Polyhydroxyalkanoates – what are the uses? Current challenges and perspectives

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Abstract

Over the past few decades, a considerable attention has been focused on the microbial polyhydroxyalkanoates (PHAs) owing to its multifaceted properties, i.e. biodegradability, biocompatibility, non-toxicity and thermo-plasticity. This article presents a critical review of the foregoing research, current trends and future perspectives on the value added applications of PHAs in the biomedical, environmental and industrial domains of life.

Keywords

Biocompatible, biodegradable, biofuel, biomedical implant, green composite, polyhydroxyalkanoates, therapeutic carrier, tissue engineering

Introduction

The prokaryotic energy storage compound polyhydroxyalkanoates (PHAs) are the polyesters of hydroxyalkanoic acids (HA). Beijerinck in 1888 first observed the PHA as granules in the bacterial cells (Chowdhury, 1963). Later on, Lemoigne (Lemoigne, 1926) described it as a homopolyester containing 3-hydroxybutyric acids (3-HB). Approximately 300 different microorganisms are reported to accumulate the PHA under the nutrient stress conditions (deficiency of nitrogen, phosphate, sulphur, potassium, tin, iron, magnesium or dissolved oxygen and surplus supply of carbon) using renewable resources (Akaraonye et al., 2010; Jendrossek, 2009; Madison & Huisman, 1999; Ojumu et al., 2004; Polyhydroxyalkanoate (PHA), 2013; Reddy et al., 2003). PHAs are biodegradable, biocompatible, non-toxic, non-linear, optically active, hydrophobic, piezoelectric, thermo-plastics and are demonstrating the high degree of polymerization (Jendrossek, 2009; Madison & Huisman, 1999; Ojumu et al., 2004; Reddy et al., 2003). The details on the types, properties, PHA producers, biosynthetic pathways and roles of PHA in microorganisms are given in Supplementary material SII.

Areas of PHAs implications

It is recently reported that the market consumption for PHA-based products will grow from an estimated 10,000 MT in 2013 to 34,000 MT by 2018 (Polyhydroxyalkanoate (PHA), 2013). The general overview of PHA applications in various sectors of life is shown in Figure 1. Henceforth, we discussed the applications of PHAs in detail.

Biomedical applications

PHA as implant material

The nature, hydrophilicity/hydrophobicity, biocompatibility, biodegradability, non-toxicity, molecular weight, surface properties or energy, interface adherence, porosity and degradation rates are essential pre-requisites for the selection of polymer as a biomedical implant material (Chen, 2009; Chen & Wu, 2005; Dhandayuthapani et al., 2011). Peng et al. stated that members of the PHA family such as; PHB, PHBV, P3HB4HB, PHBHHx and PHBVHHx are good biomaterials and stimulated the cell proliferation or tissue regeneration without tumor induction (Peng et al., 2011). Both scl and mcl-PHAs have shown good biocompatibility with epithelial cells (Shishatskaya & Volova, 2004), adrenocortical cells (Wu et al., 2004), L929 mouse fibroblasts (Liu & Chen, 2008), rabbit aorta smooth muscle cells (RASMCs), smooth muscle cells (SMC) (Qu et al., 2005, 2006), chondrocytes (Sun et al., 2005), bone marrow cells (Yang et al., 2004), osteoblasts (Wang et al., 2004), rabbit articular cartilage-derived chondrocytes (Wang et al., 2008),...
MC3T3-E1 murine osteoblasts, immortalized human keratinocyte (HaCat) cells (Liu & Chen, 2008) and human adipose-derived stem cells (hASCs) (Ye et al., 2009).

