Applied Microbiology and Biotechnology (2019) 103:2007–2032
https://doi.org/10.1007/s00253-018-09604-y

MINI-REVIEW

Biomedical applications of microbially engineered polyhydroxyalkanoates: an insight into recent advances, bottlenecks, and solutions

Akhilesh Kumar Singh[1] · Janmejai Kumar Srivastava[1] · Anuj Kumar Chandel[2] · Laxuman Sharma[3] · Nirupama Mallick[4] · Satarudra Prakash Singh[1]

Received: 28 September 2018 / Revised: 28 December 2018 / Accepted: 28 December 2018 / Published online: 15 January 2019

Abstract

Biopolymeric polyhydroxyalkanoates (PHAs) are fabricated and accumulated by microbes under unbalanced growth conditions, primarily by diverse genera of bacteria. Over the last two decades, microbially engineered PHAs gained substantial interest worldwide owing to their promising wide-range uses in biomedical field as biopolymeric biomaterials. Because of non-hazardous disintegration products, preferred surface alterations, inherent biocompatibility, modifiable mechanical properties, cultivation support for cells, adhesion devoid of carcinogenic impacts, and controllable biodegradability, the PHAs like poly-3-hydroxybutyrate, 3-hydroxybutyrate and 3-hydroxyvalerate co-polymers, 3-hydroxybutyrate and 4-hydroxybutyrate co-polymers, etc., are available for various medical applications. These PHAs have been exploited to design in vivo implants like sutures as well as valves for direct tissue repairing as well as in regeneration devices like bone graft substitutes, nerve guides as well as cardiovascular patches, etc. Furthermore, they are also emerged as attractive candidates for developing effective/novel drug delivery systems because of their biocompatibility and biodegradability with the ability to deliver and release the drugs at a specific site in a controllable manner and, therefore widen the therapeutic window with reduced side effects. However, there still remain some bottlenecks related to PHA purity, mechanical properties, biodegradability, etc., that are need to be addressed so as to make PHAs a realistic biomaterial. In addition, innovative approaches like PHAs co-production with other value-added products, etc., must be developed currently for economical PHA production. This review provides an insight toward the recent advances, bottlenecks, and potential solutions for prospective biomedical applications of PHAs with conclusion that relatively little research/study has been performed presently toward the viability of PHAs as realistic biopolymeric biomaterials.

Keywords Biopolyesters · PHAs · Polyhydroxyalkanoates · Biodegradability · Biocompatibility · Cytotoxicity · Biomaterial · Crystallinity · Drug delivery systems · Biomedical applications

Introduction

Homo- or heteropolyesters of PHAs are a type of biopolymeric substances, which are fabricated as well as assembled within the cytoplasm of different kinds of microorganisms (Kumar et al. 2015; Sharma et al. 2016; Singh and Mallick 2016, 2017; Singh et al. 2017, 2018). The PHAs accumulation in heterotrophic bacteria like *Cupriavidus necator*, etc., usually occurs under nutrient limitation, viz. ammonium, phosphate, etc. However, bacteria such as *Alcaligenes latus*, etc., can accumulate PHA polymers in active cell growth but lacking any nutrient restriction. The occurrences of biopolymeric PHAs in photoautotrophic Gram-

✉ Akhilesh Kumar Singh
aksingh2@lko.amity.edu; akhiliit@gmail.com

[1] Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus, Lucknow, Uttar Pradesh 226028, India

[2] Department of Biotechnology, Engineering School of Lorena (EEL), University of São Paulo, Lorena 12.602.810, Brazil

[3] Department of Horticulture, Sikkim University, Gangtok 737102, India

[4] Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur, West Bengal 721302, India

negative cyanobacteria with capability to carry out oxygenic photosynthesis were reported initially in 1966 in *Chlorogloea fritschii* under acetate supplemented condition (Carr 1966). The cyanobacterial PHA biofabrication route does not differ significantly from bacteria, i.e., depicts near resemblance with the bacterial PHA biosynthetic pathway (Wang et al. 2013a). Various cyanobacterial species accumulate biopolymeric PHAs photoautotrophically, whereas others need supplementation of carbon substrates (Sharma et al. 2006, 2007, 2016; Mallick et al. 2007; Samantaray and Mallick 2014, 2015; Singh et al. 2017, 2018). In cyanobacteria, limitation or deficiency of nutrient (mainly deficiency or restriction of nitrogen and phosphorus) provoke the bio-fabrication of biopolymeric PHAs (Singh et al. 2017; Singh and Mallick 2017).

The composition of biopolymeric PHAs can be altered at the monomeric level by exploiting diverse organic carbon substrates, culture conditions as well as nature/type of bacterial and cyanobacterial species (Chuah et al. 2013; Kumar et al. 2015; Singh et al. 2018). Figure 1 depicts the common structural formula of biopolymeric PHAs in which each PHA monomer holds a side-chain R group (alkyl side chain) with the ability to vary from methyl (C_1) to tridecyl (C_{13}). This R group usually represents a saturated alkyl group. It may also contain the unusual chemical structure like aromatic, substituted alkyl groups, etc., as detailed in Singh et al. (2017). In addition, the fatty acids having hydroxy group at location 4, 5, or 6 are also reported. The substituents in the R groups of PHAs could be transformed on a chemical basis through cross-linking of unsaturated linkages. The variations in the R groups as well as the competency to change their substituents are liable toward the modification of diverse biopolymeric PHAs with a range of prospective uses (Singh et al. 2017). For instance, when

$n = 1$	R = hydrogen	Poly(3-hydroxypropionate)
$n = 2$	R = hydrogen	Poly(4-hydroxybutyrate)
$n = 3$	R = hydrogen	Poly(5-hydroxyvalerate)

Biopolymeric PHAs mainly divided into three classes depending upon the size of repeating hydroxyacid (HA) monomeric units integrated to the biopolymeric chain. The first group is composed of short-chain-length PHAs (SCL-PHAs) that have HA monomeric units of three to five carbons. Poly-3-hydroxybutyrate (PHB), poly-3-hydroxyvalerate (PHV), etc., are good examples of SCL-PHAs. The second group is made up of medium-chain-length PHAs (MCL-PHAs), where HA monomers incorporate 6–14 carbons such as poly-3-hydroxyoctanoate [P(3HO)], poly-3-hydroxynonanoate [P(3HN)], 3-hydroxyhexanoate (3HHx), 3-hydroxyheptanoate (3HH), 3-hydroxydecanoate (3HD), etc. The third group comprised long-chain-length PHAs (LCL-PHAs) made up of HA monomers of 15 or more carbons. The 3-hydroxyhexadecanoic acid (3HHD) as well as 3-hydroxyoctadecanoic acid (3HOD) monomers are good examples of LCL-PHAs. These 3HHD and 3HOD monomer units are found to biosynthesize along with 3-hydroxybutyric acid (3HB) as well as 3-hydroxyvaleric acid (3HV) as constituents of SCL-LCL-PHA co-polymer (Singh and Mallick 2008, 2009a, b; Sankhla et al. 2010; Singh et al. 2013a, b, 2015, 2017, 2018). Presently, these biopolymeric PHAs gained considerable commercial interest including biomedical field globally because of their non-toxic nature, antioxidant properties, biodegradability, biocompatibility, optical activity, piezoelectric property, impermeability to gas, good ultraviolet resistance, resistance to hydrolytic degradation, and stereospecificity with unique material properties that have a promising range of features over other biopolymeric substances (Sodian et al. 2000; Singh et al. 2017, 2018).

A vast number of review articles are accessible on PHAs that furnish a comprehensive account on their common characteristics/features (Byrom 1987; Brandl et al. 1990; Steinbüchel 1991; Urtuvia et al. 2014; Kumar et al. 2015; Anjum et al. 2016; Singh et al. 2018; Obruca et al. 2018), characterization (Bonthrone et al. 1992; Brandl et al. 1990; Jendrossek and Pfeiffer 2014), biodegradation (Steinbüchel 1991; Mergaert et al. 1992; Tokiwa and Calabia 2004; Ren et al. 2010; Shah et al. 2014; Anjum et al. 2016), and potential applications including biomedical applications (Wu et al. 2009; Hazer and Steinbüchel 2007; Ren et al. 2010; Hazer et al. 2012; Kai and Loh 2014; Kumar et al. 2015; Masood et al. 2015; Obruca et al. 2018; Zhang et al. 2018). Nevertheless, very little description is available on the technical bottlenecks with various promising solutions for PHAs biomedical applications. The present article is an attempt to summarize the utmost desirable properties of microbially originated PHAs together with recent innovative research for promising biomedical applications.

