Applications of Polyhydroxyalkanoates in the Medical Industry

Christopher J. Brigham*,[1] and Anthony J. Sinskey[1,2,3]

[1]*Department of Biology;* [2]*Health Sciences Technology Division;* [3]*Engineering Systems Division, Massachusetts Institute of Technology, Cambridge, MA 02139, USA*

Abstract: The bio-based, biodegradable family of polymers, polyhydroxyalkanoates (PHA), is an attractive candidate for an environmentally friendly replacement of petroleum-based plastics in many applications. In the past decade, many groups have examined the biodegradability and biocompatibility of PHA in cell culture systems or in an animal host. Findings suggest that PHA is a suitable material for fabrication of resorbable medical devices, such as sutures, meshes, implants, and tissue engineering scaffolds. The degradation kinetics of some PHA polymers is also suggestive of drug release applications. In this review, we examine the progress, potential applications, challenges and outlook in the medical polyhydroxyalkanoate field.

Keywords: PHA, biopolymer, biodegradable, biocompatible, implant, drug release, medical device.

INTRODUCTION

Polyhydroxyalkanoates (PHA) are intracellular carbon storage mechanisms for many species of microorganism. PHA is generally produced by organisms in environments when carbon is plentiful, but other nutrients (e.g. nitrogen, phosphorus) are limiting. In 1927, Lemoine first discovered polyhydroxybutyrate (PHB), a member of the PHA family, in *Bacillus megaterium* [1]. Since that time, many discoveries have generated excitement over the potential of utilizing PHA in household, industrial, medical, and other applications. PHA is made by microorganisms and can easily be broken down by them. Many studies have shown that PHA is readily degraded by a variety of microorganisms when placed in a natural ecosystem, such as the soil or aqueous environments [2-4], thus demonstrating that PHA is a bio-based, biodegradable family of polymers. Many PHA types exhibit thermal and mechanical properties that are similar to petroleum-based plastics, such as polypropylene [5-7]. Monomer side chain length plays a role in the thermal and mechanical properties of PHA. A homopolymer of short side chain length monomers, such as PHB, exhibits stiffness and brittleness. However, a copolymer consisting of monomers of different chain lengths, such as poly(hydroxybutyrate-*co*-hydroxyhexanoate) (P(HB-*co*-HHx), see Figure **1**), exhibits pliability and flexibility [7]. Given these properties, PHA polymers are considered a biodegradable, environmentally friendly alternative to chemically synthesized plastics.

PHA can be produced commercially by fermentation of microorganisms in the presence of an inexpensive carbon source. The list of microorganisms that have been used for these processes is small, including *Ralstonia eutropha* (the paradigm of microbial PHA biosynthesis), recombinant *Escherichia coli*, *Aeromonas caviae,* and *Pseudomonas putida*, to name a few. The value of these specific organisms lies in three main aspects: (1) the ability to produce large amounts of intracellular polymer; (2) the ability to grow on a wide variety of carbon sources, including those found in agricultural waste streams; and (3) the amenability towards genetic manipulation. Thus, even though numerous species of bacteria produce PHA, only a few have been used in commercial scale fermentations to produce large amounts of the value added polymer.

For use in medical applications, materials must be biocompatible, which means they cannot cause severe immune reactions when introduced to soft tissues or blood of a host organism. PHA materials must also not elicit immune responses during degradation in the body to be considered biocompatible. Typically, PHA polymers are degraded by the action of non-specific lipases and esterases in nature [4]. This is presumably how PHA implants and other medical devices are degraded at the site of implantation in animals. Degradation of PHA matrices in the tissues of the host organism offers the possibility of coupling this phenomenon with release of bioactive compounds, such as antibiotic or anti-tumor drug. If a PHA insert is impregnated with a compound, the degradation over time will release the compound, acting as an automatic dosing agent. The kinetics of dosing of a compound from a PHA matrix can be tuned by altering the polymer properties, including using different types of PHA with different monomer side chains (Figure **1**). In this review, we examine the findings of PHA biocompatibility studies, PHA medical application

*Address corresponding to this author at the Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA; Tel: +1-617-253-5106; Fax: +1-617-253-8550; E-mail: cbrigham@mit.edu