The PHAs-based implant material does not cause an immune response in the host organisms and are considered biologically safe (Chen & Wu, 2005). The first report about the use of PHA as an implant material came with the exploitation of poly-3-hydroxyoctanoate-co-3-hydroxyhexanoate (PHOHx) based microspheres, tubes and pellets in mice as a model animal and the polyester proved to be completely non-immunogenic (Williams et al., 1999). Similarly, the PHB-based patches implanted in the gastrointestinal system of dog did not produce an inflammatory response (Lobler et al., 2003). In addition, PHA-based implants are biodegradable and are able to support the adhesion and proliferation of cells and/or tissues. (Xiao et al., 2007) reported that 3-HB being the degradation product of PHB has a stimulatory effect on the signal transduction pathway by increasing cytosolic Ca^{2+} concentration which ultimately increased the polymer surface energy and decreased hemolytic activity and less platelet adherence as blood-contact biomaterials (Qu et al., 2006). The bone and cartilage tissue engineering are examples of soft tissue engineering. While, in hard tissue engineering, a number of biodegradable polymers and bioactive ceramics demonstrating a desirable combination of biological, mechanical and surface properties are used for the fabrication of scaffolds (Knowles, 2003; Yu & Fan, 2008). The bone and cartilage tissue engineering are included in hard tissue engineering.

In the biomedical field, PHAs are pragmatic for designing of composites in combination with inorganic phases i.e., bioactive glass, glass-ceramic fillers or coatings and hydroxyapatite (HA) for tissue engineering applications for achieving the superior mechanical properties of scaffolds with excellent cells and/or tissues interaction (Chen, 2009; Chen & Wu, 2005; Misra et al., 2006; Reddy et al., 2003). Moreover, the PHA-based scaffolds are able to support the growth of cells by maintaining the proper supply of nutrients and degrade within a short period of time after inducing the tissue formation. The review of various types of PHAs used in the field of tissue engineering is given in Supplementary Table SIII 2.1.

Vascular grafting. The vascular grafting is done to repair or restore the malfunctioning arties and/or veins in the cardiovascular pathologies. The currently used methods for the treatment of last-stage vascular heart diseases are the surgical substitution of cells and/or tissues either by autografts (same individual), xenografts (species to species), homografts (same species) and/or artificial prostheses (devices made up of natural & synthetic materials used for the treatment of damaged blood vessels). However, the poor stability, immune responses, prosthetic valve endocarditis, risk of infections, thromboembolism and need for replacement are the serious shortcomings of these techniques. The PHA composites are considered valuable substitute for implications as cardiovascular patches, stents, pericardial patches, atrial septal defect repair devices and vein valves (Chen & Wu, 2005; Chen et al., 2001; Dai et al., 2009; Wang et al., 2008).

Qu et al., modified the surface of copolymer PHBBHx via plasma treatment and/or fibronectin (cell adhesion protein) coating, respectively and observed better proliferation of HUVECs and SMCs on ammonia plasma-treated PHBBHx coated with fibronectin (PFn-PHBBHx) in comparison to the fibronectin-coated (Fn-PHBBHx) or uncoated PHBBHx (Qu et al., 2005). This was due to the impairment of co-localization of fibronectin on the surface of PFn-PHBBHx which ultimately increased the polymer surface energy and

**PHAs and regenerative medicine – an open avenue**

Tissue engineering is defined as an interdisciplinary field that aims to regenerate the damaged or diseased tissues and/or promote new tissue growth using a combination of cells, signaling molecules and biomaterials (Bruder & Fox, 1999; Ma, 2004). There are two main types of tissue engineering i.e., soft and hard tissue engineering. In soft tissue engineering, scaffold made of highly porous engineered biodegradable materials is seeded with the appropriate cell type followed by *ex vivo* tissue growth. In the second step, the scaffold is implanted as a bioactive support to carry out the growth of new tissue (Du et al., 2009; Karageorgiou & Kaplan, 2005). The vascular grafting, heart valve, skin, liver and nerve tissue engineering are examples of soft tissue engineering. While, in hard tissue engineering, a number of biodegradable polymers and bioactive ceramics demonstrating a desirable combination of biological, mechanical and surface properties are used for the fabrication of scaffolds (Knowles, 2003; Yu & Fan, 2008). The bone and cartilage tissue engineering are included in hard tissue engineering.