Important properties of PHAs as biomaterials

A biomaterial involves any substance, which has been engineered to produce a structure/form that as such or as portion of a complex system is employed to direct through the regulation of contacts with constituents of biological systems during the course of any diagnostic ortherapeutic technique. In other words, biomaterials are synthetic or natural materials, which are exploited in medical devices or in interaction with the living systems for the purposes such as helping in healing, repairing/correcting deformities as well as re-establishing lost function. Considering this, there is growing concern with regard to PHA polyesters as potential polymeric biomaterials. There are

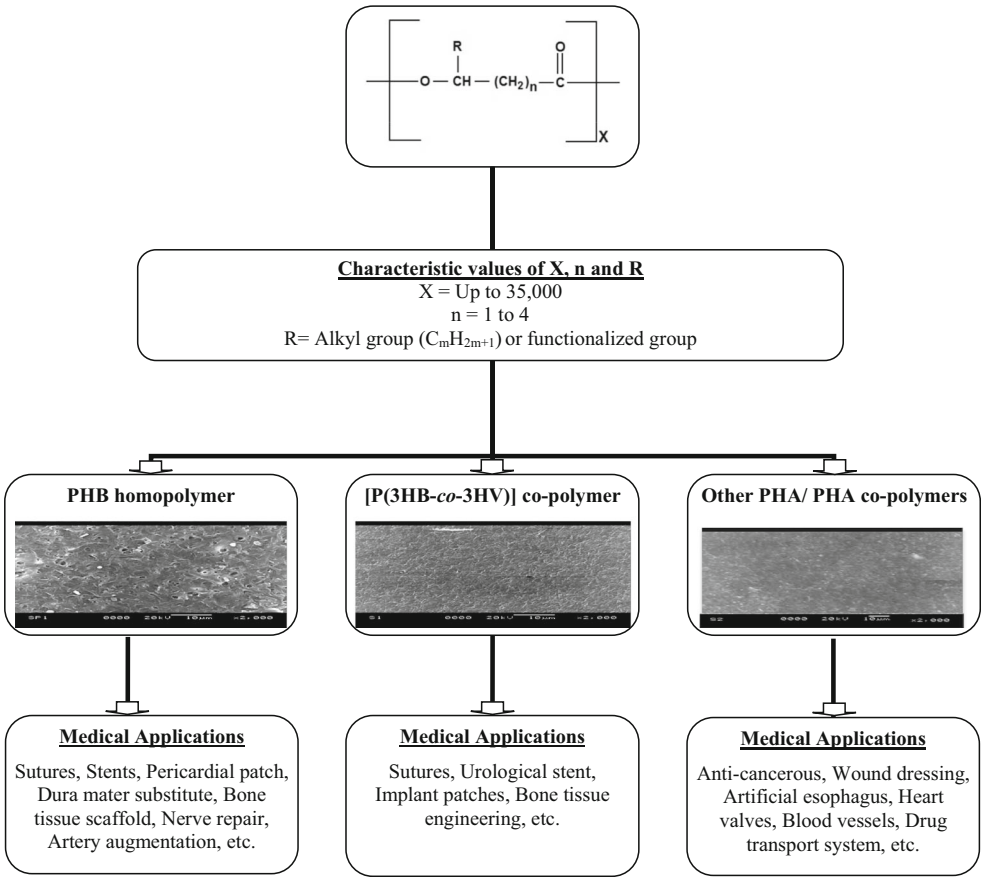

Fig. 1 General overview on structure and medical applications of PHAs (modified from Singh and Mallick 2017)

various important parameters viz. material properties, biodegradability, biocompatibility, cytotoxicity, non-teratogenicity, and non-carcinogenicity, which are responsible for determining the suitability of PHAs for use in biomedical field or as biomaterial (Ali and Jamil 2016).

Material properties of PHAs

Among all the polymeric materials, conventional plastics, viz., polypropylene (PP), low-density polyethylene (LDPE), etc., have been distinctively recognized as astonishing polymeric substances in contemporary material technology on account of their exceptional material characteristics. The material characteristics of macromolecules are broadly classified into physical as well as mechanical characteristics (Kumar et al. 2015). The glass transition temperature (T_g) as well as melting temperature (T_m) are recognized as two noteworthy thermal characteristics (physical features), which are typically investigated for PHA polymeric material to assess the temperature environments so that polymeric material can be processed as well as utilized. Differential scanning calorimetry (DSC) as well as differential thermal analysis (DTA) is exploited toward the study of T_m along with T_g of the polymers. Nonetheless, Young's modulus and tensile strength including elongation-to-break are the key mechanical characteristics assessments toward the polymeric materials (Sankhla et al. 2010; Kumar et al. 2015; Singh et al. 2017). The physical as well as mechanical characteristics of PP and LDPE are depicted in Table 1. PP showed T_m, T_g, tensile strength, Young's modulus as well as elongation to break (%) value of 176 °C, − 10 °C, 38 MPa, 1.7 GPa as well as 400%, correspondingly. However, LDPE showed values of 130 °C, − 36 °C, 10 MPa, 0.2 GPa as well as 600% in the above order. These unique properties enable traditional plastics for widespread uses in different sectors like medical, packaging materials, automobiles, construction, printers, etc. The stability, prolonged durability and resistant to degradation are found to be important properties associated with conventional plastics during their use. These properties become harmful to environment when plastic materials are out of use as they persist for many years in the form of garbage owing to mainly xenobiotic nature. Consequently, their severe environment unfriendly concerns lead to development of eco-friendly polymeric materials like biopolymeric PHA thermoplastics (Kumar et al. 2015; Sharma et al. 2016; Singh et al. 2017, 2018).

Interestingly, biopolymeric PHA thermoplastics not only depict the material characteristics akin to traditional plastics but also received considerable interest toward research and commercial ventures because of their unique properties over other

Table 1 Comparative overview of the material properties of PHAs with common plastics (Valappil et al. 2006; Kumar et al. 2015; Singh and Mallick 2016; Sharma et al. 2016; Singh and Mallick 2017; Singh et al. 2017)

Property	PHB	[P(3HB-*co*-3HV)] co-polymer (mol fraction 80:20)	[P(3HB-*co*-3HA)] co-polymer (mol fraction 94:6)	P(3HB-*co*-3HV-*co*-3HHD-*co*-3HOD)	P(4HB)	[P(3HB-*co*-4HB)] co-polymer (mol fraction 84:16)	[P(3HO-*co*-3HHx)] co-polymer (mol fraction 88:12)	PP	**LDPE**
				co-polymer (mol fraction 84.8:7.2:3.1:4.9–95.7:1.0:1.8:1.5)					
T_m (°C)	180	145	133	115 to131	53	152	61	176	130
T_g (°C)	4	− 1	− 8	− 8 to − 14	− 48	− 8	− 35	− 10	− 36
Young's modulus (GPa)	3.5	0.8	0.2	0.2–0.3	0.149	Not reported	0.008	1.7	0.2
Tensile strength (MPa)	40	20	17	17–19	104	26	9	38	10
Elongation to break (%)	5	50	680	682–723	1000	444	380	400	620

T_m, melting temperature; T_g, glass-transition temperature; 3HA, [3-hydroxydecanoate (3 mol%), 3-hydroxydodecanoate (3 mol%), 3-hydroxyoctanoate (< 1 mol%) and 3-hydroxy-*cis*-dodecenoate (< 1 mol%)]; PP, polypropylene; LDPE, low-density polyethylene

biopolyesters. The material characteristics associated with PHAs are affected by many parameters viz. their chemical structure and type of monomer units with molecular mass. Molecular mass that generally affects the physical properties of all the polymeric materials including PHA biopolymers is a very important parameter so as to assess the quality of the polymer. A perusal of the literature revealed that mechanical properties of PHAs such as co-polymeric [P(3HB-*co*-3HV)] commercialized in trademark of BIOPOL® turn out to be unacceptable when the average molecular mass $< 4 \times 10^6$ Da (Kumar et al. 2015). Among the PHA family, SCL-PHA, particularly PHB, which is the most common and well characterized, is non-flexible and fragile owing to its higher level of crystallinity. Apart from this, PHB polymers are not suitable for diverse range of commercial uses like packaging as well as biomedical applications due to their poor mechanical properties. Nevertheless, MCL-PHAs reveal higher elongation to break values and behave as semi-crystalline elastomeric materials or amorphous liquids because of their low T_g (Rai et al. 2011). MCL-PHAs act as elastomeric polymeric materials at definite side chain lengths and become more sticky or viscous polymers with further growth of the side chain length (Hazer and Steinbüchel 2007). Similarly, LCL-PHAs also behave as elastomeric materials or amorphous liquids having very low T_g and more viscosity or tacky compared to MCL-PHAs. Therefore, MCL- and LCL-PHAs as such are unsuitable for exploitation as flexible polymeric biomaterials analogous to that of SCL-PHAs, i.e., PHB polymers. These drawbacks can be overcome by the novel PHA biosynthetic pathways with the incorporation of SCL or MCL or LCL monomers to SCL-PHA (PHB) backbones, which give rise to SCL-SCL-PHA, i.e., [P(3HB-*co*-3HV)] as well as [P(3HB-*co*-4HB)] or SCL-MCL-PHA like [P(3HO-*co*-3HHx)], etc., or SCL-LCL-PHA like [P(3HB-*co*-3HV-*co*-3HHD-*co*-3HOD)] co-polymers with significantly improved material properties including ductility and processability, suitable for different commercial/biomedical applications (Kumar et al. 2015; Singh et al. 2017). Table 1 summarizes the comparative account on thermal and mechanical characteristics of biopolymeric PHAs with traditional plastic materials. Apart from these, scaffolds comprised nano-bioglass covered with polymeric PHB found to depict higher mechanical features as well as bioactivity over those made up of nano-bioglass alone (Montazeri et al. 2015). Overall, it is merely owing to their varied physical, mechanical as well as chemical characteristics, which enable PHAs to be naturally recyclable as well as biocompatible with wide-range applicability in the biomedical field as biopolymeric biomaterials (Wu et al. 2009; Ali and Jamil 2016; Singh et al. 2017).