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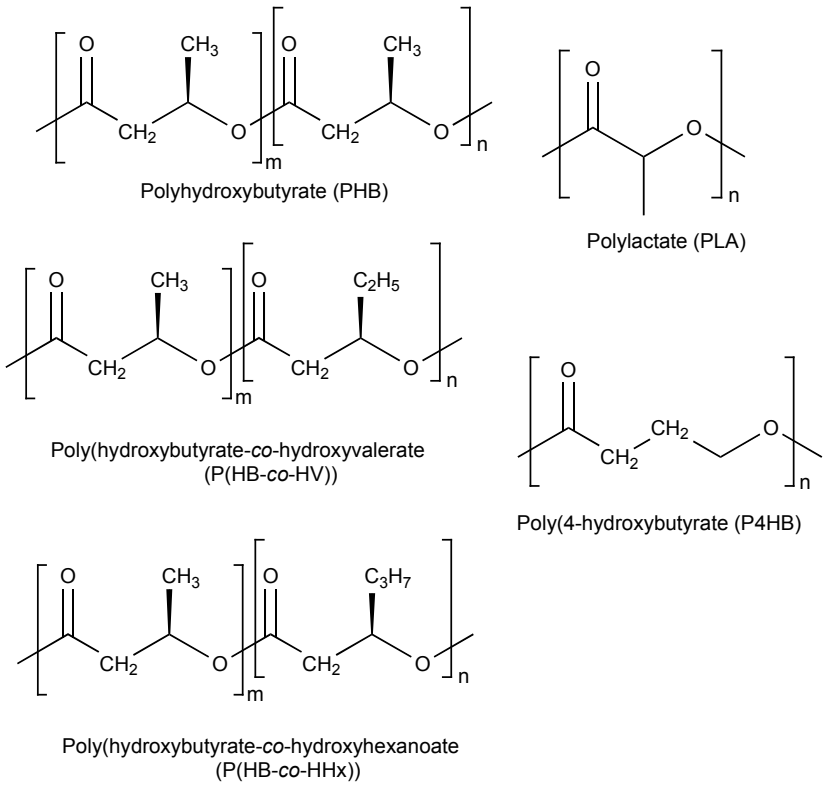

Figure 1: Schematic structures of representative polyhydroxyalkanoates. The representatives shown here are biocompatible and have been examined in different medical applications.

Figure 2: Examples of PHA matrices fabricated for medical use. Items such as meshes (top, middle) and fibers for sutures (bottom, right) show promise for surgical use. Pellets (middle and bottom, left) and microspheres (top, right) have been explored for drug delivery. Films (bottom, right) have uses in post-surgery recovery, as well as potential agricultural applications.

studies, and drug release studies. The wealth of data available currently, plus innovations occurring in the field at the present time suggest a bright future for the medical PHA field.

BIOCOMPATIBILITY OF POLYHYDROXYALKANOATES

PHB has been found in a wide range of organisms, from bacteria to higher mammals. It has been shown to

take part in formation of transmembrane ion channel complexes in some organisms [8-10]. Degradation of PHB polymer results ultimately in the production of 3-hydroxybutyrate, a natural metabolite in animal hosts that is known to be associated with ketone body formation. Given these findings, it is highly understandable that PHB, as well as other PHA types, are biocompatible. In other words, there is a strong likelihood that surgical implants, sutures, etc., produced from PHA will not result in an immune response in the host organism. Furthermore, sterilization of PHA-based materials does not appear to affect the average molecular weight (Mw), tensile strength, or other properties [11]. Surface properties of PHA films have been shown to be favorable for proliferation and attachment of tissue culture cells [11-13], suggesting that PHA is suitable for scaffolding material in tissue engineering (see below). Indeed, NIH 3T3 fibroblast cells have been shown to adhere and proliferate on PHA membranes [11]. Also, mesenchymal stem cells were shown to adhere and proliferate on several PHA substrates, with a terpolymer poly(hydroxybutyrate-*co*-hydroxyvalerate-*co*-hydroxyhexanoate) (P(HB-*co*-HV-*co*-HHx)) yielding the best results [14, 15]. The P(HB-*co*-HV-*co*-HHx) polymer exhibited the greatest surface roughness, as well as the highest water contact angle, suggesting that these characteristics are important for adherence and proliferation of cells on PHA surfaces. In one study, unusual PHA polymers were examined for their ability to facilitate adherence and proliferation of mouse connective tissue fibroblasts. Two unique PHA polymers, poly(hydroxy-10-undecenoate) (denoted in the study as PHUE) and polyhydroxyoctanoate-*co*-hydroxy-10-undecenoate) (denoted in the study as PHOUE), were shown to be able to support cell attachment and proliferation. The best results were obtained in this work with PHB, the copolymer poly(hydroxybutyrate-*co*-hydroxyvalerate) (P(HB-*co*-HV)), or an ozone treated version of the PHUE polymer [16].