**Figure 1. The applications of PHAs in the various sectors of life.**

**PHAs as valuable industrial raw material**
- Packaging application
- Biofuel

**Applications of PHAs**
- PHAs as biomedical implants
- PHAs and regenerative medicine
- PHAs as therapeutic carrier

**Biomedical applications**
- PHAs as biomedical implants
- PHAs and regenerative medicine
- PHAs as therapeutic carrier
hydrophilicity by generating new nitrogen- and oxygen-containing groups on the PHBHHx surface. The rapid proliferation of HUVECs and formation of a confluent monolayer on PHBHHx films also indicated their potential for endothelialization. The Pf/PNm-PHBHHx is demonstrating the suitability of mcl-PHA copolymer alone or their blends for vascular tissue grafting. In another study, Zhang et al., developed blends comprising of PHBHHx and poly-(propylene carbonate) (PPC) in different amounts and found that blends have similar material properties (elongation at break, elastic modulus and wettability) as required for normal blood vessel in comparison to pure PHBHHx (Zhang et al., 2007). Furthermore, the PHBHHx/PPC blend was suitable for fibronectin adsorption, adhesion and proliferation of R-SMCs. Similarly, according to Wu et al., PHBHHx was suitable for endothelialization of lumen for cardiovascular tissue engineering (Wu et al., 2008). Gaudio et al., made a comparative study using 3D tubular scaffolds of poly-caprolactone (PCL), PHBV and PCL/PHBV blend as a future biomaterial for vascular tissue grafting (Gaudio et al., 2012). The rapid adhesion and proliferation of rat cerebral endothelial cells (RCECs) were found on PCL and PCL/PHBV-based scaffold in comparison to pure PHBV scaffolds. Moreover, there was a formation of 3-D network similar to the architecture of normal vascular endothelial cells due to the high expression of adhesion proteins, actin and vinculin on PCL and PCL/PHBV scaffolds.

Heart valves tissue engineering. The scl and mcl-PHAs-based scaffold were found successful for heart valve tissue engineering in the past (Sodian et al., 2000a,b; Stamm et al., 2004). Later on, Fu et al., fabricated a composite scaffold comprising of polyglycolic acid (PGA) and poly-4-hydroxybutyrate (P4HB) and observed the rapid proliferation of human pediatric aortic cells and collagen deposition in the presence of basic fibroblast growth factor (bFGF) and ascorbic acid on this scaffold (Fu et al., 2004). In another study, Wu et al., used P4HBHHx as a coating material for decellularized porcine aortic valves and implanted this hybrid valve in pulmonary position in sheep for 16 weeks (Wu et al., 2007). Compared with the uncoated control the hybrid valve demonstrated less calcification, better proliferation of recipient endothelial cells and myofibroblasts after histological examination. Adamus et al., developed a diblock copolymer made up of atactic poly[(R,S)-3- hydroxybutyrate]-[a-PHB)] and natural PHO for coating the vascular prosthesis. The PHO/[a-PHB] blend was well penetrated in the outer surface of the prosthesis and made it impermeable and elastic in comparison to control and suggesting the suitability of this composite material for future cardiovascular tissue engineering (Adamus et al., 2012).

Liver tissue engineering. Still, the potential of PHA for liver tissue engineering is not explored in a detail. Zhu et al., fabricated the PHBV microspheres as a scaffold to guide the liver cell growth (Zhu et al., 2007b). Human hepatoma HepG2 and Hep3B cell lines were cultured on the microspheres and polymer films. In vitro cytotoxicity results demonstrated the higher cell proliferation and albumin secretion on the microspheres in comparison to films due to the improvement of hepatic function by aggregation of hepatic cells on microspheres. Further in 2007b they also studied the synergistic effect of protein complex on the proliferation and activity of Hep3B cells (Zhu et al., 2007a). For this purpose, the ECM proteins i.e., collagen (type I), laminin, and fibronectin were conjugated via covalent bonding with PHBV microsphere using cross linkers. The rapid increase in the adhesion and proliferation of Hep3B cells was found on ECM proteins-modified PHBV microsphere complex. There was also increase in albumin secretion and P-450 activity of Hep3B cells. Later on, the microsphere systems comprising of pure PHBV, PLGA and PHBV/PLGA blend were evaluated and compared for the release of BSA as a model for hepatocyte growth factor (HGF) which showed sustained release till 40 days in comparison to pure PHBV and PLGA microspheres (Zhu et al., 2009).