Biodegradability and biocompatibility of PHAs

Biodegradability involves the capacity of a substance to undergo hydrolysis, predominantly to non-hazardous products through living microbes. In this context, bacterial and fungal species are

the main agents responsible for natural decomposition process. The degradation of substances supplies microbes with precursors toward cellular constituents as well as energy for energy-demanding processes (Sharma et al. 2016). Biodegradation is reliant on various parameters, viz., activity of microbes, pH, temperature, humidity, the exposed surface area, molecular mass of the polymer, etc. Furthermore, nature of the monomers, crystallinity, and polymer composition also exhibit a significant effect on the degradation process. One of the key reasons for the continued research on PHA polymers is their biodegradability under different environments. PHA polymers are degraded when exposed to marine sediment, soil, or compost. A vast number of microorganisms secrete extracellular PHA-hydrolyzing enzymes (PHA depolymerases) to degrade PHA polymers into oligomers and monomers, which subsequently act as nutrients inside the cells (Singh and Mallick 2016). Wu et al. (2009) reported that [P(3HB-*co*-4HB)] co-polymers hydrolyze to oligo hydroxy acids by the action of both lipases and PHA depolymerases, where 4HB monomers enable them specifically more susceptible to in vivo enzymatic degradation. Moreover, co-polymers with 4HB monomers degraded more quickly compared to PHB or [P(3HB-*co*-3HV)] co-polymer.

Information regarding the degradation rate of a polymeric material (apart from biocompatibility) such as PHA polymer is important for its exploitation as biomaterial. PHA polymers could be exploited as absorbable sutures, surgical pins and staples, retarded drug release, drug carrier, etc., on account of their biodegradable nature as well as disintegration by surface erosion. Interestingly, PHB polymer depicted comparatively more in vitro disintegration and bio-disintegration in living mammalian cells than the other polymeric materials like polylactic acid (PLA) as well as poly(DL-lactide-*co*-glycolide) (PLGA) (Ali and Jamil 2016). Disintegration of PHA biopolymers within the cells of host organism provides the opportunity of combining this process with liberation of bio-active substances, for instance antibiotic and/ or antitumor drug. When a PHA polymer is incorporated with a substance, the disintegration eventually will liberate the substance, behaving as a mechanized dosing representative. The kinetics of a substance dosing from a PHA matrix could be adjusted through changing the characteristics of polymer in conjunction with the exploitation of various kinds of PHAs having varied monomeric side chains (Fig. 1). The degradation of PHA matrix within human tissue differs from cell to cell and also reliant on the approach employed for making its diverse profiles like scaffolds, PHA nanofibers, etc. (Brigham and Sinskey 2012). One most recent study reported functionalization of co-polymeric [P(3HB-*co*-3HV)] with ascorbic acid (AC) in the presence of enzyme lipase (Bhatia et al. 2019). The resulted functionalized [P(3HB-*co*-3HV)]-AC acts as an antioxidant active biomaterial having lesser extent of crystallinity and high thermal decomposition temperature together with hydrophilicity over [P(3HB-*co*-3HV)] co-polymer alone. This novel biomaterial exhibited 14% scavenging impact on 1,1-diphenyl-2-picryl-hydrazyl with 1.6-fold enhancement toward biodegradability over [P(3HB-*co*-3HV)] co-polymer alone. Therefore, such enhancement in PHAs polymeric features through addition of functional groups might be an excellent strategy so as to enhance their biodegradable temperament and cost-effectiveness including potential uses in the medical field.

Biocompatibility involves the capability of a substance to carry out with a suitable host response in a particular use. An extremely improved biocompatibility is usually needed before the introduction of foreign substances into the soft tissues/blood of a host organism. Surface porosity, shape, and the tissue environment including chemical build-up of the materials play a significant function in biocompatibility (Shishatskaya et al. 2004; Williams et al. 1999). Biocompatibility that can be evaluated in vivo and in vitro assays involves wound healing, the foreign body response, inflammation, in vivo evaluation of tissue compatibility, in vitro evaluation of tissue compatibility, and regulatory issues accompanied with medical device development. Furthermore, no substance/material can be "biocompatible" if it releases cytotoxic substances (excluding possibly a drug delivery system designed to carry cytotoxic substances specifically to cancerous cells). Thus, the appropriateness of PHA polymer for involvement in drug carrier together with other biomedical uses will reliant on their biocompatibility in addition to biodegradability. A review of literature revealed that investigators have devised technologies for PHA production, recovery, and purification to develop high-purity polymers (Peptu and Kowalczuk 2018). Specimens of these biopolymeric PHA materials were evaluated on physiological, toxicological, medical, and histological parameters of the in vitro cell cultures and laboratory animals, where PHAs were found to depict high biocompatibility at the levels of cells, tissues, and organism (Shishatskaya et al. 2004, 2005). He et al. (2014) carried out investigation on the [P(3HB-*co*-3HHx)] and [P(3HB-*co*-3HV)]/PLA fibers for assessment of their mechanical characteristics, degradability as well as biocompatibility for their promising use as medical sutures. The [P(3HB-*co*-3HHx)] fiber depicted significant biocompatibility through H&E staining with negligible effect on the neighboring cells. The other [P(3HB-*co*-3HV)]/PLA fibers also revealed high biocompatibility as well as comparatively high degradability. Pramanik et al. (2019) conducted investigation concerning functionalization of [P(3HB-*co*-3HV)] co-polymer with Fe_3O_4/graphite oxide (GO) that resulted in the formation of Fe_3O_4/GO-*g*-[P(3HB-*co*-3HV)] composite. This composite depicted more bactericidal properties toward Gram-negative bacterial strains/species over Gram-positive strains/species. This composite surface was also found to support fibroblast cell (NIH 3T3) substantially with respect to adhesion as well as growth up to 85% and, thus indicated toward its improved biocompatibility with enhanced cell physiology of NIH 3T3.

Broadly, the biocompatibility of PHA materials can be differentiated into two categories, viz., immunocompatibility and non-allergic response. The materials essentially are immunocompatible for use in medical applications, i.e., these should not elicit harsh immune responses upon introduction into soft tissues or blood of a host organism (Shrivastav et al. 2013). Immunocompatibility involves the extent of antigenic resemblance between the tissues of various individuals that determines the acceptance or rejection of allografts. The monomeric constituent of PHB and R-3-hydroxybutanoate is a result of cellular metabolism, which formed owing to oxidation of fatty acid within the liver cells. These are ketone bodies, which act as source of fuel for the brain. Interestingly, 3HB is a usual component of blood of human being with magnitude ranging from 0.3 to 1.3 mM (Zinn et al. 2001). PHA biopolymeric materials such as PHB homopolymers and co-polymers of [P(3HB-*co*-3HV)], [P(3HB-*co*-4HB)], [P(4HB)], [P(3HB-*co*-3HHx)], and [P(3HHx-*co*-3HO)] have been subjected to animal testing as well as in vivo assessments toward tissue reaction and have been reported to be immunocompatible in different host systems. However, very little data are accessible pertain to human beings on products bearing such substances, which has ultimately hampered the approval of in vivo medical application of PHA polymers. In this context, there is need of intensifying research work on animal testing of PHA materials along with associated products so as to make them grand success as biomedical materials (Valappil et al. 2006).

An allergic response involves a hypersensitive immune reaction to a substance, which generally is innocuous or would not elicit an immune response in everyone and the phenomenon is known as allergenicity. An allergic response may cause undesirable symptoms such as inflammation or itching or tissue injury. Allergies are an abnormal immune reaction that elicit by substances called allergens. The immune system of human is intended to defend the body from potential harm. However, the immune system found to reacts with allergens in those individuals who have allergies. The allergic response is associated with the production immunoglobulin E, i.e., IgE antibodies for each allergen. The IgE antibodies stimulate cells to produce histamines that act on different parts of the body such as nose, eyes, skin, throat, gastrointestinal tract, or lungs to produce symptoms of an allergic reaction. In these contexts, investigations have been conducted so as to evaluate the allergic/non-allergic responses of biopolymeric PHA materials. The inflammatory reactions and other unwanted responses in studies of innovative biomaterials as well as constructions can be correlated not merely to composition and type of the implant including specific site of implantation but also to the extent of chemical purity of the substance/material. This is supported by fact that leachable contamination along with lower molecular mass PHB are at least partially accountable toward enhanced accumulation of collagen in in vivo investigation of subcutaneous PHB implants in rats (Tang et al. 1999). Shishatskaya et al. (2002) conducted an investigation on allergic reaction of PHA materials using mice and rabbit as animal model. In this study, aqueous extracts of PHB and [P(3HB-*co*-3HV)] holding 4% and 18% 3HV were applied to the skin of white mice and instilled into eyes of rabbit to find out whether these polymeric materials triggered an irritating action. Interestingly, in either variant, no irritating effect was detected. The sites of skin applications showed no edema, hyperemia, flaking, xeroderma, and other response analogous to control. The extract of PHA materials instilled into the conjunctival sac of the animals did not cause any irritating response on mucous membranes. No instant allergic reaction was detected in either case. In another investigation, PHB sheets were evaluated for inflammatory response by employing the chorioallantoic membrane of the developing egg, where it was observed that the polymeric material, i.e., PHB sheets did not elicit any inflammation (Saito et al. 1991). Thus, the study conducted so far revealed that PHA material did not elicit an allergic response, which is an important parameter for its use as biomaterial.

