the approval of *in vivo* medical use of PHAs. This further emphasizes the urge for research on animal testing of PHAs and related products in the near future.

Table 1. Material and thermal properties of PHAs.

Polymer	Melting temperature (^{0}C)	Glass transition temperature (^{0}C)	Tensile strength (MPa)	Tensile modulus (GPa)	Elongation to break (%)
P(3HB)	177	4	40	3.5	6
P(4HB)	60	-50	104	0.149	1000
P(3HB-*co*-16%4HB)	152	-8	26	N.D	444
P(3HB-*co*-20%3HV)	145	-1	32	1.2	50
P(3HB-*co*-17%3HHx)	120	-2	20	0.173	850
P(3HO-*co*-12%3HHx)	61	-35	9	0.008	380
Polypropylene	176	-10	38	1.7	400
Polyurethane	195	20	38	0.004	550

ND: Not detected; P(3HB): Poly(3-hydroxybutyrate); P(4HB): PHA: Polyhydroxyalkanoate; Poly(4-hydroxybutyrate); P(3HB-co-16%4HB): Poly (3-hydroxybutyrate-co-16% 4-hydroxybutyrate); P(3HB-co-20%3HV): Poly (3-hydroxybutyrate-co-20% 3-hydroxyvalerate); P(3HB-co-17%3HHx): Poly (3-hydroxybutyrate-co-17% 3-hydroxyhexanoate); P(3HO-co-12%3HHx): Poly (3-hydroxyoctanoate-co-12%3-hydroxyhexanoate). Several reports have demonstrated variation of the measured values, hence an average value has been quoted.

Table 2. A summary of polyhydroxyalkanoates used for animal testing and measuring *in vivo* responses of tissues.

PHA	Application	Animal model	Ref.
P(3HB)	Sutures	Rat	[13,14]
	Conduits	Rat	[20]
	Carrier scaffold	Rat	[19]
	Stents	Rabbit	[24]
	Soft tissue repair	Rat	[25–27]
	Dura mater substitute	Pig	[45]
	Bone tissue scaffold	Cat	[16,17]
	Nerve repair	Calves, human trial	[46–48]
	Pericardial patch	Sheep	[49]
	Artery augmentation		
P(4HB)	Blood vessels	Sheep	[50]
	Artery augmentation	Sheep	[51]
	Heart valves	Sheep	[52]
	Vascular graft	Sheep	[50,53]
	Nerve guides	Rat	[104]
P(3HB-*co*-3HV)	Sutures	Rat	[13,14]
	Implant patches	Dog	[28]
	Bone tissue engineering	Rabbit, rat	[31–39]
	Urological stent	Dog	[28]
P(3HB-*co*-4HB)	Wound dressing	Rats	[105]
	Pericardial patch	Dog	[106]
	Vascular graft	Dog	[106]
	Nerve guides	Rat	[104]
P(3HB-*co*-3HHx)	Artificial esophagus	Dog	[8]
P(3HHx-*co*-3HO)	Heart valves	Sheep	[51,54,55]
	Vascular grafts	Sheep, rat	[56–58]

PHAs reported in drug delivery are described in Table 3.
P(3HB): Poly(3-hydroxybutyric acid); P(3HB-co-3HV): Poly (3-hydroxybutyrate-co- 3-hydroxyvalerate); P(3HB-co-4HB): Poly (3-hydroxybutyrate-co-4-hydroxybutyrate); P(4HB): Poly(4-hydroxybutyrate); P(3HB-co-3HHx): Poly (3-hydroxybutyrate-co-3-hydroxyhexanoate); P(3HHx-co-3HO): Poly(3-hydroxyhexanoate-co-3-hydroxyoctanoate).

Medical devices based on PHAs

Sutures & wound dressings

The desirable features of an ideal surgical suture are high *in vivo* tensile strength during the critical wound-healing period, rapid absorption rate once the critical period is over, minimal tissue reactivity, no memory (the tendency of the suture to revert to its original form even after being twisted or bent), predictable performance, easy suture handling and knot security [59]. There are two types of sutures: nonabsorbable and absorbable. Various natural absorbable (catgut) and synthetic absorbable materials (polygalactin-910 [Vicryl®], polydioxanone [PDS], polyglyconate, polyglecaprone-25 [Monocryl] and polygalactin-910 rapide [Vicryl Rapide®]) are available for the applications as sutures. However, the use of natural gut sutures are restricted because of the risk of transmission of Creutzfeldt-Jakob Disease (CJD) [59].

As early as the mid-1960s, it was suggested that P(3HB) could be used as an absorbable suture [103]. P(3HB) and P(3HB-*co*-3HV) sutures were reported to possess the strength necessary for the healing of myofascial wounds [13,14]. These were compared with other natural absorbable (catgut) and nonabsorbable (silk) sutures. A typical prolonged (throughout the postsurgery monitoring period) and pronounced macrophagal stage was observed in the female wistar rats implanted with P(3HB) and P(3HB-*co*-3HV) sutures. Shishatskaya and colleagues extended their *in vivo* study up to a year and found no acute vascular reaction at the site of implantation or any adverse events, such as suppurative inflammation, necrosis, calcification of the fibrous capsule or carcinogenesis [14]. The reaction of tissues to the implantation of P(3HB) and P(3HB-*co*-3HV) sutures fitted into the usual scheme characteristic of the wound process and of the reaction to a foreign-body invasion. In addition, no differences were recorded between the control and the test animals in the entire set of physiological biochemical parameters, weights of animals and their internal organs, blood morphology and biochemistry and lymphoid tissue response [14]. Most importantly, unlike in the previous *in vivo* studies [15], they noted that the presence of hydroxyvalerate in the polymer did not affect the duration and intensity of the inflammation phase and the development of a fibrous capsule around sutures. Hence, overall, these *in vivo* studies demonstrated that PHAs could be further developed as future natural absorbable sutures.

