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REVIEW ARTICLE

# Biomedical Applications of Polyhydroxyalkanoates

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**Abstract** Polyhydroxyalkanoates (PHA) are produced by a large number of microbes under stress conditions such as high carbon (C) availability and limitations of nutrients such as nitrogen, potassium, phosphorus, magnesium, and oxygen. Here, microbes store C as granules of PHAs—energy reservoir. PHAs have properties, which are quite similar to those of synthetic plastics. The unique properties, which make them desirable materials for biomedical applications is their biodegradability, biocompatibility, and non-toxicity. PHAs have been found suitable for various medical applications: biocontrol agents, drug carriers, biodegradable implants, tissue engineering, memory enhancers, and anticancer agents.

**Keywords** Antibacterials · Biocontrol agents · Biodegradable implants · Drug carriers · Memory enhancer · Tissue engineering

**Abbreviations**

| | |
|---|---|
| PHA | Polyhydroxyalkanoate |
| PHB | Polyhydroxybutyrate |
| 3HB | 3-Hydroxybutyric acid |
| 3HV | 3-Hydroxyvaleric acid |
| 3HO | 3-Hydroxyoctanoate |
| 3HD | 3-Hydroxydecanoic acid |
| 4HB | 4-Hydroxybutyric acid |
| P(3HB-3HV) | Poly-3hydroxybutyrate-co-3hydroxyvalerate |
| P(3HB-4HB-3HV) | Poly-3hydroxybutyrate-co-4hydroxybutyrate-co-3hydroxyvalerate |
| P(3HB-3HV-3HHx) | Poly-3hydroxybutyrate-co-3hydroxyvalerate-co-3hydroxyhexanoate |
| P(3HB-3HO) | Poly-3hydroxybutyrate-co-3hydroxyoctanoate |
| P(3HB-3HV-DHB) | Poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-2,3-dihydroxybutyrate) |
| 3HA | Hydroxyalkanoic acid |
| OA | Octanoic acid |
| UA | Undecanoic acid |

## Introduction

Plastics are synthetic polymers which find wide usage in our daily lives. The major limitation associated with plastics is their non-biodegradable nature. The production of plastics in large quantities makes their disposal a major issue to worry about among Environmentalists and Health Departments. The most well envisaged alternative is to produce biodegradable plastics. In Nature, microbes have been bestowed with ability to withstand environmental stress. Under stress caused by high carbon: nitrogen (or potassium, oxygen, magnesium, phosphorus) ratio, microbes manipulate their metabolic activities in such a manner that rather than following the tricarboxylic acid cycle, the acetyl-CoA is diverted towards the synthesis of

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polyhydroxyalkanoates (PHAs), which can be categorized as completely degradable natural bioplastics [1–14].

The use of PHAs as biodegradable polymers has gained attention because of their biological (microbial) origin and non-toxic nature compared to synthetic plastics, which may be toxic [15]. Biocompatibility of PHA polymers—PHB and PHBV is an issue which has been the focus for their use in medical applications. In fact these polymers have caused prolonged and acute inflammatory responses. It was realized that we need PHAs which will be appropriate for engineering biological tissues and various applications in medical field [16, 17].

## Antibacterials

R-3HAs are chiral compounds, which have potential for being used as building blocks for compounds, for use in pharmaceutical industry [18]. 3HA can be transformed into different hydroxycarboxylic acids such as 2-alkylated 3HB and β-lactones. These can be employed as oral drugs [19]. R-3HAs can be formed by the degradation of PHAs [18]. The most important compounds are carbapenem or macrolide antibiotics [20, 21]. Depolymerase enzyme of *Pseudomonas fluorescens* GK13 encoded by gene *phaZGK13* can depolymerise PHAs to monomers [22, 23]. These monomeric units can reduce bacterial infection such as those by *Staphylococcus aureus* [24] and those conjugated with D-peptide prove anticancerous [25]. P3HB/P4HB helps in enhancing angiogenic properties of skin and wound healing (Table 1) [26, 27].

## Biocontrol Agents

Antibiotics are commonly used as feed supplement for animals. At low concentration, these antibiotics have been reported to influence the growth of the animals—livestock and aquaculture [28]. As the incorporation of antibiotic in a consistent manner can be a risky affair and the gastrointestinal microflora is likely to develop resistance [29, 30]. One has to ensure that there is complete elimination of antibiotic from the digestive system of the animal. It has been observed that Short-chain fatty acids (SCFAs) are effective in controlling agents against pathogens [31]. As PHAs are biopolymers of β-hydroxy SCFAs, these can be metabolized in the intestinal tract. The metabolites can be exploited as biocontrol agents for giant tiger prawn *Penaeus monodon* [32, 33].