Non-teratogenicity and non-carcinogenicity of PHAs

Teratology deals with the study of abnormal development in embryos including the sources of birth defects or congenital deformities. These anatomical or structural malformations are exist by birth though they cannot be identified until later in life. Remarkably, only a rare reports are available regarding the effect of biopolymeric PHA materials pertaining to the growth of embryos as well as larvae. Li et al. (2016a) performed interesting research to assess the effect of PHAs on developmental as well as exploratory profile of zebrafish larvae. Developmental results revealed no adverse impacts of PHA materials with reference to growth and progress of zebrafish embryos as well as larvae, except the incidence of variation of *krox20* expression. *krox20* is a transcription factor that participated in the segmentation of vertebrate hindbrain, mainly in the development and specification of rhombomeres (r) 3 and 5 as noteworthy marker of zebrafish brain. Such report indicated toward the non-teratogenicity of PHA materials and thus increasing their further importance as unique biomaterial.

Cancer commonly involves an abnormal growth of cells with a tendency to multiply or proliferate in an uncontrolled way and, in some cases, to metastasize, i.e., spread. In another word, it is a “disease of the genes.” Cancer can occur in any tissue of the body and have various different kinds in each body part. Carcinogenesis involves a complex phenomenon, where its initiation needs assembly of various independent mutations (Dumaz et al. 1993). It is initiated as a result of incitement of oncogenes like c-Fos, which involves initiation, promotion as well as progression (Dumaz et al. 1993). Many

investigations found to reveal that inhibition or deactivation of tumor suppressor genes is leading stage for the formation of cancers in human being (Hollstein et al. 1991). Among such genes, mutation of p53 gene has been shown to be the utmost usual genetic transformation occurred in human being malignancies (Dumaz et al. 1993). Thus, oncogene action by means of deactivated p53 plays a substantial function toward the process of carcinogenesis. The unrestricted growth, invasion as well as metastasis are the characteristics features associated with cancerous cells. The capability of cells to undergo unrestricted growth and development might be critically vital in the carcinogenesis process (Noble et al. 2004).

PHA have been exploited as drug transport matrices and medical implants including devices for supporting cell growth owing to their superior elastic property, flexible mechanical strengths, biocompatibility, and biodegradability. However, a critical review of literature revealed that so far, there is no report or insignificant report on the probability of whether fast cell growth and development upon PHA matrices might stimulate the development of cancerous cell. In this context, Peng et al. (2011) did an interesting investigation to find out the impact of fast cell growth and development on PHA matrices toward tumor development. It was found that proliferating rat osteoblasts cultivated upon different PHA biopolymers together with PHB, [P(3HB-*co*-3HV)], [P(3HB-*co*-4HB)], [P(3HB-*co*-3HHx)] as well as [P(3HB-*co*-3HV-*co*-3HHx)] did not cause cancer induction. In this study, the cell multiplication was assessed through the integration of 5-bromodeoxyuridine (BrdU) and the transcript expression of cancer linked genes Ki67, p53, and c-Fos was studied using quantitative real-time PCR. This study depicted that the cells multiplying upon the PHA polymers were under control of normal cell cycle. Furthermore, DNA aneuploid as well as telomerase activity was merely observed in the positive control UMR-108 cells. The UMR-108 cells found to have longer telomeres when compared to cells grown on films. This observation further revealed the normal condition of cells growing and developing on the PHA polymers. This study showed that such PHAs might be exploited for supporting cell growth without sign of vulnerability toward tumor formation. In another investigation performed by Tezcaner et al. (2003), it was concluded that in vitro assessment of [P(3HB-co-3HV)] biopolymeric materials targeting retinal pigment epithelial cells of human depicted healthy cell growth and development under regulated rate of growth without any uncommon cell spreading. Similarly, Abdelwahab et al. (2019) conducted an investigation regarding the antibacterial as well as anti-cancerous activities of functionalized PHB with various amino compounds. Among amino-PHB polymeric materials, PHB-piperazine (PHB-P) displayed promising anti-cancerous activities toward in vivo Ehrlich ascetic carcinoma–bearing mice in vivo with no toxic effect, i.e., improved biocompatibility. The effect of biopolymeric PHA materials on cell growth and development as well as biocompatibility has been found to confirm in different investigations. Nevertheless, there is still lack of clear reports on PHA polymers regarding their non-carcinogenic effects in all cell lines (Ali and Jamil 2016). Based on this information, it is clear that so far, polymeric PHAs depicted non-carcinogenicity as one of the most important properties in addition to other important properties. Furthermore, advancing and intensifying research work is a major compulsion to explore completely their non-carcinogenicity toward all cell lines. These advance research work unquestionably result in prolific exploitation of PHA biopolymer as unique and wonder biomaterial in near future.

Biomedical applications of PHAs

Medical sutures and heart valves

Sterile fibers, for example, medical sutures including absorbable/non-absorbable, are employed toward hemostasis, wound healing, and tissue attachment with ligation (He et al. 2014). The absorbable sutures were found to lose their tensile power under the duration of 2 months followed by degradation with non-toxic products. The suitability to tie and grip, resistance toward growth of bacteria, capability to sterilize without difficulty, smooth/uniform superficial texture, biocompatibility, high in vivo tensile strength, ultimate absorbance as well as disappearance are the desirable features needed for absorbable sutures. Such anticipated characteristics for the exploitation as biomedical sutures were showed by [P(3HB-*co*-3HV)]/PLA and [P(3HB-*co*-3HHx)] co-polymers, where [P(3HB-*co*-3HHx)] co-polymers depicted improved tensile power and elasticity than the exceptional biocompatibility of [P(3HB-*co*-3HV)]/PLA (He et al. 2014).

Wang et al. (2004) and Li et al. (2005) carried out investigations on [P(3HB-*co*-3HHx)] co-polymers, where these co-polymeric materials depicted improved osteoblasts attachment, propagation as well as differentiation than the PHB. These co-polymeric PHAs were also found to promote the chondrogenesis of bone marrow–derived MSCs of humans (Yan et al. 2011). Furthermore, [P(3HB-*co*-4HB-*co*-3HHx)] co-polymers revealed greater potential toward differentiation of MSC than the [P(3HB-*co*-3HHx)] co-polymer (Wei et al. 2009). In another investigation, [P(3HB-*co*-3HHx)] scaffolds were mingled along with collagen and then exploited effectively to grow MSCs (Lomas et al. 2013). But [P(3HB-*co*-3HV-*co*-3HHx)] films depicted a large effect toward encouraging apoptosis of rat osteoblasts with expression of various different integrins. These osteoblasts were treated with various biopolymeric PHA films such as PHB, [P(3HB-*co*-3HV)], [P(3HB-*co*-3HHx)], and [P(3HB-*co*-3HV-*co*-3HHx)] for support, fixation/attachment as well as growth and development.

Remarkably, the [P(3HB-*co*-3HV-*co*-3HHx)] polymers revealed a lesser level of fixation and late cell division with more apoptosis (Wang et al. 2013b). It could be presumed that these are the features of biopolymeric PHAs, which play a significant function in the cell response. However, further investigation is needed pertaining to the tissue engineering (TE) of cartilage cells along with PHAs (Ali and Jamil 2016). In order to attain the anticipated chemical and physical features, electrospinning technique could be employed to process and assess biomedical PHAs, viz., PHB, [P(3HB-*co*-4HB)], [P(3HB-*co*-3HV)], and [P(3HB-*co*-3HHx)]. The electrospinning technique involves an electric field to regulate the formation as well as deposition of polymer fibers on a substrate. Sheets and cylindrical shapes can be fabricated with this technique. It was shown that electrospun PHB scaffoldings decreased cell growth in addition to the differentiation of the skeletal myotube development ability of myoblastic cell lines (C2C12 and H9c2 cells) (Ricotti et al. 2012). In another study, PHA scaffolds were developed by electrospinning, where it was found that they did not influence the growth and development, sustainability as well as fixation of NIH 3T3 fibroblast cells of mice, signifying their promising application in TE (Volova et al. 2013). Most recently, Rajaratanam et al. (2018) conducted an investigation in which the usual eco-friendly polymer poly(L-lactic acid) was employed for blending with superheated steam hydrolyzed [P(3HB-*co*-3HHx)] oligoesters matrix of high biocompatibility in the weight proportion of 80:20 so as to enhance the mechanical characteristics of the resulting polymers. Interestingly, the mechanical characteristics of the blended polymers were found to be near a requisite robustness range of medical sutures, thereby opening new possibility toward the development of resorbable medical sutures, which needs quick healing. These studies showed that PHAs might be exploited as promising natural absorbable sutures in the near future.