A highly flexible P(3HB-*co*-4HB) film was used in the abdominal cavity of rats between incisions in the skin and intestine to prevent adhesions [105]. After 1 month, the incisions had substantially healed and no adhesion had occurred, although no *in vivo* degradation of the film was observed even after 1 year. This example emphasizes the need to tailor polymers to control their degradation rate in order to be able to use them for a range of applications. This tailoring of the degradation rate is quite readily possible with polymers of the PHA family.

Nerve repair

Conduits

The four central components of constructs for nerve regeneration include: a scaffold for axonal proliferation, support cells, such as Schwann cells, growth factors and an extracellular matrix [60]. An ideal conduit used to bridge nerve gaps must be easily available, biodegradable, readily vascularized, have a low antigenicity, be porous for oxygen diffusion and avoid long-term compression. Nerve conduits used for peripheral nerve and spinal-cord injuries are typically termed as guidance channels and bridges. Guidance channels have been fabricated from a range of natural (e.g., collagen) and synthetic polymers (e.g., silicone, ethylene vinyl coacetate, ethyl vinyl acetate co-polymer, poly(lactide-*co*-glycolide) [PLG]). Conduits made from these materials possess a range of degradation rates, porosities and mechanical properties. The porosity of the conduit material affects the accessibility of soluble growth-promoting factors or nutrients from the surrounding environment [61]. Additionally, the mechanical properties of the conduit must prevent channel collapse, which would limit neurite outgrowth and regeneration [62]. Although many materials have been tested, the distance that these conduits can bridge has traditionally been limited [60].

The use of a nonwoven P(3HB) sheet as a wrap to repair transected superficial radial nerves was evaluated in cats for up to 12 months [16,17]. The axonal regeneration was demonstrated to be comparable with closure with an epineural suture for a nerve gap of 2–3 mm and the inflammatory response was normal. In a subsequent study, the same material was used to bridge an irreducible gap of 10 mm in rat sciatic nerve and the results were compared with an autologous graft [18]. The rate and amount of regeneration in the P(3HB) conduit did not fully match the autologous nerve graft but it demonstrated good axonal regeneration with a low level of inflammatory infiltration over 30 days.

In another report, P(3HB) conduits were used to bridge a 10-mm gap in the rat sciatic nerve [20]. The conduits were filled with alginate hydrogel and were incorporated with genetically labelled Schwann cells (SC) and cultured allogeneically or syngeneically. It was noted that both allogeneic and syngeneic SC equally enhanced the axonal regeneration distance but the number of axons was greater when syngeneic SC were used. In both cases, a deleterious immune response was not reported [20].

Recently, use of a nerve guide conduit made of P(4HB) increased the axonal regeneration as well as improved the degree of restoration of motor and/or sensory function compared with the previously described conduits made of P(3HB) [53]. The P(4HB), also referred to as PHA4400, conduits used to bridge 10-mm gap in 30 male Sprague-Dawley rats sciatic nerve demonstrated an axonal regeneration rate of at least 0.8 mm per day with no evidence of wound infections, inflammation or anastomotic failures over 20 days.

Carrier scaffolds

Injuries to the spinal cord that result in disruption of axonal continuity have devastating consequences for injured patients [63]. The two basic components for structure supporting axonal migration available currently are either natural or synthetic materials. A variety of natural materials have been used for axonal migration, such as vein, laminin, fibronectin and collagen [64]. Although natural materials are ideal, there are some inherent difficulties, including undesirable immune responses and batch to batch variation [65]. On the other hand, synthetic polymers, such as those based on poly(α-hyroxy acids), for example, poly(L-lactic acid) or poly(glycolic acid), facilitate cell attachment and directed neurite growth and have the advantage that they can be easily used to form fibers [66]. However, the degradation products of these polymers sometimes cause an inflammatory reaction owing to the acidic end products. This can be reduced substantially by using PHAs, such as P(4HB), which offer better mechanical properties and less *in vivo* tissue reaction owing to less acidic end products.

P(3HB) fibers have been used as a carrier scaffold for matrix components and cell lines supporting neuronal survival and regeneration after spinal-cord injury. Novikov used a graft consisting of P(3HB) fibers coated with alginate hydrogel and fibronectin [19]. The fiber was implanted in the lesion cavity after cervical spinal-cord injury in adult rats. In control groups, P(3HB) was omitted and only alginate hydrogel or fibronectin, or their combination, were used for grafting. In addition, comparisons were made with animals treated intrathecally, after spinal-cord injury, with neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) or neurotrophin-3 (NT-3). The neurons of the rubrospinal tract served as the experimental model. It was found that 45% of the injured rubrospinal neurons were lost at 8 weeks postoperatively in untreated animals. However, implantation of the P(3HB) graft reduced cell loss by 50%, a rescuing effect similar to that obtained after treatment with BDNF or NT-3. In the absence of P(3HB) support, implants of only alginate hydrogel or fibronectin, or their combination, had no effect on neuronal survival. After the addition of neonatal Schwann cells to the P(3HB) graft, regenerating axons were seen to enter the graft from both ends and to extend along its entire length [19]. These results demonstrate beyond doubt the potential of the PHAs as carrier scaffold materials in nerve repair.

Drug-delivery systems

Conventional drug therapy, administered either intravenously or via an extravascular route (oral, nasal, sublingual or rectal), typically does not maintain drug concentrations within a desired therapeutic window at the target site for extended periods of time [67]. Controlled-release drug therapy circumvents fluctuations inherent to the conventional dosage forms by achieving a continuous release of drug within the therapeutic window for the required time of treatment. Additionally, the placement of such a system at the target location also results in site-specific drug delivery. The potential use of P(3HB) and P(3HB-*co*-3HV) in drug delivery has been evaluated in a number of studies (TABLE 3)

Table 3. A summary of polyhydroxyalkanoates reported in drug delivery studies.