A survey of *in vivo* PHA biocompatibility studies is shown in Table **1**. PHA (non-PHB) scaffolds implanted in rats exhibited a mild tissue response. However, PHB implants showed more of a tissue response, potentially because PHB is so rigid that it exerts a mechanical stimulus to the tissues surrounding the implant. The bioabsorption rate of the implants decreased in correlation with the 3HB content [17]. When implanted into an animal host, fibers made from PHB or P(HB-*co*-HV) were observed to elicit a tissue response that was similar to implants made from silk or catgut, two materials currently used in surgical procedures [18]. In fact, most inflammation seen in these experiments was related to post-traumatic inflammation following surgical procedures. Following implantation, there were statistically no immunological differences between reactions to the PHA implants or the silk and catgut implants [18, 19]. In subcutaneous implantations in rabbits, tissue response was measured for polylactate (PLA), PHB and P(HB-*co*-HHx) implants. The P(HB-co-HHx) copolymer implants elicited a mild tissue response, less pronounced than even the PHB and PLA implants [20]. Furthermore, rabbit smooth muscle cells tended to proliferate better on P(HB-co-HHx) surfaces that contained larger quantities of HHx monomer [21]. Another work also examined the copolymer poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) (P(3HB-*co*-4HB)) implanted subcutaneously in rats. This study found that with higher 4HB content in the copolymer, a milder tissue response was seen [17]. In these works, while the immunological response towards the PHA substrate was minimal, prolonged dwell time in the host organism resulted in degradation of the polymer, as measured by loss of sample weight and loss of polymer Mw. This suggests that each host organism employed the means by which to biodegrade PHA, indicating that this family of polymers is an ideal material for implantable, resorbable medical devices.

Table 1. Survey of *In Vivo* Biocompatibility Studies of PHA Matrices

PHA polymer and matrix/geometry	Host organism	Reference
P(HB-*co*-HV) membranes	Dog	[38]
PHB and P(HB-*co*-HV) fibers/sutures	Rat	[19, 48, 49]
PHB and P(HB-*co*-HV) films	Human blood	[22]
PHB, P(HB-*co*-HHx), PLA, and P(HB-*co*-HHx)/PEG blend discs	Rabbit	[20]
PHB, P(HB-*co*-HHx), P(3HB-*co*-4HB) electrospun films	Rat	[17]
PHB microspheres	Rat	[50]

PHA matrices have also been tested for hemocompatibility by inspecting the response of mammalian blood when incubated with polymer films. It was shown that PHB or P(HB-*co*-HV), when in contact with blood, did not affect platelet responses, nor did the polymer activate complement system. However, more involved polymer purification procedures had to be followed to significantly reduce the amount of bacterial cell wall material associated with the purified PHA [22]. Since PHA is typically produced in large quantities by Gram-negative bacteria, removal of lipopolysaccharide and other cell wall material is necessary for medical applications, especially for contact with blood. Typically, PHA is repeatedly re-dissolved in solvent and re-precipitated to remove protein, carbohydrate, and lipid impurities that could affect tissue responses to the polymer material. This re-purification can be performed several times following initial recovery from cells, depending on the presence of contaminants [22].