Valvular heart disease is an important reason for serious morbidness along with individual death in the world. Traditional replacement surgery includes the grafting of mechanical valves or biological valves. Presently exploited heart valve prostheses are non-viable; therefore, child patients outgrow substitution valves that require repetition of surgeries to substitute them. However, for the adult population, increased valve longevity is needed. A potential technique involves the formation of tissue engineering–based heart valve frameworks with a higher level of maturity before grafting (Valappil et al. 2006). The TE of heart valves emerged as an innovative process for the improvement of present methods of therapy in valvular heart surgery. Biopolymeric PHA TE (frameworks of soft/hard tissues) has an advantage, viz., the tailor-made flexible features with a widespread variety of amalgamations as well as building blocks by various production techniques compared to other procedures (Ali and Jamil 2016). Sodian and colleagues grabbed the benefit of this capability of PHA biopolymeric material to implant a complete tri-leaflet heart valve by means of seeded autologous cells with [P(3HHx-*co*-3HO)] co-polymer in a lamb model that functioned efficiently for 3 months with no thrombus observed except mild stenosis (Sodian et al. 2000; Levine et al. 2015). Nevertheless, non-progressive heart valve regurgitation was detected over 6 months after material implantation when PGA was employed to mingle with [P(3HHx-*co*-3HO)] co-polymer (Stock et al. 2006). Qu et al. (2005) reported surface modification of a co-polymeric [P(3HB-*co*-3HHx)] with fibronectin layer as well as/or ammonia plasma treatment of human umbilical vein endothelial cells including smooth muscle cells of rabbit aorta that caused improved growth due to formation of a confluent monolayer. Altogether, these investigations certainly point toward the potential outcomes of heart TE with high feasibility to develop a heart valve for the adult population (with improved valve durability) and children, which can grow and, therefore does not require to be substituted.

Nerve repair and regeneration

Current developments/progresses of TE have brought expectation for patients with brain impairment or injury or damage for the restoration/repair as well as regeneration of impaired/injured/damage neurons. Earlier, it was viewed impossible toward the restoration as well as regeneration of impaired/injured/damage neurons. However, the discovery of some neural stem cells as well as neural progenitor cells of the central nervous system enables us to carry out nerve repair along with regeneration by TE approach (Ali and Jamil 2016). Interestingly, there are four important components of constructs, i.e., a scaffold toward axonal multiplication, support cells like Schwann cells, growth factors as well as an extracellular matrix responsible for nerve regeneration (Yang et al. 2005). An ideal conduit employed to link nerve gaps should certainly be decomposable, depicts lower antigenicity, permeable toward oxygen diffusion, readily vascularized, easily accessible as well as circumvents long-term compression. Nerve conduits employed for peripheral nerve and spinal-cord wounds are known as guidance channels as well as bridges. Guidance channels are developed from natural substances such as collagen as well as non-natural polymeric materials, for example, PLGA, ethyl vinyl acetate co-polymer, silicone, and ethylene vinyl co-acetate. Remarkably, conduits developed from these substances have a variety of disintegration rates, mechanical properties as well as porosities. The porous characteristic of the conduit influences the obtainability of soluble growth-stimulating parameters or nutrients from the neighboring surroundings. Furthermore, the mechanical characteristics of the conduit material essentially forbid the disintegration of channel that otherwise would limit neurite outgrowth as well as regeneration (Schmidt and Leach 2003).

Increasing attention/interest has arisen for the exploitation of biopolymeric PHA material (such as PHB etc.) for nerve repair owing to the piezoelectric properties of PHAs. The elementary method is based on aligning detached nerve ends inside a tiny tube of PHB without the requirement of sutures. Hazari et al. (1999a) as well as Ljungberg et al. (1999) assessed the application of a non-woven PHB film as coating to restore transected superficial radial nerves in cats over a period of 1 year. Axonal regeneration was not only found to be analogous with closure with an epineural suture for a nerve gap of 2–3 mm but also the inflammatory reaction developed by PHB was comparable to that established in primary epineural repair. In a consecutive investigation, the biopolymeric PHB was exploited to connect an irreducible gap of 10 mm in rat sciatic nerve in which the outcomes were compared with an autologous nerve graft (Hazari et al. 1999b). The axonal regeneration was found to be good in the PHB conduits with a little inflammatory infiltration over a period of 30 days. However, the rate as well as amount of axonal regeneration in the PHB conduit does not completely match with that of the nerve graft. In another study, exploitation of a nerve guide conduit composed of [P(4HB)] not only improved the axonal regeneration but also enhanced the level of repair of motor and/or sensory function over earlier reported conduits composed of PHB (Opitz et al. 2004). The [P(4HB)] (also known as PHA4400) conduits employed to link a 10 mm gap in 30 male Sprague-Dawley rats' sciatic nerve revealed an axonal regeneration rate of not less than 0.8 mm per day without wound infections, inflammation, or anastomotic failures under a period of 20 days.

Synthetic polymeric materials such as poly(α-hyroxy acids), for instance, PLA or polyglycolic acid, enable cell attachment as well as directed growth of neurite with the benefit that they can be without difficulty employed to produce fibers. Nevertheless, the disintegration products of these polymeric materials not often elicit an inflammatory response because of the acidic end products. This can be decreased significantly as a result of exploitation of PHAs like [P(4HB)] that provide improved mechanical features as well as lower in vivo tissue response on account of less acidic end products. Homopolymer PHB and co-polymers of [P(3HB-*co*-3HV)], [P(3HB-*co*-4HB)] as well as [P(3HB-co-3HHx)] depicted good outcomes toward improved neural survival, elicitation of better axon–dendrite separation including growth of neural stem cells, viability, and proliferation (Xu et al. 2010; Chen and Tong 2012). It may be concluded that for neural repair and regeneration, excellent surface characteristics associated with desired bio-functions should include the utmost appropriate approach (Lu et al. 2013), and the exploitation of various PHA blends in this approach could offer an answer to the issue (Lizarraga-Valderrama et al. 2015). The treatment of NaOH with [P(3HB-*co*-3HHx)] co-polymers resulted in a substantial improvement in the hydrophilicity of these biopolymeric materials, which increased the fixation of neural stem cells as well as neural progenitor cells in the presence of small amounts of serum (Lu et al. 2014). All these outcomes reveal beyond doubt the potential of the PHA materials for nerve repair and regeneration.

Osteogenic and chondrogenic impacts

It was reported by Pelham Jr and Wang (1997) that biomaterials have a tendency to begin a transformation in cell conduct. Therefore, various biopolymeric PHA materials associated with particular surface properties can cause corresponding cell retaliation. This cell response was observed with the planar as well as grooved co-polymeric [P(3HB-*co*-3HHx)], where these planar and grooved films depicted commencement/initiation of chondrogenic as well as osteogenic activities, correspondingly on bone marrow–derived MSCs (Li et al. 2015). Remarkably, the involvement of such substances played an important role toward enhancing or reducing the expression of some particular gene markers, which are accountable for modifications in cell reaction. Such results depicted that surface variation could be a most important parameter toward the stimulation of a preferred signaling transduction process for different tissue substitutions. Cells are capable to react/respond to a specific engineered surface on account of detecting/sensing the substance by means of some integrin clusters as well as formation of focal adhesion (Geiger et al. 2009). Therefore, cell multiplication stimulating substances, especially PHA materials, can be exploited as extracellular matrix (Gardel and Schwarz 2010). In addition, incorporation of a biopolymeric PHA binding protein with an MCL-PHA can also be an efficient approach for increasing the multiplication of these cell lines. However, further intensifying research work is needed for finding the expression of integrins on the engineered surfaces of PHA materials so as to describe the corresponding cellular behaviors such as motility, variations, shape, growth patterns, and attachment (Ali and Jamil 2016).

Infection-resistant biomaterials

In medical practice, biomaterial-associated bacterial infections are presently receiving as a serious concern/threat. The antibiotic resistant bacterial species, for example, *Staphylococcus aureus*, which is methicillin resistant, are of great apprehension. Biomaterial-mediated inflammatory reaction/response could be regulated as a result of different techniques, viz., reactive oxygen species imaging, the hydro-indocyanine green dye approach as well as near-infrared fluorescence imaging probes (Dinjaski et al. 2014). Kuroda and Caputo (2013) reported antimicrobial polymeric materials as potential substances for substitution of traditional drugs, not only for antimicrobial mechanisms but also decreased possibility toward development of resistance. For instance, Abdelwahab et al. (2019) conducted interesting investigation in which the

functionalized PHB-ethylenediamine exhibited promising antibacterial properties toward *Pseudomonas aeruginosa*, *E. coli*, and *Klebsiella pneumoniae* including *S. aureus* over standard drug ciprofloxacin as control. Association of implants with antimicrobial peptides (anti-MPs) like tachyplesin I (TPI) may inhibit disease-causing microorganisms including decreasing the threat of producing resistance toward antibiotics. Considering this, Xue et al. (2018) performed an interesting investigation in which TPI was associated to PHA granule linked protein (PGLP) followed by immobilization on [P(3HB-*co*-3HV)] co-polymer by hydrophobic linkage. The PGLP-TPI covering not only prevents the growth of Gram-negative as well as Gram-positive bacterial strains/species but was also found to enhance the healing of injury as a result of reducing the bacterial strain/species counts less than 10^5 colony-forming units g^{-1} tissue in a deep-wound mouse model in vivo.