PHA drug carrier form	Animal model	Drug/hormone/vaccine	Target disease or effect of treatment	Ref.
P(3HB) implants or tablets	Mice	HET	Model drug	[68,69]
	Mice	Celiprolol-HCl	Beta-blocker	[70]
	Human trial	Tetracycline	Gingivitis	[71,72]
	Sheep	GnRH	High incidence of ovulation	[73]
P(3HB) microparticulate carriers	Mice	CCNU	Lewis lung carcinoma	[74]
	Dog	Sulphamethizole	Model drug	[75]
	Mice	5-fluoro-2'-deoxyuridine	P388 leukemia	[76]
	Mice	Coumarin	Good absorption in the payers patches	[77]
	Dog	Rifampicin	Chemoembolization agent in renal arteries	[78]
P(3HB-*co*-3HV) implants or tablets	Rabbit	Sulperazone	Osteomyelitis	[79]
	Cattle	Metoclopramide	Fescue toxicosis	[80]
	Rabbit	Sulbactam-cefoperazone	Osteomyelitis	[81]
P(3HB-*co*-4HB) implants or tablets	Rabbit	Sulperazone or Duocid	Osteomyelitis	[82]

CCNU: Lomustine [N-(2-chloroethyl)-N'-cyclohexyl]-N-nitrosourea; GnRH: Gonadotropin-releasing hormone; HCI: Hydrochloric acid; HET: 7-hydroxyethyltheophylline; P(3HB): Poly(3-hydroxybutyric acid); P(3HB-co-3HV): Poly (3-hydroxybutyrate-co- 3-hydroxyvalerate); P(3HB-co-4HB): Poly (3-hydroxybutyrate-co-4-hydroxybutyrate).

and reviewed several times (for a recent review, see [7]). Studies have included investigations of these polymers as subcutaneous implants, compressed tablets for oral administration and microparticulate carriers for intravenous use. Although there are exceptions, the following observations are fairly typical, as reported by Williams and Martin [7]:

- Increased valerate content in copolymers of P(3HB-*co*-3HV) usually slows down the rate of drug delivery;
- Smaller particle sizes decrease loading capacity but increase the rate of drug release;
- Large changes in polymer molecular weight lead to enhanced release rates;
- Drug release from both P(3HB) and P(3HB-*co*-3HV) is entirely diffusion controlled;
- Lower drug loading reduces the release rate.

The use of P(3HB-*co*-4HB) rods as antibiotic carriers for the treatment of osteomyelitis using Sulperazone™ or Ducoid was evaluated and compared with P(3HB-*co*-3HV) [82]. P(3HB-*co*-4HB) was preferred as it was less rigid and easier to handle as compared with P(3HB-*co*-3HV) [80]. Recently, Hasirci and Keskin reported the use of P(4HB), referred to as PHA4400, and its copolymers, such as P(3HB-*co*-4HB), referred to as PHA3444, and P(4HB-*co*-glycolate), referred to as PHA4422, as matrices for sustained drug delivery [107]. The release of tetracycline from these devices demonstrated that it had desirable physical properties, including strength, modulus and elongation compared with previously reported drug-delivery systems [107].

Stents

The development of the recent concept of drug-eluting stent technology has revolutionized the field of interventional cardiology, which had been dominated previously by bare-metal stents (BMS). One of the main problems with the use of metallic stents in cardiovascular applications is the subsequent restenosis that can result from excessive growth of the blood vessel wall [67].

The polymer carrier, as a drug-delivery coating on the stent, needs to meet several criteria. These include compatibility with the drug, ability to withstand processing, sterilization and storage, adjustable formulation and drug-release properties. Moreover, good mechanical integrity during handling and throughout the clinical deployment procedure in the tortuous vessel anatomy, as well as through its residence in the mechanically and biologically demanding coronary artery is essential along with its vascular compatibility. Recently a lot of attention has focused on the use of PHAs as an alternative to mechanical stents where the PHAs can prevent reocclusion of the vessel in the short term but can then be absorbed so that it does not cause any persistent irritation of the vessel wall. However, when P(3HB) biodegradable stents and tantalum stents were implanted into the iliac arteries of New Zealand white rabbits for up to 30 weeks, it was found that P(3HB) instigated intense inflammatory reactions with an increase in collagen (2.4- to 8-fold vs native segments), thrombosis and in-stent lumen narrowing [24]. The elastic membranes were destroyed in all specimens. By contrast, the tantalum stents increased the in-stent lumen progressively, penetrated the external elastic membrane and increased mural collagen

content (6- to 8.6-fold vs native segments) without restenosis or thrombosis. Thus, it was concluded that P(3HB) stents caused intensive inflammatory vascular reactions in the rabbit iliac artery, which saw them banned from clinical use [24]. Although the *Alcaligenes eutrophus*-derived P(3HB) used in this study was gas sterilized with ethylene oxide prior to implantation, endotoxin levels in the P(3HB) used were not quantifie owing to the presence of endotoxins known to copurify with P(3HB), which emphasizes the importance of the pharmacological purity of PHAs for medical applications.

Soft-tissue repair

Implant patches

Surgical repair of soft tissues, such as hernias, was proposed as a potential use of P(3HB) by Baptist and Ziegler as early as 1965 [17]. P(3HB) patches for the gastrointestinal tract utilizing the rat experimental system were tested [25]. Asymmetric patches made from P(3HB) sutured onto the stomach wall did not induce a strong inflammatory response or fibrosis [25]. In another study, implants of P(3HB) patches produced better results in the stomachs of rats as they adhered well to the gastric wall compared with the Vicryl patches used normally [26].