Skin tissue engineering. Skin is the largest organ in the human body. Skin regeneration is reported to occur partially after a substantial laceration, diabetic injuries or burn. To address this problem, the significant research has been done with the applications of PHA-based scaffolds for skin tissue regeneration (Tang et al., 2008; Volova et al., 2003). Ji et al., stated the terpolyester poly-3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyhexanoate (PHBVHHx) was excellent support to the growth of HaCaT cell line due to its better thermo- and mechanical properties (Ji et al., 2008). Kuppan et al., evaluated and compared the adhesion, proliferation and gene expression of human skin fibroblasts on PHBV fibers, 2-D PHBV films and tissue culture polystyrene (TCPS, control) (Kuppan et al., 2011). The comparable number of cell adhesion and proliferation with higher expression of collagen I and elastin gene was found on both PHBV fibers and 2-D PHBV films in comparison to TCPS. However, the expression of collagen III gene was down regulated on PHBV fibers and 2-D PHBV films. Moreover, the wound healing capacity of PHBV fibers was evaluated in the presence and absence of an angiogenesis factor (R-Spondin 1) using the rat as a model animal. It was found that the presence of R-Spondin 1 stimulated the wound healing process. In another study, Veleirinho et al., fabricated the hybrid nanofibrous mats comprising of PHBV/Chitosan for skin tissue engineering (Veleirinho et al., 2012). The good adhesion, proliferation of L929 cell lines and remarkable wound healing process was found in rat using PHBV/Chitosan nanofiber mats. Sankar et al., developed a 3D macroporous chitin/PHBV hydrogel blend for fabrication of scaffold for skin tissue engineering (Sankar et al., 2012). The cytocompatibility studies were done using human dermal fibroblast cells (HDF). The hydrogel blend appeared to be biocompatible in nature due to twofold increase in adhesion and proliferation of HDF cell.