POLYHYDROXYALKANOATES AS MEDICAL SCAFFOLDING MATERIAL

Given the biodegradability and biocompatibility of PHA, an obvious medical application for the polymer is for scaffolding material in tissue engineering. In earlier studies, blends of PHB and hydroxyapatite (HA) were used as scaffolds to treat bone defects [12, 23-25]. Also, a copolymer of polyglycolic acid (PGA) and PHB was used to produce pulmonary valve leaflets and pulmonary artery scaffolds in sheep [26]. This study was followed up by construction of a PHA-based heart valve scaffold, which was again surgically inserted into sheep [27]. Both of these studies illustrated that tissue engineering using biopolymer scaffolds is possible. Since then, PHB has been used successfully as a graft matrix for neuronal generation after spinal cord injury in rats [28]. PHB films were also found to provide scaffolding to patch a large bowel defect in rats and were shown to degrade more readily *in vivo* [29].

Polymer crystallinity has been shown to be a factor in the interactions of PHA with cartilage chondrocytes. Maturational differentiation of chondrocytes was shown to be affected by the amount of PHB in a PHB/P(HB-*co*-HHx) blend present on a surface [30]. Scaffolds produced from unblended P(HB-*co*-HHx) were also shown to be effective in cartilage repair [31]. Matrices fabricated from P(HB-*co*-HV) implanted into cartilage defects in rabbits exhibited better healing response than scaffolds fabricated from collagen impregnated with calcium phosphate [32]. Scaffolds produced from PHA copolymer and implanted in rats showed mild tissue response. However, PHB implants showed more of a tissue response, potentially because PHB is so rigid that it exerts a mechanical stimulus to the tissues surrounding the implant. The bioabsorption rate of the implants decreased in correlation with the 3HB content [17]. Another study has shown that PHA matrices allow proliferation of neural stem cells. P(HB-*co*-HHx) allows for the most penetration of stem cells into the polymer matrix, presumably due to the porosity of P(3HB-*co*-3HHx) [33]. Osteoblasts were also demonstrated to adhere, proliferate, and deposit calcium on PHA substrates [34]. It was further demonstrated that PHB and P(HB-*co*-HHx) polymers were the most biocompatible, with osteoblasts preferring a lower percentage of HHx monomer (12 mol%) compared to fibroblasts (20 mol%) [35]. A recent study [36] has examined geometry of P(HB-*co*-HV) platforms for use in tissue repair. Laser microperforation did not inhibit cell proliferation or migration through the micropores, suggesting that the technique could be used for production of tissue engineering scaffolds.

POLYHYDROXYALKANOATES AS SURGICAL MATERIAL

For use in sutures, a polymeric material must exhibit exceptional tensile strength in order to be effective in wound closures. PHB and P(HB-*co*-HV) sutures were shown to be able to facilitate healing of muscle-fascial wounds [19, 37]. P(HB-co-HV) films facilitated wound healing following oral surgery in dogs [38]. A common PHA type used for fabrication of surgical material is poly(4-hydroxybutyrate) (P4HB). As suture material, oriented P4HB fibers (545 MPa) are stronger than polypropylene sutures (410-460 MPa). Also, the Young's modulus of P4HB sutures is significantly lower than other monofilament sutures, produced from other substances, that are on the market [39]. Tepha, Inc. in Cambridge, MA, USA manufactures several medical devices from PHA. The most well-known product, and the first approved by the US Food and Drug Administration (FDA), is the TephaFLEX® suture fabricated from P4HB. Tepha, Inc. also produces surgical meshes and films fabricated from PHA. All products have been demonstrated to have favorable mechanical properties for use in surgical procedures (www.tepha.com).

DRUG RELEASE

Given the numerous experimental evidence that PHA is tolerated well by mammalian systems, including the human body [40, 41], various forms of PHA are

Table 2: Survey of Drug Release Studies from PHA Matrices

Polymer/composite	Drug	References
P(HB-*co*-HV)	Tetracycline	[51]
PHB	Rifampicin	[52]
P(HB-*co*-HV)	Sulbactam-cefoperazone	[53]
P(HB-*co*-HV)	Gentamicin or Sulperazone	[45]
P(HB-*co*-HV)/wollastonite composite	Gentamicin	[54]
P(HB-*co*-HV)	Gentamicin	[42]
P(HB-*co*-HHx)	Rhodamine B isothiocyanate	[47]
PHB	Rubomycin	[46]