Improved mechanical properties of P(3HB), such as higher flexibility and elongation at break point, were achieved by blending bacterial P(3HB) with atactic P(3HB) (at-PHB, prepared from β-butyrolactone with potassium acetate as catalyst), while the crystallinity and glass transition temperature decreased. These blends have the potential to be used as a new biomaterial for temporary soft-tissue applications because of their suitable mechanical properties and tailorable degradation behavior [27]. P(3HB)/at-PHB blend was evaluated for repair of a bowel defect in Wistar rats using a patch film of the blend that was fabricated by a dipping/leaching method [27]. A total of 26 weeks postimplantation, material remnants were found in only one of the four animals. The bowel defects were closed in all cases, demonstrating that the patch material resists intestinal secretions for a sufficiently long time before it degrades completely [27].

P(3HB-*co*-3HV) membranes reinforced with polygalactin 910 (Vicryl) fibers have been used as an occlusive barrier over implants [28]. This was placed into fresh extraction sockets of ten dogs having the third and fourth mandibular premolars extracted bilaterally. The P(3HB-*co*-3HV) membranes used in this study interfered with the marginal bone healing adjacent to the immediately placed implants [40]. An increased inflammatory reaction and significantly less marginal bone healing was registered near the P(3HB-*co*-3HV) membrane compared with the control side [29]. The nonporous P(3HB-*co*-3HV) film was also tested to keep the mucoperiosteum and bone separated until the transition of teeth was completed [28]. After 8 and 12 weeks, the films were unimpaired and surrounded by fibrous capsules. Thus, it was concluded that P(3HB-*co*-3HV) films were more suitable for this procedure in terms of mechanical properties and tissue response than other synthetic polymers, such as polylactic acid or polycaprolactone.

Urological stent

Bowald and Johansson described the use of a tube for urethral reconstruction [102]. A solution of the P(3HB-*co*-3HV) was used to coat thin, knitted tubes made of Vicryl material and implanted into four dogs to replace the urethra. After 6–9 months, a fully functional urethra had been reconstructed in all test animals, hence proving the promise of PHAs in urology.

Artificial esophagus

Corrosive injury of the esophagus in children is a serious condition with many possible complications, including perforation, mediastinitis, fistulization to the greater vessels, penetration to the stomach and stricture formation [83]. Recently, some studies using P(3HB-*co*-3HHx) as artificial esophagus material demonstrated that P(3HB-*co*-3HHx) can stimulate the regeneration of the removed esophagus of dogs. This result demonstrates that P(3HB-*co*-3HHx), which has good elasticity and strength, could be a good candidate material for use as an artificial esophagus [8].

Dura mater substitute

Significant technical advances, such as imaging and technical refinements, in recent years have helped the base of the skull to be routinely approached for various symptoms, such as malignant head and neck tumors invading the skull base [45]. Bacterial P(3HB) with synthetic at-PHB has been used as a dura mater substitute [45]. Such patches are of interest since no ideal dura substitute is available currently, although numerous experimental and clinical studies have been performed to identify a suitable material. In the past, lyophilized human dura or substitute materials from animal origin, such as bovine pericardium or dura mater, were used. The availability of these materials is limited owing, to reports of transmission of prion diseases such as CJD or HIV [84–86].

P(3HB)-based patch materials fulfill the requirements that are necessary for a dura substitute, including defect closure, stability and biocompatibility. The patch material should help in sealing the tissue defect, provide support for tissue regeneration, should not adhere to the brain and must be flexible. Blends of PHB/at-PHB 70/30 and 50/50 have a smooth surface that prevents the adhesion of brain in addition to being porous (in contact with the rhinobasal skull) enough to support bone regeneration. The material was tested in minipigs since they have the great frontal sinus near the brain (similar to human paranasal sinuses). Experiments were conducted to cover rhinobasal-skull defects, including a dura-mater lesion, using the blends of PHB/at-PHB. Histological examination after 3, 6 and 9 months of implantation confirmed defect closure, prevention of adhesions to brain tissue and no inflammation or malignant degeneration [45]. Hence, PHA-based materials are promising dura mater substitutes.

Hard-tissue regeneration

PHAs on their own or as composites in combination with inorganic phases (to further improve the mechanical properties, rate of degradation and also to impart bioactivity) are being

considered for hard-tissue engineering applications. P(3HB), P(3HB-*co*-3HV) and P(3HB-*co*-3HHx) are some of the polymers that have been studied extensively to fabricate composites in combination with hydroxyapatite, bioactive glass and glass-ceramic fillers or coatings, as reviewed recently [87].

Kostopoulus and Karring successfully used P(3HB-*co*-3HV) membranes to create spaces for bone fill to treat jaw-bone defects in rats [31]. During a 6-month study, P(3HB-*co*-3HV) membranes helped in increasing the bone fill compared with an uncovered control that demonstrated in growth of other tissues. The same group also successfully used P(3HB-*co*-3HV) membranes to increase the height of the rat mandible [32].

Several studies of the use of PHA polymers for internal-bone fixation devices have been reported. For example, the use of compression molded T-plates prepared from P(3HB) reinforced with carbon fibers (7%) to fix osteotomies of the tibial diaphysis in rabbits were described [33]. The implants were fixed to the tibia with absorbable polyglycolic acid (PGA) sutures and compared with implants prepared from reinforced Vicryl. After 12 weeks, the reinforced P(3HB) gave better results than the Vicryl plates, with the latter frequently leading to nonunion of the osteotomies, breakage and angulation. Later, Doyle and colleagues studied the behavior of P(3HB) filled with hydroxyapatite (HAp) *in vivo* [34]. No significant difference between implants derived from P(3HB) or P(3HB) filled with HAp were observed when rivets were prepared from these materials and inserted into rabbit femurs. The overall tissue responses were favorable and some indication of osteogenic activity of P(3HB) was noted [34].