Several macromolecules associated with positive charge were found to exploit as biocidal polymeric materials as they can fight the target cells on account of net negative charge. Dinjaski et al. (2014) carried out an interesting investigation, where a functionalized type of bacterial PHA materials depicted antibacterial activities toward such kind of infections. PHA materials holding a thioester group in the side chains exhibited greater antibacterial potential than the poly-3-hydroxyoctanoate-*co*-3-hydroxyhexanoate [P(3HO-*co*-3HHx)] and polyethylene terephthalate (PET) both within and outside of living mammalian cells. Furthermore, the attachment of bacterial species to biomaterials is reliant on the kind of monomeric units incorporated together with the purity of the material. The antimicrobial action of poly(3-hydroxyacetyllthioalkanoate-*co*-3hydroxyalkanoate) co-polymer could be improved through integration of a large number of thioester ligands into the main chain of PHA material. Apart from this, the integration of other active groups could be very supportive for initiation of antibacterial actions toward bacterial species apart from *S. aureus*. The actual mechanism of the antibacterial action of poly(3-hydroxyacetyllthioalkanoate-*co*-3hydroxyalkanoate) co-polymer is so far not clear owing to fact that this co-polymeric material is specific toward some bacterial species/strains. Changing the composition of thioester along with molecular mass of the polymeric material enhances the possibility of antimicrobial action of these PHA biomaterials toward more microbes. Further, the incorporation of several metals in the monomeric units of PHA materials could be of definite application for fighting disease-causing agents on account of their anti-microbial properties (Ali and Jamil 2016).

Drug carrier systems

Traditional drug treatment that involved intravenous or extravascular route such as oral, sublingual, etc., failed to maintain drug concentrations in anticipated frame in the target area toward prolonged duration. Regulated drug transport systems include discharging therapeutic device at anticipated rate for anticipated period in such a way that the drug concentration in the body within the therapeutic frame can be sustained (Nair and Laurencin 2006; Rathbone et al. 2010). The drug carrier system was established for discharging, activating, uptaking, maintaining, targeting, bringing as well as localizing the drugs at the right time, place, and dose including duration (Hazer et al. 2012; Inan et al. 2016).

Among drug delivery systems, PHAs emerged as one of the potential attractive candidates owing to their biodegradability and thermoprocessability, which enable them to be exploited as biomaterials for use as drug carrier in addition to traditional medical devices including TE. Biodegradable polymeric materials possessing an encapsulated drug could be targeted/introduced inside the body. These are employed toward localized drug transport for the regulated discharge of a drug over a period of months (Lenz and Marchessault 2005). The disintegration of biopolymeric PHA materials in the tissues of the host organism provides the likelihood of coupling this process with the liberation of bioactive substances, for example, antitumor drug or antibiotic (Shrivastav et al. 2013). The drug discharge kinetics could be regulated by appropriately engineering the biopolymeric PHA matrix factors to attain desired rates of degradation. Among PHA materials, SCL-PHAs undergo degradation by means of surface erosion, which enable them to be an attractive polymeric material toward drug carriers. Several pores are developed on the surface of SCL-PHAs because of their crystallinity and hydrophobicity, which are responsible for the discharge of drugs very rapidly without any polymer degradation. Also, PHB microspheres toward vaccination of staphylococcal enterotoxin B for gut-related lymphoid cells were found to be efficient in targeted drug delivery system formalization (Winnacker and Rieger 2017). In contrast, MCL-PHA co-polymers are much more appropriate for drug delivery as they are associated with low crystallinity along with low melting point (Fava et al. 2015). Furthermore, the drug transport systems can be developed with diverse shapes, i.e., microcapsules, gels, polymeric micelles, microspheres, films, polymer linked drugs, porous matrices, and nanoparticles. The physical linkages are usually chosen to attach the drug to the polymeric material without any impairment/damage to the molecular structure of the drug (Ueda and Tabata 2003). Apart from this, it has been also established that homopolymeric PHB could be exploited toward transdermal drug delivery (Alves et al. 2016).

The PHB polymers and [P(3HB-*co*-3HV)] co-polymers have been evaluated in many investigations pertaining to their potential drug delivery systems (Shrivastav et al. 2013). For instance, Pramual et al. (2016) conducted a study on nanomaterial (NMs) of co-polymeric [P(3HB-*co*-3HV)] as transporters of a hydrophobic photosensitizer, 5,10,15,20-tetrakis(4-hydroxy-phenyl)-

21*H*, 23*H*-porphine (pTHPP) toward photodynamic treatment. The in vitro photo-cytotoxic assessment was carried out on adenocarcinoma cell line HT-29 of colon, where pTHPP-laden PHA NMs depicted higher photo-cytotoxic impact toward the same cancerous cells. Likewise, glycerol 1, 3-diglycerolate diacrylate (GDGD) that contains OH– groups was grafted onto MCL-PHA co-polymer, i.e., [P(3HO-*co*-3HH$_X$)], which resulted in the formation of [P(3HO-*co*-3HH$_X$)-*g*-GDGD]. The GDGD was found to improve the hydrophilic characteristic of [P(3HO-*co*-3HH$_X$)-*g*-GDGD] polymer and is therefore, extremely efficient not only toward drug delivery but also cellular interaction (Ansari and Annuar 2018).

PHA material could be potential candidates for combating extremely challenging infections since PHA drug transport systems depicted capability for provision along with upholding of sufficient concentration of antibiotics in infection sites (Gould et al. 1987; Gursel et al. 2001). PHB, [P(3HB-*co*-3HV)], and [P(3HB-*co*-4HB)] co-polymers were found to be beneficial for the development of decomposable, implantable rods in local transport of antibiotics for chronic osteomyelitis treatment (Turesin et al. 2001). It was reported that anticancer agent lomustine was liberated rapidly from the PHB microsphere compared to PLA microsphere during in vitro and in vivo studies of PHB and PLA microspheres as promising transporters toward drug targeting (Bissery et al. 1985). Furthermore, the PHB microspheres incorporated with ethyl or butyl esters of fatty acids exhibited enhanced rate for the drug discharge. Lu et al. (2011) conducted an investigation on the biopolymeric PHA material as a drug delivery carrier, where continued discharge system of P13 K inhibitor (TGX221) based on PHA nanoparticle (NMs) was formed with the intention to inhibit the growth of cancerous cell lines. Interestingly, TGX221 was slowly liberated from PHA-based NMs and the proliferation of cancerous cell lines was considerably sluggish for cells treated with TGX221 NMs. These studies advocate toward the feasibility of the biopolymeric PHAs as attractive drug delivery systems.

The tailored properties of the newly designed PHA materials in thermal and mechanical properties, hydrophilicity as well as disintegration rate have met some special needs toward particular biomedical uses (Kai and Loh 2014). Recently, Shah et al. (2012) carried out a study for evaluating the efficacy as well as bioavailability of a chemotherapeutic agent active toward a different type of tumor called cisplatin in the form of cisplatin-loaded self-assembled amphiphilic [P(3HV-*co*-4HB)-*b*-mPEG] block co-polymer nanoparticles. Bacterial non-crystallized P(3HV-*co*-4HB) co-polymer was used as hydrophobic portion in order to enhance the drug loading efficiency. This novel block co-polymer was developed as a result of transesterification reaction between bacterial [P(3HV-*co*-4HB)] co-polymer and monomethoxypolyethylene glycol in the presence of *bis*(2-ethylhexanoate) tin catalyst. The emulsification–solvent evaporation technique was exploited toward the formation of core–shell particles in the range of nanometer size. The loading of cisplatin in the core hydrophobic domain of NMs depicted sustained discharge in vitro. Furthermore, transmission electron microscopy (TEM) and confocal microscopy investigation not only showed the internalization of cisplatin-loaded NMs into the tumor cells but also exhibited a significant repression impact along with the increase of apoptotic process of the tumor cells mediated through the NMs over freely administered cisplatin. Therefore, it is clear that drug entrapment within the amorphous polymeric nanocarriers might hold more drugs in the target site for sustained duration over free drug in solution. These novel amorphous nanoparticles may increase the bioavailability of cisplatin to the cancer cells, hence, reducing the toxicity associated with multiple dose of the drug. The PHA NMs are also reported to be the appropriate choice toward the therapy of diseases concerning the nervous system, primarily for dealing with cancerous patients with their effective target drug delivery system as it decreases the threat associated with the utilization of drugs having a cytotoxic effect. This system helps in the accumulation of particles inside the target tissues when it is liked with the drug delivery particles (Zubairi et al. 2016). Overall, all these studies revealed that the future of biopolymeric PHAs will utmost unquestionably be linked to their uses as drug delivery systems against different microbial triggered diseases including cancer therapy and tumor treatment. Nevertheless, the appropriate design of the microsphere or microcapsule or NMs of PHA holding anticipated drugs remains an aspect of foremost apprehension to confirm that the features of the drug and/or PHA material are not influenced and that the discharging rate of the drug is near to its target site (Ali and Jamil 2016).