Nerve tissue engineering. Nerve tissue engineering is mainly used for the treatment of peripheral nervous system (PNS) injuries and neurodegenerative diseases. A nerve autografting technique used for the treatment of PNS injuries has suboptimal functional revived. The ideal nerve conduit should be biodegradable and porous for nutrient exchange. Therefore, nerve conduits comprising of biodegradable natural polyester are a good choice for the regeneration of peripheral nerves. The scl-PHAs are used for nerve tissue...
regeneration in many studies (Mohanna et al., 2003; Young et al., 2002). Other studies provided the evidences that nerve conduits made up of PHBHHx are also ideal for Schwann cell regeneration in addition to PHB (Armstrong et al., 2007; Bian et al., 2009). Yet in another study, the potential of PHBHHx for fabrication of nerve conduits was increased by it fabricating its blend with poly(ε-lactide) (PDLLA) (Gao et al., 2006). The crystallinity of PHBHHx/PDLLA composite was reduced in comparison to PHBHHx alone. Furthermore, the adsorption of fibronectin was higher on PHBHHx/PDLLA composite than on neat PDLLA mat. The creation of highly aligned micro-architecture capable of mimicking native nerve tissues is a pre-requisite for the efficient regeneration of injuries across the long nerve gap. For this purpose, Yucel et al., fabricated a nerve conduit using porous micropatterned film comprising of tubular PHBV, poly(L-lactide-co-D,L-lactide) P(L-D,L) LA and PLGA as exterior component which were wrapped around the aligned electrospun mat of PHBV–PLGA for healing of injured nerve tissues (Yucel et al., 2010). The tensile strength of this nerve conduit was 3.13 MPa and Young’s Modulus was 0.08 MPa. The nerve conduit was excellent at stimulating the neurons and supporting cell alignment. The configuration of nerve conduit provided the maximum advantage to utilize the topographic cues from both the electrospun fibers and micropatterned films for the relocation, arrangement of nerve cells and stimulated the endogenous nerve healing process. Last year, Chen and Tong reported the differentiation of neural progenitor cells (NPCs) into neurons with the better axon–dendrite segregation using PHBV microspheres (Chen & Tong, 2012). The collagen is the most abundant ECM protein in nerve and exhibited nanofibrous architecture. For this purpose, Prabhakaran et al., fabricated random, aligned PHBV and composite PHBV/collagen (PHBV/Coll) nanofibers to evaluate their potential for nerve tissue regeneration using PC12 nerve cells (Prabhakaran et al., 2013). Comparatively the higher nerve cell proliferation was found on composite PHBV/Coll50:50 nanofibers than aligned PHBV or PHBV/Coll75:25 nanofibers. They stated that aligned nanofibers of PHBV/Coll provide orientation as well as bipolar neurite extensions for nerve tissue regeneration. While, the cell showed a multipolar phenotype on random PHBV/Coll nanofibers. Masaeli et al., designed electrospun scaffolds by blending PHB and PHBV nanofibers for myelinic membrane regeneration (Masaeli et al., 2013). The increase in PHBV content in blend resulted in a decrease in the melting and glass temperatures as well as the crystallization degree of the blends. Moreover, random and aligned PHB/PHBV nanofibrous scaffold was designed with or without type 1 collagen. The better adhesion and differentiation of Schwann cells were found in aligning PHB/PHBV/collagen fibers due to the high expression of GDNF and NGF neurotrophic factors.

Bone tissue engineering. Tissue engineering is a superb approach for the treatment of damaged or lost bone and cartilages. For this purpose the scaffold material must have strong mechanical properties and ability to regulate the development, proliferation, differentiation of osteoblasts/ chondrocytes and bone ECM. In the human body, the bones are mainly composed of 40% (w) type I collagen (Col 1) and 60% (w) HA. Initially, the stiff and brittle scl-PHAs are considered more suitable for use in hard tissue engineering. However, in the last few decades the significant research has also been on mcl-PHA due to their elastomeric nature and improved mechanical properties. Wang et al., evaluated and compared the adhesion and proliferation of rabbit bone marrow cells on 3D scaffolds of PLA, PHB and PHBHHx (Wang et al., 2004). The excellent biocompatibility of the PHBHHx-based scaffold was observed for adhesion, proliferation of osteoblasts with typical morphology, higher Ca2+ deposition and collagen synthesis in comparison to PLA and PHB scaffolds. Similar results were also reported by Li et al., (Li et al., 2005). The PHBV containing nano-sized HA (n-HA) promoted the higher levels of mineralization as well as low inflammatory responses in comparison to the other biomaterials (Cool et al., 2007). Francis et al., developed a novel multi-functional PHB microsphere/45S5 bioglass-based composite scaffold containing HA particles and used this scaffold for gentamycin delivery during bone tissue engineering (Francis et al., 2011). Hayati et al., developed PHB/nHA composite scaffolds having porosity and mechanical properties equivalent to the native spongy bones (Hayati et al., 2012). Baek et al., immobilized Coll I on the PHBV/HA scaffold and found the better adhesion, proliferation and differentiation of osteoblast cells (MC3T3-E1) on PHBV/HA/ Col I scaffold than on the PHBV/HA based scaffolds (Baek et al., 2012). Sultan reported the design of the scaffold material as well as the addition of high content of HA into PHBV scaffold produced the highly porous, irregular and isotropic composite matrix which could provide a more suitable environment for the osteoblast adhesion and proliferation (Sultan, 2012).