## Drug Carriers

In order to improve the efficacy of the drugs, it has been envisaged that they should be delivered in a controlled and targeted manner. PHAs have been recognized as biomaterials which have desirable physical properties with high biocompatibility. Hence, their usage as raw material for producing tablets, nano-particles and as scaffolds for eluting drugs can be effective [34, 35].

3HB monomers have proved helpful in the synthesis of novel polymers such as Dendrimers. These polymers are biodegradable, have monodispersity, and surface-functional moieties. These features make them as potent drug carriers [19, 20]. Monomers—3HB and 4HB have been exploited for preparing novel β- and γ-peptides, which are resistant to the action of peptidases. This enables them to stay longer in mammalian serum i.e., improving its suitability for cargo-drug delivery. 3HB can inhibit glycolysis during haemorrhagic shock. These monomers are helpful in the synthesis of sex hormones and fragrance (S-citronellol). A few other properties of these monomers include antibacterial, anti-proliferative and haemolytic activities [36].

PHB microspheres carrying rifampicin were used as hemoembolizing agent. The microspheres proved effective in releasing drug. Implantable rods prepared with PHB, PHBV and their copolymers such as P(3HB-4HB) were used for delivery of antibiotics [37]. mcl-PHAs are more effective candidate for drug delivery as they have lower crystalinity and melting point. mcl-PHA have been employed for transdermal drug delivery. PHA copolymers containing 3HO and 3HHx shows adhesion to the python reticulatus skin and was used for dispersing three respective drugs such as tamsulosin, ketoprofen and clonidine. This drug shows good permeability through PHA matrix [38].

*P. fluoroscenes* produces mcl-PHAs, which helps in drug delivery, protein purification and immobilizing agent in clinical diagnostics (Table 1) [39]. These PHAs are bioengineered as biologically active beads for various medical applications. PHA beads are used in clinical diagnostics such as in recombinant protein production, vaccine delivery, bio-imaging, endotoxin removal, etc. (Table 1) [40–50]. PHA beads are also used as tuberculin skin test reagent for diagnosis of bovine tuberoculosis (Table 1) [51, 52].

**Table 1** Potential applications of polyhydroxyalkanoates and their derivatives in medical industries

| Bioproducts | Source | Applications | References |
|---|---|---|---|
| Polyhydroxybutyrate | Not known | Stomach wall patch | [77] |
| | *Staphylococcus aureus* | Peripheral nerve guide | [54] |
| Polyhydroxyalkanoate (mcl) | *P. fluorescens* | Drug delivery, protein microarray, protein purification, antibody immobilization in clinical diagnostics | [39] |
| Polyhydroxyalkanoate beads (antigen displaying polyester beads) | *Escherichia coli* | Tuberculin skin test reagent for diagnosis of bovine tuberculosis (TB) | [51, 52] |
| Polyhydroxyalkanoate beads | *E. coli* | Recombinant protein production | [47] |
| | *E. coli* | Protein purification | [49] |
| | *E. coli* | Vaccine delivery agent | [43] |
| | *Lactococcus lactis* | Hepatitis C vaccine delivery agent | [44] |
| | *Alcaligenes latus* | Microbeads | [40] |
| | *E. coli* | Nano/microdevices for bioimaging in medical approaches | [42] |
| | *E. coli* | Fused to specific antigen and applied as beads in Fluoroscence activated cell sorting based diagostics | [41] |
| | *E. coli* | Displays foreign antigens are immunogenic and presents a delivery system for vaccination against Hepatitis C virus | [50] |
| | *Ralstonia eutropha* | Endotoxin removal and protein purification | [48] |
| Polyhydroxybutyrate-valerate | *Ralstonia eutropha* B5785 | Suture | [79] |
| | Sigma-Aldrich Company | Myocardial patch | [78] |
| | Not known | Bone regeneration | [57] |
| Poly-3-hydroxybutyrate, Poly-4-hydroxybutyrate | *Cupriavidus eutrophus* B-10646 | Elastic nonwoven membranes-helps in reducing inflammation, enhancing angiogenic properties of skin, facilitate wound healing capacity | [74] |
| Polyhydroxybutyrate-hydroxyhexanoate | Shantou Lianyi Biotech Company | Osteoblast attachment, proliferation and differentiation | [55, 56] |
| | Not known | Bone regeneration | [55] |
| | *Aeromonas hydrophila* 4AK4 | Vessel stent, hemocompatibility and cytocompatibility | [58, 59] |
| | *A. hydrophila* 4AK4 (Recombinant) (20%) | Smooth muscle cells related graft scaffolds for tissue engineering | |
| | Not known | Cartilage tissue engineering | [56] |
| | – | Nerve conduit | [60] |
| | Not known | Scaffold for cartilage tissue engineering | [61] |
| | Not known | Nanoparticles | [34] |
| Polyhydroxybutyrate | *Biomer and Roth* | Scaffolds for tissue engineering | [67] |
| Polyhydroxyvalerate | *Goodfellow* | | |
| Poly-3-hydroxyoctanoate-co-3-hydroxyundecanoate | *Procter and Gamble* | | |
| | *EMPA* | | |
| Poly-3-hydroxy-acetyllthioalkanoate-co-3-hydroxyalkanoate | *P. putida* KT2442 | Antibacterial activity against methicillin resistant *S. aureus* | [24, 26] |
| | *P. putida* KT2442 fadB; *P. fluorescens* GK13 | | |
| 3Hydroxybutyrate | *E. coli* | Immobilized cell factories for biocatalysis and bio-transformation, Chaperone protein levels | [46] |
| 3Hydroxyalkanoic acid | *Pseudomonas* sp. | Helps in establishing PHA producers in soil and rhizosphere, and improves metabolism | [23] |
| 3-hydroxydecanoic acids conjugated to D-peptide | *P. putida* CA-3 | Anti-cancer activity | [25] |
| Polyhydroxybutyrate-valerate microspheres | Sigma-Aldrich Company | Neural tissue engineering | [66] |
| PHB-Hydroxyapatite composite | *R. eutropha* B5786 | Bone regeneration | [74] |
| | Not known | Bone bonding between implants and biological tissue | [73] |