Gastrointestinal system, urological stent, and artificial esophagus

Biopolymeric PHA materials could be employed as organ wall patches in gastrointestinal system. Thus, PHA materials must reveal strong structural properties viz. thick, hydrophobic as well as not brittle (Löbler et al. 2002). The same group conducted investigation to evaluate the uses of biodisintegrable patches in the gastrointestinal tract, where asymmetric patches composed of PHB were sutured on the rat stomach wall and systemic together with local immune responses were emphasized (Löbler et al. 2002). It is revealed that C-reactive protein (CRP) level that is a strong systemic marker of acute inflammation is produced in large quantity during acute inflammatory response locally around the implantation site on the 14th day of implantation. Bowald and Johansson (1990) discussed the application of a conduit/tube for urethral reconstruction. A solution of the [P(3HB-*co*-3HV)] co-polymer was employed to coat thin, knitted conduits composed of vicryl substance and implanted into four

dogs to substitute the urethra. After a period of 6–9 months, a completely functional urethra had been reconstructed in all test animals, therefore showing the potential of PHAs in urology.

In children, corrosive impairment/injury of the esophagus is a serious condition, which is associated with several likely complications together with fistulization to the greater vessels, mediastinitis, perforation and penetration to the stomach, and stricture formation (Janousek et al. 2006). Investigation has been carried out on co-polymeric [P(3HB-*co*-3HHx)] as artificial esophagus material, where the same co-polymer can promote the regeneration of the eliminated esophagus of dogs. This study reveals that [P(3HB-*co*-3HHx)] co-polymer, which has higher strength and elasticity might be a better polymer toward application as a synthetic esophagus (Chen and Wu 2005).

Other biomedical applications

In addition to the above potential applications of biopolymeric PHAs in the medical field, they also have been exploited for meniscus repair devices, adhesion barriers, skin substitutes, slings, hemostats and wound dressings, vein valves, orthopedic pins, bone filling augmentation material, bone dowels, etc. (Kumar et al. 2015; Ali and Jamil 2016; Singh et al. 2017; Peptu and Kowalczuk 2018). Table 2 summarizes the various kinds of PHAs employed for specific medical application.

Current bottlenecks and solutions for PHAs as realistic biomaterials

Biopolymeric PHAs are recognized as promising biomaterials for upcoming uses as these are biodegradable, biocompatible, easy to fabricate, etc. In the current scenario, PHAs have been investigated toward biomedical uses because of their unique and desirable features. However, there still remain some bottlenecks related to PHA purity, mechanical properties, functionalities, cytotoxicity, biodegradability, etc., which need to be addressed so as to make PHAs a realistic biomaterial. This section addresses the following bottlenecks and solutions for PHAs as realistic biomaterials:

Purity issues

Biopolymeric PHA of high purity is required toward biomedical uses. Specifically, bioactive contaminants such as proteins, DNA, lipids, lipopolysaccharides (LPS), different reagents exploited in extraction such as surfactants/antifoaming agents, etc., associated with PHAs should be decreased to extremely low level since these might elicit immunological responses. The type of impurities associated with PHAs is depending on the biological route and downstream processing. Among such contaminants, the LPS being potent endotoxins can cause severe problems by eliciting immunoreactions. Remarkably, all Gram-negative PHAs producing strains/species including cyanobacteria found to fabricate LPS as an important component of outer membrane during growth of cell biomass. Endotoxins are not only heat stable but also not easy to get rid of. Therefore, the occurrence of endotoxins upon the surface of biomaterials causes a higher immunological response making PHA biopolymers extremely unsuitable in medical uses (Chen and Wu 2005; Furrer et al. 2008; Durai et al. 2015; Peptu and Kowalczuk 2018). As per US Food and Drug Administration (FDA) regulations, the content of endotoxin associated with medical devices should not increase 20 US Pharmacopeia (USP) endotoxin (E) units (U) per device. However, in case of medical devices, which are in association with the cerebrospinal fluid, the endotoxin content should not enhance 2.15 USP EU per device. Attaining such purity in case of biopolymeric PHAs toward biomedical applications is difficult task and requires additional efforts (Williams et al. 1999; US Department of Health and Human Services, FDA 1997). For instance, usually values of more than 100 EU per gram of polymer were found in commercially available biopolymeric PHAs like PHB (Williams et al. 1999). The LPS in small amounts can interact with blood and elicit immune system leading to an undesirable effect. Biopolymeric PHA contamination with endotoxins is a severe issue that mainly results through fermentation of Gram-negative bacterial strains/species since LPS is associated with the external membrane. In cell lysis as well as recovery of product, the LPS were found to contaminate PHAs as a result of liberation from the external membrane. Hence, purification of PHAs from such bioactive contaminants (endotoxins) should be cautiously carried out for biomedical applications concerning medical devices. The usual approaches employed toward the PHAs purification are redissolution as well as precipitation, washing with non-solvent, chromatographic purification, treatments with chemical agents as well as filtration. The purity of PHAs can also be improved as a result of washing the biomass before PHAs extraction or through aqueous digestion for removal of major contaminants (Furrer et al. 2008; Peptu and Kowalczuk 2018).

The probable range of PHAs impurities from PHAs free biomass is widespread. However, the extraction solvent essentially decide which contaminants will be carried over. For example, protein as well as DNA molecules are often observed when PHAs are recovered through aqueous chemical digestion. The LPS are observed with aqueous digestion and solvent extraction. These are dissolved in water; nevertheless, their polar nature causes them to undergo some extent dissolution in hydrophobic solvents. Repetitive dissolution and precipitation strategies are generally employed for attaining purity close to 100%. The effectiveness of this approach is reliant on various factors such as concentration of the polymer solution, temperature, etc.; nevertheless, generally, huge quantities of non-solvents are exploited (Furrer et al. 2008). Jiang et al.

Table 2 Various types of PHAs exploited as biomaterials during the last decade

PHA material	Applications	Model	References
[P(3HB-*co*-3HV-*co*-3HHx)] co-polymer	Skin tissue engineering	Human keratinocyte cell line HaCaT	Ji et al. (2008)
[P(3HB-*co*-3HV-*co*-3HHx)] co-polymer	Bone tissue engineering	Human bone marrow mesenchymal stem cells	Hu et al. (2009)
PHB polymer, [P(3HB-*co*-3HV)] co-polymer and [P(3-hydroxy-10-undecenoate)] polymer	Tendons and ligaments tissue engineering	L929 murine fibroblast cells	Rathbone et al. (2010)
[P(3HB-*co*-3HHx)] co-polymer	Candidate for islet transplantation	Murine islet beta-cell line, NIT-1 cells	Yang et al. (2010)
PHB nanoparticles	Targeted cancer therapy	MDA-MB 231 breast cancer cells	Lee et al. (2011)
PGA meshes coated with [P4HB] polymer	Heart valve tissue engineering	Non-human primates	Weber et al. (2011)
Mono-methoxy-poly(3HB-*co*-4HB)-graft-hyper-branched polyethylenimine co-polymers	Human gene therapy	A549-Luc and MCF-7-Luc cells	Zhou et al. (2012)
Blend of P(3HB-*co*-3HV) co-polymer with poly(Ɛ-caprolactone)	Vascular tissue engineering	Rat cerebral endothelial cells	Del Gaudio et al. (2012)
3HB methyl ester	Drug candidate against Alzheimer's disease	Alzheimer's disease mice	Zhang et al. (2013)
3HB and 3HB methyl ester	Drug candidates against osteoporosis	Mice	Cao et al. (2014)
Various PHA4400	Axonal regeneration	Rat	Bugnicourt et al. (2014)
PGA meshes coated with [P4HB] polymer	Heart valve tissue engineering	Sheep	Emmert et al. (2014)
[P(3HB-*co*-3-hydroxy-10-undecenoate)] co-polymer	Tissue engineering scaffold	Lamb	Levine et al. (2015)
[P(3HB-*co*-3HHx)]-Ligand	Drug delivery system	Cancer cell lines	Nigmatullin et al. (2015)
[P(3-hydroxybutyrate-co-3-hydroxyvalerate-*co*-2,3-dihydroxybutyrate)] co-polymer containing hydroxyl groups	Scaffolds for tissue engineering	Human mesenchymal stem cells	Insomphun et al. (2017)
[P4HB]/gelatin hybrid	Heart valve tissue engineering	Sheep	Capulli et al. (2017)
PHA or [P(3HB-*co*-3HHx-*co*-3HV)] co-polymer	Injectable scaffolds for tissue regeneration	Mice	Wei et al. (2018)
Fe_3O_4/GO-*g*-[P(3HB-*co*-3HV)] composite	Antimicrobial properties	Gram-negative and Gram-positive bacteria	Pramanik et al. (2019)
PHB functionalized with piperazine	Anti-cancerous activities toward *in vivo* Ehrlich ascetic carcinoma	Mice	Abdelwahab et al. (2019)

(2006) revealed that the concentration of UV absorbing substances was significantly reduced through acetone mediated solubilization of MCL-PHAs followed by precipitation in cold methanol. Likewise, SCL-PHAs were repetitively solubilized in chloroform followed by precipitation in ethanol so as to eliminate bioactive materials. Very small amounts of fatty acids in the range of C6 to C18 were removed with enhancement in hemocompatibility (Sevastianov et al. 2003). These researchers suggest that such fatty acids produced by lipopolysaccharides. Temperature-controlled solubilization followed by precipitation in 2-propanol was carried out for purifying some MCL-PHAs like polyhydroxyoctanoate (PHO) (Furrer et al. 2007). A purity of approximately 100% with endotoxicity of below 10 EU/g of PHAs was attained by this approach. The endotoxin content was further decreased to acceptable ranges, e.g., < 10 EU/g of PHAs as a result of treatment with oxidizing agent like hydrogen peroxide or benzoyl peroxide (Williams et al. 2001). The LPS was also disintegrated under application of basic environments (Lee et al. 1999; Sevastianov et al. 2003). The concentration of base as well as treatment period are important factors toward ideal detoxification. Else, the disintegration of LPS is inadequate or biopolymeric PHA undergo depolymerization that result in reduction of its molecular mass. Overall, despite the technological advancements in the purification techniques of extracted PHAs, the purity of PHAs (as per FDA regulations) is still away from medical grade for successful exploitation as biomaterials. Therefore, further intensifying research is needed in the coming years so as to improve the purification techniques of extracted PHAs in such a way that PHAs purity reaches up to prescribed medical grade for various biomedical applications. Figure 2 summarizes the various contaminants as a major barrier in the way of PHAs as potential biomaterials.