In another study by Misra et al., PHB foams having 85% porosity was prepared and bioactive glass (BG) particles of 45S5 Bioglass® grade were introduced in these scaffold microstructures (Misra et al., 2010). The PHB/BG composite foams were found suitable for attachment and proliferation of MG-63 osteoblast cells and showed no immune response in rats after one week of implantation. Moreover, the multifunctional PHB scaffolds containing BG, Vitamin E and carbon nanotubes having bactericidal, bioactive, electrically conductive, anti-oxidative behavior were also developed. Wang et al., developed a 3-D PHBV/β-Ca3SiO4 composite scaffold to imitate the internal environment of natural ECM for better adhesion, proliferation and differentiation of human osteoblast-like MG-63 (Wang et al., 2013). The addition of β-Ca3SiO4 nanoparticle promoted the transcription of transforming growth factor-β1 (TGF-β1) and bone morphogenetic protein-7 (BMP-7) genes which ultimately led to the early differentiation of human osteoblasts during the bone tissue engineering.

Cartilage tissue engineering. Cartilage being avascular tissues is hard to regenerate. The substantial research has been carried on PHA based scaffold for cartilage tissue engineering in the past decades. PHB, PHBV, PHBHHx and PHBHHx/PHB blends are considered suitable candidates for cartilage tissue engineering due to good adhesion, enhanced proliferation and better differentiation of chondrocytes on them.
and cartilage formation in nude mice was found during in vitro proliferation of chondrocyte on PLCL/PHBV microsphere were increased. Moreover, the good adhesion, fold to enhance the mechanical properties of PHBV microsphere were increased after 24 weeks of implantation without remnants of PHB/PHBHHx degradation products. Similarly, Liu et al., investigated the proliferation potential of in vitro chondrogenic differentiated hASCs on PHBV scaffold for the formation of neocartilage in a heterotopic animal model (Li et al., 2010). No cartilage formation was observed using non-differentiated cell/PHBV scaffold. While, the chondrogenic pre-differentiated hASCs produced the neocartilage having a good mechanical strength in nude mice after 16 weeks of implantation. Li et al., incorporated poly (L-lactide-co-ε-caprolactone) (PLCL) scaffold to enhance the mechanical properties of PHBV microspheres for cartilage tissue engineering (Li et al., 2013). Both the compressive and young moduli of resulting PLCL/PHBV microsphere were increased. Moreover, the good adhesion, proliferation of chondrocyte on PLCL/PHBV microspheres with an increase in GAG, type II collagen contents and cartilage formation in nude mice was found during in vitro and in vivo chondrogenesis.

Periodontal tissue engineering. Wang et al., fabricated a blend comprising of flexible and hydrophilic Ecoflex with PHBV for periodontal tissue engineering and observed the faster adhesion and proliferation of periodontal ligament stem cells (PDLSCs) and periosteum-derived stem cells (PC) on a PHBV/Ecoflex mat in comparison to pure PHBV mat (Wang et al., 2012). Still, the limited research work is done on the use of PHAs for periodontal tissue engineering and need to be explored further.

Therapeutic carrier

The poor oral bioavailability, toxicity, inconsistency in circulation, inadequate tissue distribution and non-specific delivery of therapeutics to the both targeted and non-targeted tissues are the main disadvantages of the conventional therapeutic system (Gabriel, 2007; Llombart et al., 2006). The drug delivery systems comprising of polymeric matrix are used to deliver the pharmacologically active substance, i.e. antibiotics, contraceptives, immunogens, hormones, anti-inflammatory drugs, gene therapy agent, anti-apoptotic agent, antioxidants, neurotrophic factors or other substance to site of action by increasing their bioavailability to prevent infections, apoptosis and necrosis of tissue while evading therapeutic toxicity in the normal cells.