Since the homopolymer of P(3HB) is piezoelectric, its role in inducing local bone formation was studied in subsequent years. Knowles and colleagues demonstrated that the piezoelectric potential output of P(3HB-*co*-3HV) composites with glass fibers is fairly close to that of bone [35]. Furthermore, the same group implanted P(3HB-*co*-3HV)-phosphate glass composites subcutaneously and as nonload bearing femoral implants in rats [36,37]. Initially, relatively high cellular activity was observed that was attributed to ions being released from the glass. At 4 weeks, cells were seen entering the surface pores in the femoral implants formed by the solubilizing glass and, over time, new bone was seen developing on the implant surface. However, when composites of P(3HB-*co*-3HV)/HAp and P(3HB-*co*-3HV)/HAp-glass were implanted in rabbit femurs, the former bonded better than the latter. This result was attributed to the release of ions from the P(3HB-*co*-3HV)/HAp-glass composites causing a soft-tissue reaction [38,39]. More recently, the interface between P(3HB)/HAp composites and bone when implanted *in vivo* was studied in rabbits [88]. After 1 month of implantation, bone apposition occurred along the whole length of the implant interface. At 3 months after the implantation, bone formation with an interlocking structure was observed on the exposed HAp particles at the interface, followed by dense bone formation after 6 months of implantation.

In addition to composites incorporating HAp and bioactive glass in PHAs, Jones and colleagues tested composites of P(3HB-*co*-3HV) with tricalcium phosphate filler in subcutaneous and femoral implants [89]. These implants were compared with composites derived from PLA and tricalcium phosphate and found to degrade approximately four times slower *in vivo*. Recently, an *in vivo* study of porous P(3HB-*co*-3HV) and calcium phosphate-loaded collagen (CaP-Gelfix) foams in rat femurs was reported [30]. Both cell-loaded and cell-free P(3HB-*co*-3HV) matrices elicited minimal fibrous-tissue formation during the 6-week implantation. Moreover, macroscopic and radiological studies demonstrated better healing with P(3HB-*co*-3HV) matrices than with CaP-Gelfix in 3 weeks. However, histologically, fibrous connective tissue establishment and inflammation scores were significantly higher in the CaP-Gelfix group when compared with the P(3HB-*co*-3HV) group [30]. Research is presently being carried out in our laboratory to create novel PHA composites containing bioactive glass (Bioglass®) particles. Initial attempts have been carried out to form 3D porous P(3HB)/Bioglass scaffolds. This is the first time that Bioglass of type 45S5 (45 wt% SiO_2, 24.5 wt% Na_2O, 24.5 wt% CaO and 6 wt% P_2O_5) has been combined with PHAs for tissue engineering applications (MISRA AND COLLEAGUES, UNPUBLISHED DATA).

Overall, therefore, PHAs and their composites are promising new materials for hard-tissue regeneration.

Heart tissue engineering

Cardiovascular diseases are the most common causes of death and serious morbidity in the world [90]. In most cases, surgical treatment is necessary and patch closure, reconstruction of the defect or revascularization is required [91]. The patch materials used currently are limited to prosthetic materials, autologous pericardium and allogenic or xenogenic (glutaraldehyde-fixed) pericardium [92]. These materials are unable to regenerate, repair and remodel, leading to aneurysm formation in patch aortoplasty, inelasticity and a high risk of hemolysis induced by contact with blood. An optimal cardiovascular patch material should have long durability, lack thrombogenicity, be resistant to infections, lack immunogenicity, have a potential for cell growth and the ability to prevent patch dilatation and thinning.

Pericardial patch

One of the most advanced applications of PHA polymers in cardiovascular products has been the development of a regenerative P(3HB) patch that can be used to close the pericardium after heart surgery, thus preventing adhesions between the heart and sternum [46,47]. However, the P(3HB) patch generated a strong immunogenic reaction in sheep models with the polymer being phagocytosed by polynuclear macrophages. However, in this study, the sheep were not subjected to bypass surgery, which is considered more clinically relevant. Promising results were, however, obtained in the first clinical study of P(3HB) patches carried out in a set of 50 human patients who had undergone bypass surgery and valvular replacement [48]. Using computed tomography (CT), 39 of these patients (19 with patch and 20 without) were

examined for the presence of adhesions at 6 and 24 months. The group with PHA patches developed lesser adhesions, thus indicating that these biodegradable polymers could be used as pericardial patches.

Artery augmentation

Studies on P(3HB) nonwoven patches, as transannular patches, were carried out by implanting them into the right ventricular outflow tract and pulmonary artery in 13 weaning sheep [49]. Regeneration of a neointima and a neomedia, comparable to native arterial tissue, was observed in the test group after 3–24 months. In the control group, a neointimal layer was present but no neomedia comparable to native arterial tissue was found. These results demonstrate the potential of P(3HB) nonwoven patches as scaffolds for tissue regeneration in low-pressure systems.

P(4HB) has been used successfully as a scaffold for preparing autologous cardiovascular tissue [65]. Patches of P(4HB) with 95% porosity and pore sizes in the range 180–240 µm were used as scaffolds seeded with autologous endothelial, smooth muscle and fibroblast cells to augment the pulmonary artery in a sheep model [51]. Echocardiography and explant examination revealed progressive tissue regeneration with no evidence of thrombus, stenosis or dilation in the patch containing sheep. In comparison, a slight bulging and lesser tissue regeneration were found at 20 weeks for the control patch. An additional positive feature of the P(4HB) patching material was a self-sealing property preventing blood leakage compared with a polytetrafluoroethylene (PTFE) patch, which leaves a hole for blood to leak.