**Table 1** continued

| Bioproducts | Source | Applications | References |
|---|---|---|---|
| | Not known | Bone tissue engineering | [75] |
| Fibronectin and alginate coated PHB-fiber | Astra Tech Sweden | Spinal cord injury | [94] |
| PHBV-PLGA | Aldrich, UK | Nerve tissue engineering | [76] |
| Unsaturated m- and lcl-copolyesters: PHO-Sy series | *P. oleovorans* octanoic acid (OA) and soya oily acids (Sy) | Subcutaneous patches in rats | [92] |
| Gold embedded poly (3-OH octanoate-co-3-OH-10-undecenoate) | *P. oleovorans* (OA and 10-undecanoic acid, UA) | Subcutaneous implantation in rats | [93] |
| Polyhydroxyoctanoate +PGA conduit | PHO 3836; TEPHA Inc., Cambridge, MA | Vascular tissue engineered structures | [72] |
| | PHO 3836; TEPHA Inc., Cambridge, MA | | |
| | PHO 3836; TEPHA Inc., Cambridge, MA | | |
| | PHO 3836; TEPHA Inc., Cambridge, MA | | |
| | PHO3836 TEPHA Inc, Cambridge, MA | | |

## Tissue Engineering

PHAs available in general were not targeted for use as medical implants and were thus lacking the quality which can get approval of the Drug Administrators. The need is to produce PHAs of high purity, check their biodegradation in vivo, fabrication of scaffolds, modify their surface [53]. PHAs with necessary modifications hold great potential to contribute to tissue engineering, developing tissue products for medical and therapeutic applications: (1) vascular grafts, (2) heart valves, (3) nerve tissue engineering, etc. (Table 1) [54–65].

PHAs can be used to produce scaffolds, which have higher mechanical strength. These scaffolds promote growth of the cells by supplying nutrition (Table 1) [66]. These products are available as screws, pins, sutures, films, etc. (Table 1) [67]. P(3HB-4HB-3HV) has been exploited for fiber meshes by providing support to stem cell growth for proliferation and cell adhesion [68]. P(3HB-3HV-3HHx) can be employed as scaffolds for engineering liver tissue [69]. On the other hand, 3-D scaffolds had been developed by using PHA nanofibers [70]. P(3HB-3HO) have found usage for scaffold formation for cartilage repair [71]. The new P(3-HB-3HV-2,3-diHB) produced by recombinant *Ralstonia eutropha* have also been exploited [53].

Further to enhance mechanical strength and flexibility of PHAs, inorganic bioceramics have been combined with PHAs, which produce novel composites for using them for engineering tissues. Composites of PHA and ceramic are employed to form different blends. Hydroxyapatite and PHA are also used in tissue engineering (Table 1) [72–76].