The PHA-based microspheres, microparticles and nanoparticles are considered to be ideal for use in drug delivery applications due to their biocompatibility, non-toxicity and biodegradability (Francis et al., 2011; Lu et al., 2010; Mendes et al., 2012). The survey of PHA applications as a drug carrier is given in Supplementary Table SIII 2.2. The non-specific lipases and esterases are involved in the degradation of PHA in nature. PHAs are also hydrolytically degradable biomaterials (Akaraonye et al., 2010; Mukai et al., 1993). Initially the scl-PHAs are used as a drug carrier. But, the rapid release of drug from scl-PHA matrix due to their crystalline nature made them an inappropriate choice for controlled release studies (Gursel et al., 2002; Li & Chang, 2005). The mcl-PHAs are considered more suitable for controlling release studies due to their low crystallinity. Moreover, recently the use of PHA-based nanoparticle formulations grows significantly to increase the bioavailability of hydrophobic therapeutics as well as for sustained drug release studies (Lu et al., 2010, 2011; Pignatello et al., 2009; Yao et al., 2008). According to ISO 10993, PHB-based nanoparticles are safe to be used in animals due to its biocompatibility (Pötter & Steinbüchel, 2005). The PHB and PHBHHx nanoparticles are effectively used for intracellular drug release (Xiong et al., 2010). The release of drug kinetics from PHA based matrix can be easily controlled by tailoring their surface properties, type of monomeric contents and molecular weight. In our study, poly-(3-hydroxybutyrate-co-5 mol% 3-hydroxyvalerate) (PHBV-S), poly-(3-hydroxybutyrate-co-11 mol% 3-hydroxyvalerate) (PHBV-11) and poly-(3-hydroxybutyrate-co-15 mol% 3-hydroxyvalerate) (PHBV-15) nanoparticles were used to increase the bioavailability of anticancer drug ellipticine (EPT) (Masood et al., 2013a). The EPT loaded PHBV-15 nanoparticles with higher valerate content and smaller molecular weight showed a higher degradation rate in comparison to EPT-loaded PHBV-S and PHBV-11 nanoparticles (Masood et al., 2013a). The degradation rate of PHA copolymer was dependent on the length of its side-chain, i.e. PHB > PHBV > PHBHHx (Li et al., 2007; Numata et al., 2007). There was also another report that PHBV samples containing 8 mol% and 12 mol% of HV monomeric subunits were degraded at a faster rate in comparison to pure PHB (Phithakrotchanakoon et al., 2009). The hydrophobicity is another critical factor which needs to be addressed before the wide-spread use of PHA based nanoparticles in biomedical field as a drug carrier. Therefore, Shah et al., modified the hydrophobic PHA via non-toxic, blood compatible and hydrophilic monomethoxy poly(ethylene glycol) (mPEG) to develop the amphiphilic drug delivery system (Shah et al., 2010). The sustained release of thymoquinone from the amphiphilic PHA-mPEG nanoparticles was checked using prenatal rat neuronal hippocampal cells and the amphiphilic nanoparticles showed good biocompatibility independent of the presence of co-monomeric units in the PHA (Shah et al., 2010). Later on Shah et al., encapsulated cisplatin (a chemotherapeutic agent) within amphiphilic block copolymer poly-3-hydroxybutyrate-co-4-hydroxybutyrate monomethoxy poly(ethylene glycol) (P3HV4HB-b-mPEG) nanoparticles to reduce the toxicity associated with the multiple drug dosing (Shah et al., 2012). The significant suppression effect on the tumor cell growth and augmentation of apoptotic process was found for drug loaded nanoparticles in comparison to the free drug treated cells. The surface modification of nanoparticles can also be done with the other...
hydrophilic polymers such as poly(vinyl alcohol) (PVA), PLA and PLGA to prevent the process of opsonization (Masood et al., 2013b; Reis et al., 2006; Xiong et al., 2010).