Vascular grafts

Vascular grafting is used to repair or replace malfunctioning blood vessels in the arterial or venous systems due to damage or disease [7]. Large diameter blood vessels are generally replaced with synthetic grafting materials, usually Dacron™ (polyethylene terphthalate) or expanded PTFE (ePTFE). However, these materials do not perform well for a small-diameter graft since the grafts rapidly close. Instead, when surgeons require small diameter grafts, such as coronary bypass procedures, they use blood vessels from the patient. Such autologous grafts can be compromised or be in short supply if the patient has had multiple procedures. A synthetic graft was investigated with P(3HB-*co*-4HB) as a graft coating in dogs for up to 10 weeks [106]. The degradation of the polymer started after 2 weeks of implantation. However, when P(3HHx-*co*-3HO) was used as an impregnation substrate in rat models, the results demonstrated very slow degradation of the polymer (30% reduction in molecular weight after 6 months) [57,58]. Further, P(3HHx-*co*-3HO) was evaluated as a component of an autologous cell-seeded tissue engineered vascular graft that also contained a nonwoven PGA mesh on the inside layers and was studied in lambs [56]. All the P(3HHx-*co*-3HO)-PGA grafts were found to allow non-restricted blood flow except for one structure and no aneurysms or inflammatory responses were observed.

Recently, P(4HB) scaffolds were used to create tissue engineered viable ovine blood vessels and implanted them in a sheep model [50]. The study revealed that the mechanical properties of the bioreactor cultured tissue engineered blood vessels were very similar to those of the native aorta [50]. In another similar study, tissue engineered blood vessels (TEBV), autologous cells seeded in P(4HB) scaffolds and cultured in bioreactors were implanted in the descending aorta of juvenile sheep and found to be fully functional for up to 3 months. However, at 6 months, the graft remained functional but significantly dilated, caused by an insufficient elastic fiber synthesis [53].

Heart valves

Recently, some of the most promising results in heart valve development have been obtained with PHA polymers [52,54,55]. Heart valve replacement surgery is particularly acute for the pediatric population. Currently used heart valve prostheses are nonviable, hence child patients outgrow replacement valves and need repeat surgeries to replace them. Further, for the adult population, improved valve durability is required. A promising approach is the development of tissue engineered heart valve scaffolds with a high degree of maturity before implantation.

In early studies, several synthetic absorbable polyesters, such as PGA and PLA, were evaluated as potential scaffolding materials for heart valves [7]. However, the synthetic polyesters were too stiff to function as flexible leaflets inside a trileaflet valve [7]. Interestingly, the leaflets replaced with porous and relatively more flexible P(3HHx-*co*-3HO)-PGA mesh were found to be more suitable in the *in vivo* study in lambs [54]. The postoperative echocardiography of the vascular cells seeded P(3HHx-*co*-3HO) constructs revealed no thrombus formation with mild stenosis and trivial regurgitation for up to 17 weeks after implantation in a lamb model [55]. It was concluded that tissue engineered heart valve scaffolds made from P(3HHx-*co*-3HO) can be used for implantation in the pulmonary position for 120 days in lambs [55].

In order to achieve more rapid tissue remodeling *in vivo*, recently, a porous scaffolding material in the form of a trileaflet heart valve was produced from a PGA nonwoven mesh solvent coated with P(4HB) [52]. In an *in vivo* study, implantation of the tissue engineered heart valve in place of the native pulmonary valve of juvenile sheep was found to function well and echocardiography of the implanted valves demonstrated functioning mobile leaflets without any stenosis, thrombus or aneurysm. Just 8 weeks after implantation it was reported that the composite scaffolds had completely degraded and by 20 weeks it had been replaced with a new tissue engineered heart valve that closely resembled the native valve. This work has been reviewed recently and the following points were emphasized [41]:

- The tensile mechanical properties of the valve were almost indistinguishable from the native valve;
- Biochemical analysis revealed a similar make up to the native counterpart;

- Histological analysis of the leaflet structure (perhaps the most striking result) demonstrated that the new structure was made up of three distinct organized layers: a fibrous layer of collagen, a loose layer rich in glycosaminoglycans and a layer of elastin that is characteristic of the native leaflet structure;
- The size of the valve had increased from 19 mm at implant to 23 mm at 20 weeks, as the lamb had grown.

This is certainly one of the most promising results of heart tissue engineering since it indicates that it should be possible to develop a valve for children that can grow and therefore does not need to be replaced.

Relevance of pharmacologically pure PHAs

The outstanding positive biological response to PHA polymers *in vivo* represents the most important property of these biomaterials if a medical application is being contemplated. Most of the previously reported studies have been based on the use of industrial rather than medical grades of PHA polymers and hence pyrogenic contaminants copurified with PHAs might have been the cause of adverse tissue responses as opposed to the monomeric composition of the PHAs.

In general, the PHA recovery processes from bacteria can be divided into two categories: solvent extractions and aqueous digestions. Both processes have to fulfill several conditions: high purity of the end-product, avoidance of halogenated solvents, no degradation of the polymer and high efficiency. Currently, PHAs are produced at an industrial scale using Gram-negative bacteria exclusively (such as *Wautersia eutropha*, *Methylobacterium organophillum*, *Pseudomonas oleovorans* and recombinant *Escherichia coli*). However, PHAs isolated from Gram-negative organisms, such as *E. coli*, contain the outer membrane lipopolysaccharide (LPS) endotoxins, which are pyrogens known to copurify with the PHAs. The presence of LPS induces a strong immunogenic reaction and is therefore undesirable for the biomedical application of the PHAs [8]. Owing to the serious risks associated with endotoxin, the US FDA has set guidelines for medical devices and parenteral drugs [93]. Current limits are such that eluates (endotoxin free water where the medical devices are immersed for at least 1 h at room temperature) should not exceed 0.5 EU/ml of endotoxin, unless the device comes into contact with cerebrospinal fluid, where the limit is then 0.06 EU/ ml [94].