## Medical Devices

PHA have been envisaged to prove useful in making medical devices, as they are biocompatible, biodegradable and have strong mechanical characteristics. Some of the most potential devices are: adhesions barriers, articular cartilage repair, cardiovascular patch grafting, meniscus repair device, orthopedic pins, repair patch, rivets and tacks, staples and screws, stents, surgical mesh, sutured fastener, etc. (Table 1) [77–80]. Vessel stent produced from PHA copolymer (PHB-HHx) from *Aeromonas hydrophila* 4AK4 has high hemo- and bio-compatibility (Table 1) [58, 59]. The preparation of cardiovascular patches should have very high quality features, primary being resistance to infection and degradation. In addition, these bioproducts should be durable, lack immunogenicity and should not be toxic.

## Biodegradable Implants

Implants are used on a large scale in a very skilful manner. However, invariably the issue of their getting infected with pathogens comes as a major hurdle. Now-a-days biomaterials are also used as implants. Biomaterial associated infection is a serious health issue. In order to meet the functional demand, materials with desirable properties must be selected [81]. For example, their physical, chemical, biological, biochemical properties. Use of biopolymers as biodegradable implants has greatly influenced the modern medicine [82].

The use of PHA degradation product HAs for preparing biodegradable implants, and to fabricate systems to deliver antibiotics like Duocid and Sulperazone, in chronic osteomyelitis therapy have been gathering importance [19]. Rod of PHA biopolymer having antibiotics, such as combinations of sulbactam-ampicillin/cefoperazone, gentamicin, were prepared with the help of copolymer—P(3HB-3HV) as matrix [83–86]. Mixtures P3HB and P3HO were employed as a matrix system for coronary stents needed for eluting drugs. This can prove effective in reducing the reoccurrence of the blockages in the arteries [87].

To avoid biofilm formation and bacterial adhesion, there is a need to improve implant surfaces [88]. PHB and its copolymer P(3HB-3HHx) sheets loaded with lysozyme are being used for biofilm inhibition and in fabrication of wound dressing [89]. Implants coated with PHB copolymer fastens the degradation process for a stable drug release within a given time period as compared to its co-polymer [90, 91].

Gold-catalyzed oxidation of bacterial polyester produced by *Pseudomonas oleovorans* supplemented with octanoic acid and 10-undecanoic acid was employed for biodegradable subcutaneous implantation in rats (Table 1) [92, 93]. PHB fiber covered with fibronectin and alginate was used as implant in spinal cord injury (Table 1) [94].

## Anti-osteoporosis Effect

Ketoacidosis is induced in human beings by the accumulation of high concentration of 3HB [95]. Oligomers of 3HB have properties to act as good energy substrate for patients, where these compounds undergo rapid diffusion within peripheral tissues. It also prevents brain damage as it can enhance cardiac efficiency by regenerating mitochondrial energy. 3HB can potentially cure Parkinson's and Alzheimer's diseases, where they act by reducing the death rate of neuronal cells [96]. 3HB enhances osteoblasts growth and anti-osteoporosis activity, by rapidly depositing calcium and its strong serum alkaline phosphatase activity. It helps in prevention lowering of bone mineral density and reducing the level of serum osteocalcin [97, 98].

## Memory Enhancer

Memory loss and related abilities are serious enough to disturb our daily routine. Among the different forms of dementia, Alzheimer's disease is the most common. As a consequence of memory loss, there are problems of thinking and behaviour. Derivatives of 3HB such as 3-hydroxybutyrate methyl ester (HBME) have the potential to act as drug against Alzheimer's disease. HBME acts by protecting mitochondrial damages [99]. During ketogenesis, D-β-HB prevents neuronal death, which is induced by glucose deprivation [100]. HA monomers derived from PHAs can stimulate the $Ca^{2+}$ channels, which can help in enhancing memory [98, 101–104].

## Challenges for Industrial Scale Production of PHAs

PHAs have unique characteristics, which make them suitable for commercial production. However, there are still a few challenges, which limit their upscaling [3]. The physical and chemical properties which demand attention include: lowering of melting point and glass transition temperature, elastic modulus, tensile strength and elongation. These characteristics depend up on the monomeric composition and the molecular weight of the polymer. Copolymers of PHAs with high molecular weights have the potential to overcome these limitations [3]. In addition, strategies to manipulate feed composition [14], culture conditions such as independence from nutritional imbalance [8, 107], increased microbial biomass, high expression of polymerase genes and genetic modifications to synchronize termination of PHA biosynthesis with cell lysis [1] will certainly help in economic production of PHAs on an industrial scale.

## Future Prospects

PHAs have been finding their applications in diverse fields [105–107]. The economic feasibility of using PHAs and their derivatives for medical purposes stands at the highest level. The usage of PHAs can be extended to other health related issues such as cancer therapy, fighting malnutrition, neurodegenerative and metabolic disorders, anti-diabetics agent, monitor environmental health, etc.

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