being studied for use as drug delivery devices [41-44]. In an *in vitro* study of antibiotic release, P(HB-*co*-HV) rods impregnated with either gentamicin or Sulperazone, sustained release of drug into aqueous solution was seen over the course of 2 weeks. Furthermore, using a higher HV content copolymer (20% HV, compared to 7% or 14% HV), sustained release of Sulperazone was seen for over 60 days. Kinetics of release could be altered by changing the amount of drug loading. Higher levels of cumulative release could be seen using a copolymer with higher HV content [45]. In a study of antibiotic delivery, gentamicin was incorporated into P(HB-*co*-HV) discs, and release of the drug was measured over time. Polymer containing higher HV content released more antibiotic into solution (12% 3HV v. 8% HV). These P(HB-*co*-HV) discs containing gentamicin incubated in normal human blood samples were shown not to cause proliferation of white blood cells, red blood cells or platelets, indicating no adverse affects of the polymer/antibiotic combination [42]. We can further this research by examination of different drug/PHA combinations, including use of different copolymers. Given the increase in drug delivery to the surrounding solution by P(HB-*co*-12 mol% HV) compared to P(HB-*co*-8 mol% HV) [42], we can increase the HV content of the copolymer and examine if drug delivery increases. Also, P(HB-*co*-HHx) copolymers can be studied, as well as terpolymers and 4-component polymers. Earlier studies have suggested release of antibiotics, such as rifampicin and tetracycline, from PHB microspheres results in too rapid a release of these drugs, and that lower crystallinity PHA is required for better timed release of drug into the surrounding tissue [41]. For example, the drug tamulosin was mixed with poly(hydroxyhexanoate-*co*-hydroxyoctanoate (P(HHx-*co*-HO)) polymer and shown to facilitate permeation of the drug through the polymer into skin. PHB, on the other hand, was less suited to this task both due to its higher crystallinity and its inability to adhere to skin in the system tested [44]. P(3HB-*co*-4HB) polymer has also been shown to be effective at releasing drug in solution [43].

A study using PHB microspheres demonstrated that release of the anti-tumor drug rubomycin inhibited proliferative activity of Ehrlich's carcinoma in mice [46]. Pseudo-PHA granules (i.e. nanoparticles), fabricated *in vitro,* have also been shown to be effective drug delivery devices. In a recent study, rhodamine B isothiocyanate (RBITC) was targeted to cancer cells or macrophages by incorporating with P(HB-*co*-HHx) and associating with a recombinant PhaP phasin protein. These recombinant phasins were protein *N*-terminal fusions consisting of human epidermal growth factor (hEGF) or human α1 acid glycoprotein (hAGP) for targeting cancer cells or macrophages, respectively. Proper targeting of PHA/RBITC nanoparticles to each cell line was demonstrated by fluorescence microscopy [47]. For commercialization of PHA drug delivery devices, Tepha, Inc. is currently developing TephELAST® and TephaFLEX® materials into drug delivery systems (http://www.tepha.com/pipeline-drug-delivery.htm). A survey of PHA-based drug release studies is shown in Table **2**.

MEDICAL POLYHYDROXYALKANOATES – OUTLOOK

Matrixes produced from PHA or PHA blends are biocompatible have the physical properties to be fabricated into medical devices and consumables. As fabrication procedures become more refined, several novel applications for PHA in medicine will emerge. With the continuing interest in tissue engineering, PHA applications as scaffolding material will continue to grow. Bioactive compound delivery by PHA matrixes

will continue to generate interest, as PHA drug delivery systems offer unique methods by which to control release (e.g. type of monomers present in copolymer or terpolymer and their relative percentages). One can envision systems for delivery of growth factors or immunomodulators using PHA systems. Processes for fabrication of such devices could involve surface modification of the PHA matrix and directed attachment of bioactive molecules. For large scale production of PHA for medical use, purification challenges will have to be addressed, as polymer with close to 100% purity is needed. Continuous dissolution and re-precipitation of polymer is acceptable at laboratory scale, but industrial processes must be formulated that are cost-effective and environmentally friendly. Regardless, given the versatility of PHA polymers, the applications in the medical field are numerous, and demand for PHA devices will continue to grow, especially given the continued research breakthroughs in the field.

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