PHAs produced by the fermentation of Gram-negative bacteria can be treated with an oxidizing agent, such as hydrogen peroxide or benzoyl peroxide, to reduce the endotoxin content to less than 20 endotoxin units/gram of PHA to produce PHA that does not elicit an acute inflammatory response when implanted in an animal [42]. Lee and colleagues have demonstrated that sodium hydroxide can also be used to remove endotoxin [95]. Tepha, Inc. (MA, USA) produces P(4HB), which meets the standards set by the FDA for endotoxin and biocompatibility tests recommended in 1995 by the Office of Device Evaluation in their general program memorandum #G95–1 'Biological Evaluation of Medical Devices' [41]. Gram-positive bacteria lack LPS and are hence potentially better sources of PHAs to be used for biomedical applications, as reviewed recently [96]. Recently, supercritical fluid extraction (SFE) with CO_2 was suggested as a promising approach for the purification of PHAs because it does not use toxic solvents or a drying step and the process does not degrade the polymer sample [201].

Expert commentary

The many successful studies using various animal models have clearly demonstrated that PHAs, represented by P(3HB), P(3HB-*co*-3HV), P(4HB), P(3HB-*co*-4HB), P(3HB-*co*-3HHx) and P(3HHx-*co*-3HO), possess the biodegradability, biocompatibility and thermoprocessibility required for various medical applications. Some of the patents granted since the year 2000, for a variety of biomedical devices based on PHAs, are tabulated in TABLE 4. Depending on the property requirement for different applications, PHAs can be blended, surface modified or composited with other polymers, enzymes or even inorganic materials (as fillers or coatings) to further adjust their mechanical properties or biocompatibility. Even blending among the several PHA themselves can change the material properties and biocompatibility. The enormous possibility to tailor-make PHAs for specific medical devices has made this class of materials promising as tissue engineering scaffolds. For example, one of the most promising PHAs are P(4HB) [41] fibers, which are stronger than typical polypropylene sutures (410–460 MPa) and at least comparable in strength to commercial absorbable sutures, such as Maxon™ (540–610 MPa) and PDS II™ (450–560 MPa) [92]. What may potentially set the P(4HB) suture fiber apart from current absorbable synthetic fibers is a lower Young's modulus resulting in improved handling and a different breaking strength retention profile upon implantation. The Young's modulus of oriented P(4HB) fibers (670 MPa), for example, is significantly lower than that of other monofilament sutures, such as Maxon (2930 MPa), PDSII (1380 MPa) and Biosyn™ (1000 MPa) [97].

From initial studies, it appears that P(4HB) degrades faster than other PHAs, such as (P3HB), in a subcutaneous environment [41]. In one implantation study, the loss of mass from a P(4HB) implant was found to vary with porosity [51] and surface area. P(4HB) polymers are likely to undergo gradual changes in mechanical properties rather than the more abrupt changes observed with other synthetic absorbable polymers, such as PGA. This could potentially be advantageous in applications where a sudden loss of a mechanical property is not desirable and a gradual loss of implant mass with concomitant growth of new tissue is beneficial. Further, owing to its lower pK_a and tendency to lactonize, 4HB is less acidic than the α-hydroxy acids, such as glycolic and lactic acids, that are released from PGA and PLLA implants, causing lesser irritation. Moreover, P(4HB), unlike PGA, is relatively stable to moisture even during processing and has a satisfactory shelf life. Finally, all currently used cardiovascular prostheses (namely, PTFE, Dacron, fixed pericardium, xenografts, mechanical heart valves and homografts) do not have any endothelial coverage owing to poor adherence to prosthetic grafts. However, seeding of autologous cells and *in vitro*

Table 4. List of patents granted, since 2000, for medical applications of polyhydroxyalkanoates.

Patent (year)	Inventors	Title
WO 0015216 (2000)	Veech RL	Therapeutic compositions
WO 0004895 (2000)	Martin DP, Peoples OP, Williams SF, Zhong L	Nutritional and therapeutic uses of 3-hydroxyalkanoate oligomers
US 6013590 (2000)	Noda I	Fibers, nonwoven fabrics and absorbent articles comprising a biodegradable polyhydroxyalkanoate comprising 3-hydroxyalkanoate and 3-hydroxyhexanoate
US 6043063 (2000)	Kurdikar DL, Strauser FE, Solodar AJ, Paster MD, Asrar J	Methods of PHA extraction and recovery using nonhalogenated solvents
WO 0051662 (2000)	Williams SF	Bioabsorbable, biocompatible polymers for tissue engineering
WO 0119361 (2001)	Williams SF, Martin DP	Therapeutic uses of polymers and oligomers comprising γ-hydroxybutyrate
US 2001009769 (2001)	Williams SF, Martin DP, Gerngross T, Horowitz DM	Polyhydroxyalkanoates for *in vivo* applications
US 2002141967 (2002)	Williams SF, Martin DP	Polyhydroxyalkanoate compositions for soft-tissue repair, augmentation and viscosupplementation
US 6867247 (2002)	Williams SF, Martin DP, Skraly FA	Medical devices and applications of polyhydroxyalkanoate polymers
US 6623749 (2003)	Williams SF, Martin DP, Gerngross T Horowitz DM	Medical devices containing polyhydroxyalkanoate treated with oxidizing agent to remove endotoxin
WO 2004101002 (2004)	Martin DP, Rizk S Ahuja A, Williams SF	Polyhydroxyalkanoate medical textiles and fibers
WO 2004065608 (2004)	Yanagita Y, Ogawa N, Ueda Y, Osakada F, Matsumoto K	Method of collecting highly pure polyhydroxyalkanoate from microbial cells
US 6828357 (2004)	Martin DP, Skraly FA, Williams SF	Polyhydroxyalkanoate compositions having controlled degradation rates
WO 2005020825 (2005)	Terenghi G, Mohanna PN, Martin DP	Polyhydroxyalkanoate nerve regeneration devices
WO 2005007195 (2005)	Hasirci VN, Keskin DS	Poly-4-hydroxybutyrate matrices for sustained drug delivery
WO 2005121206 (2005)	Kenmoku T, Fukui T, Mihara C, Kusakari A, Yano T	Polyhydroxyalkanoate having vinyl group, ester group, carboxyl group and sulfonic acid group and production method thereof
WO 2006015276 (2006)	Rizk S	Noncurling polyhydroxyalkanoate sutures
WO 2006004814 (2006)	Narasimhan K, Yee K, Cearley AC, Levengood D, Chen GQ	Solvent extraction of polyhydroxyalkanoates from biomass
US 20060084161 (2006)	Yanagita Y, Ogawa N, Ueda Y, Osakada F, Matsumoto K	Method of collecting highly pure polyhydroxyalkanoate from microbial cells
US 20060084155 (2006)	Huisman GW, Skraly F, Martin DP, Peoples OP	Biological systems for manufacture of polyhydroxyalkanoate polymers containing 4-hydroxyacids