The drug partition coefficient in interior hydrophobic and exterior aqueous phase has controlled drug encapsulation efficiency (Mittal et al., 2007). Moreover, the drug encapsulation efficiency is also dependent on both the molecular weight as well as the type of monomeric content of polymer. “In our study, the loading efficiency of EPT in different formulations of PHBV nanoparticles with variable molecular weights did not show a regular pattern. The encapsulation efficiency of EPT for PHBV-S, PHBV-11 and PHBV-15 nanoparticles was 45.55, 39.32, and 45.65% respectively. This was due to the fact that the molecular weight and percentage of valerate content in PHBV nanoparticles control the encapsulation efficiency of the EPT. The high encapsulation efficiency of EPT in PHBV-S nanoparticles was due to its molecular weight which could entrap more EPT within its structure. But, in case of PHBV-15 nanoparticles the lower molecular weight and high valerate content as compared to PHBV-S nanoparticles developed a strong hydrophobic interaction between drug and polymeric molecular chain which resulted into higher drug encapsulation efficiency. Whereas, in case of PHBV-11 nanoparticles, the lower molecular weight in comparison to PHBV-S nanoparticles and lower valerate content in comparison to PHBV-15 nanoparticles was responsible for its low encapsulation efficiency (Masood et al., 2013a)”. According to Reis et al., “the polymeric composition (hydrophobicity, surface charge, and biodegradation profile) of the nanoparticles, any adjuvant substances, and the associated drug (molecular weight, charge, localization in the nanospheres by adsorption or incorporation) have a great influence on the drug absorption, biodistribution pattern, and elimination” (Reis et al., 2006).

The fabrication of targeted drug delivery systems comprising of targeting ligand, carrier and model drug has gained a significant attention in the recent past especially for the cancer therapy. Yao et al., reported the receptor-mediated targeted drug delivery system based on PHA nanoparticles containing Rhodamine B isothiocyanate (RBITC) as a model anticancer drug, PHA granule binding protein PhaP and polypeptide or protein ligands fused to PhaP (Yao et al., 2008). The resulting RBITC-loaded PHBHHx nanoparticle were effectively endocytosed by tumor cells during in vitro and in vivo trials using mice as a model animal and showed significant anticancer activity on transplantable murine hepatoma22 (H22) model cells. Zhang et al. developed a targeted drug delivery system based on poly-3-hydroxybutyrate-co-poly-3-hydroxyoctanoate (PHBHO) as a carrier of model anticancer drug doxorubicin (DOX) using folic acid (FA) a targeting ligand. It was observed that DOX/FA–PEG–PHBHO nanoparticles were taken up by HeLa cells more efficiently and showed good anticancer activity in comparison to non-folate-mediated PHBHO nanoparticles during in vivo anti-tumor activity trials (Zhang et al., 2010). Similar results were also obtained by using PHBHHX nanoparticles as a carrier of anti-neoplastic drug etoposide (Kiliçay et al., 2011).

Hence, the current section concludes with the anticipation that the future in vitro, in vivo and computational modeling based approaches can offer new insights for the better understanding of the complex interplay of cellular, biochemical and biomechanical processes while using the PHA-based biomaterials as an implant, tissue engineering scaffold and drug carrier. Furthermore, chemical modification of PHA in order to make them amphiphilic and antimicrobial can also be a good approach to expand its applications in the biomedical area.

The details on applications of PHAs in industrial and environmental fields are given in Supplementary material SIV.

Future prospective

There is still an open avenue for the isolation of new bacterial strains capable of giving novel monomeric compositions of PHA homopolymers, copolymers, block copolymers either directly or through metabolic engineering and need to be fully characterized them which will broaden their applications in the near future. Moreover, metabolic engineering approaches including manipulation of the host cell genome and/or external substrates, addition of inhibitors, recombinant gene expression and protein engineering of PHA biosynthetic enzymes can be applied either directly or in combination to fabricate the robust microbial plastic factories. Mathematical modeling strategies can also be used for the intelligent target prediction as well as to explicate these metabolically engineered systems. There is a need of active collaborative research among people of diverse expertise to get the immense benefits of human welfare from PHAs.

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Declaration of interest

The authors report no declarations of interest.

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