conditioning of a PHA graft was found to be promising since the cells attach themselves readily to the PHA scaffold [98]. Hence, further experiments on these new constructs might improve endothelial coverage, which will be highly beneficial in a tissue engineered cardiovascular construct.

Five-year view

In vivo *studies*

Early results summarized in this review thus indicate that certain polymers of the PHA family, such as P(3HB), P(3HB-*co*-3HV), P(4HB), P(3HB-*co*-4HB), P(3HB-*co*-3HHx) and P(3HHx-*co*-3HO), are filling an unmet need for new absorbable biomaterials with properties unavailable in alternative materials for medical product development. Among all other applications investigated in the past 5 years, PHAs have become one of the materials of choice for the development of tissue-engineered cardiovascular products because they can offer properties not available in other synthetic absorbable polymers, such as PGA, PLLA or polycaprolactone. Successful animal testing has helped to develop one of the most advanced applications of PHAs, the development of a regenerative P(3HB) patch that can be used to close the pericardium after heart surgery. Although clinical study

of these patches in human patients took place a decade ago, cardiovascular products made of PHAs, such as tissue-engineered heart valves, are expected to become a clinical reality only within the next decade. Recently, one of the very promising early studies that attempted to create human cardiovascular tissue *in vitro* using P(4HB) was reported [98]. Although tissue engineering of autologous human cardiovascular patches appears promising, issues, such as endothelial coverage and control of extracellular matrix formation, have to be addressed in future *in vivo* experiments before a final human implantation can take place. However, the developments in the cardiovascular application of PHAs and its market impacts are growing at a fast pace. Tepha Inc. (MA, USA), the world's leading company developing PHAs for medical applications, emphasizes that the market, just for heart valves and related products, increased from US$420 million in 2001 to $573 million in 2006 [202]. Moreover, the company forecasts dramatic gains in the future in the cardiovascular application of PHAs as the market for a transcatheter segment alone is predicted to potentially exceed $500 million by 2010.

Availability of PHAs

Scaling up the production of pharmacologically relevant PHAs, such as P(4HB), needs more attention as the currently employed fermentation strategies often result in less pure polymers. Progress is being made in using plants as the source of pharmacologically pure PHAs. However, research advances need to be made to improve low PHA contents in plant cells, overcome the compartmentalization of PHA biosynthesis gene expression in plant hosts and establish an efficient method of isolation of PHAs from plants. We forecast that it will be at least 10–15 years before PHAs can be obtained commercially from agriculture.

Novel PHAs

Another aim must be the detection of novel PHAs, which might exhibit features not demonstrated by other synthetic polymers and are therefore of extra value. The physical and mechanical properties of PHAs are dependent on the compound's monomeric composition. Recently, the Gram-negative PHA accumulating bacterium *Ralstonia eutropha* synthesized a copolymer of 3-hydroxybutyrate and 3-mercaptopropionate, P(3HB-*co*-3MP), which might be used, for example, as a skin substitute because these new polymers may exhibit antifungal and antibacterial properties [99]. In future, we suggest that PHAs incorporating antimicrobial ions, such as copper and silver, could be produced. These materials would act as antimicrobial ion-releasing implants that would fight bacterial infections by releasing ions, thus avoiding the need for antibiotics in postoperational healing. To this end, we think that some strains of the PHA-accumulating bacterial genera, *Pseudomonas*, which are also known to accumulate metals (e.g., *Pseudomonas stutzeri* AG259), could be used for the production of metal-containing PHAs.

Overall, the review of the recent literature presented here reveals that PHAs are promising new materials in the ever evolving area of materials for biomedical applications. PHAs have the potential to meet a large number of requirements for the design and fabrication of biomedical devices in the near future. These polymers, if used judiciously and innovatively, have the scope of revolutionizing the use of biodegradable polymers in medicine.

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Key issues

- Production of cost-effective and pure, medically relevant polyhydroxyalkanoates (PHAs) is discussed along with the possibilities of producing new PHAs with novel properties for diverse medical applications.
- PHAs are becoming increasingly important in medical applications owing to desirable properties, such as biodegradability, biocompatibility and mechanical characteristics, they offer *in vivo*. This fact, coupled with the feasibility of producing medically relevant PHAs in a pharmacologically pure grade, have contributed to significant developments in this field during the past 6 years.
- Tailored physical, biological and mechanical properties, as well as predictable degradation behavior, of PHAs can be achieved by choosing the right microorganism and carbon source or by employing genetically modified organisms to produce PHAs.
- *In vivo* tissue responses during various medical applications of PHAs are discussed in relation to some of the alternative available materials.

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