Cytotoxicity issues

The prefix "cyto" refers to cell and "toxic" to poison. Cytotoxic can be defined as a substance or process that causes

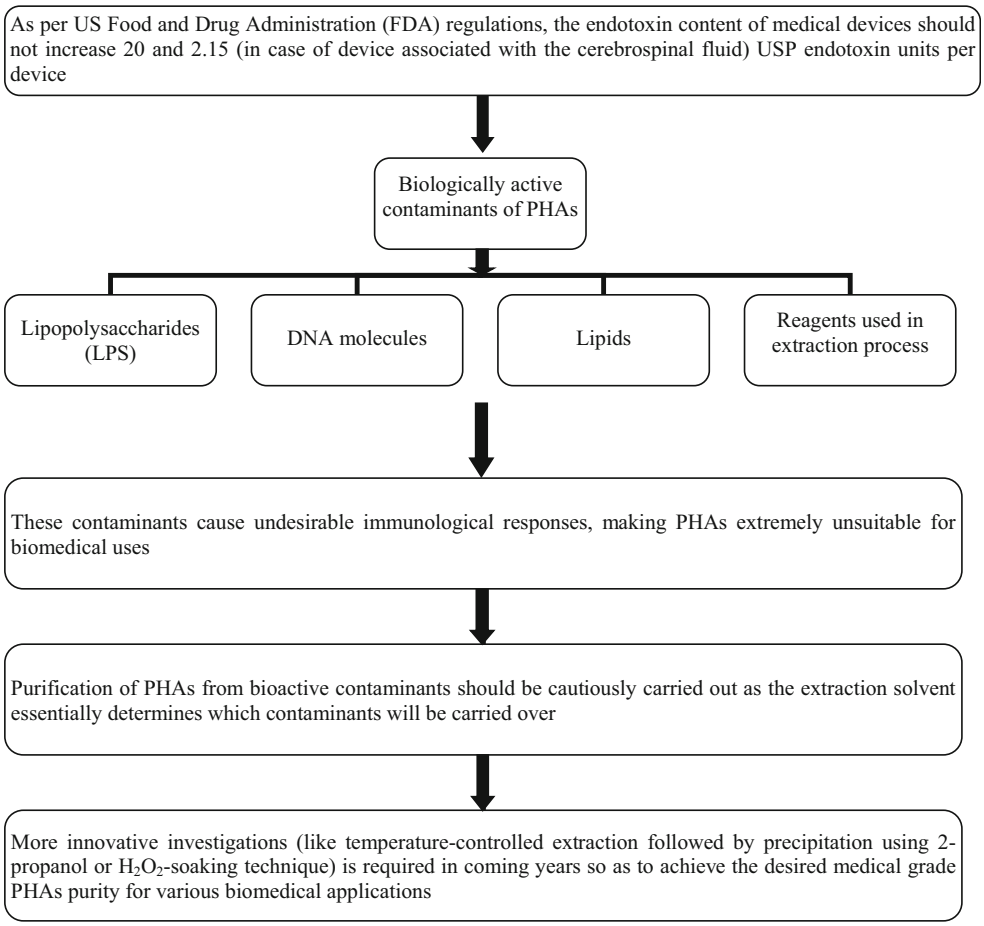

Fig. 2 Impact of purity of PHA on its biomedical applications

cell damage or cell death and the phenomenon is known as cytotoxicity. The cytotoxicity analysis, one of the biological assessments as well as screening evaluations, exploits tissue cells in vitro so as to detect the cell growth, reproduction as well as morphological impacts through the medical devices/biomaterials. Considering this, the impacts of monomers of biopolymeric PHAs on the overall shape, physiology, growth, cytotoxicity of target as well as surrounding cells have been studied. In one report, it was found that different monomeric units of PHB homopolymer, [P(3HB-*co*-4HB)] as well as [P(3HB-*co*-3HHx)] with concentration < 20 mg/L were unable to affect murine fibroblast L929 cells. Nevertheless, upon doubling the concentration, these monomeric units found to decrease the cellular growth followed by apoptosis. The cytotoxicity linked to oligomers reduced inversely with the length of the side chain. Similarly, viability analysis of mesenchymal stem cells (MSCs) of human depicted that the (P[(R)-3HB-*co*-3-hydroxypropionate-*co*-5HV]) co-polymer was associated with little cytotoxicity as well as found to support cell growth and development, thereby emphasizing this co-polymer as promising biomaterials (Chuah et al. 2013). Furthermore, Basnett et al. (2013) carried out investigation and demonstrated that amalgamation of PHAs can reduce their cytotoxic characteristics. In this investigation, it was found that a novel blend of [P(3HO)] along with PHB exhibited more potential with reference to enhanced biocompatibility as well as decreased cytotoxic effect on microvascular endothelial cells of human being than the [P(3HO)]. Such blend undergoes degradation through surface erosion but not by bulk disintegration that resulted into more regulated disintegration, although still upholding the main assembly. This PHA blend exhibited very promising perspective for the design and fabrication of novel biopolymeric stents (Basnett et al. 2013). Vieyra et al. (2018) conducted another study on the cytotoxicity of PHB film blended with various concentrations of cellulose nanowhiskers by employing peripheral blood mononuclear cells from healthy donors. Culturing of healthy donors cells were carried out in the presence of films of the biomaterial for the period of 24 h and 7 days followed by evaluation of cell viability as well as production of TNFα and IL6 as proinflammatory cytokines. This investigation established that the amalgamations of PHB with nano-whiskers are not only harmless but also devoid of cytotoxicity in human cells that enable them attractive candidates toward implant substances. Likewise, Rajaratanam et al. (2018) carried out a study with respect to in vitro cytotoxicity impact of superheated steam treated [P(3HB-*co*-3HHx)] samples toward the design of functional biomaterial, particularly resorbable medical sutures. Untreated

as well as superheated steam treated (150 and 190 °C) [P(3HB-*co*-3HHx)] samples having low as well as high % of unsaturated chain ends were devoid of cytotoxicity toward the proliferation of mouse fibroblast cell line NIH 3T3, where the cell viability % was > 95%. Apart from this, PHA monomeric units including 3HB, 3HV, and 4HB found to be slightly harmful over commonly exploited scaffolding polymeric materials like PLA as well as poly-glycolic acid (PGA) owing to their lesser acidity along with decreased bioactivity. Further, [P(3HB-*co*-3HHx)] having greater tensile strength as well as elasticity over PHB as well as [P(3HB-co-3HV)] polymeric materials depicted good hemocompatibility with higher affinity for different types of tissues. This makes co-polymeric [P(3HB-*co*-3HHx)] even more beneficial for the application as medical sutures (Wu et al. 2009; Yang et al. 2002). Masood et al. (2013) conducted a study on the [P(3HB-*co*-5 mol% 3HV)] and [P(3HB-*co*-11 mol% 3HV)] including [P(3HB-*co*-15 mol% 3HV)] as a nano-delivery system toward the wrapping of ellipticine (EPT) as a model anticancer drug. In vitro cytotoxicity assays confirmed that the blank [P(3HB-*co*-5 mol% 3HV)], [P(3HB-*co*-11 mol% 3HV)] as well as [P(3HB-*co*-15 mol% 3HV)] nanomaterials (NMs) were depicting substantial biocompatibility but without influencing the survival of cancerous cell line A549. The encapsulation effectiveness of EPT in [P(3HB-co-3HV)] NMs was varied in the range of 39.3–45.7%. However, the % inhibition of cancerous cell line A549 varied between 64.3 and 67.8% compared to EPT alone, where % inhibition reached ≤ 45.11%. This system revealed an enormous possibility so as to enhance the cytotoxicity of EPT via enhancing its bioavailability. Chaput et al. (1995) studied the cytotoxicity response of three [P(3HB-*co*-3HV)] co-polymers with composition of 7, 14 as well as 22% 3HV units by direct contact along with agar diffusion cell culture techniques. It was observed that these polymers caused slight to moderate cellular reaction. Nevertheless, the cytotoxicity of these polymeric materials fluctuated with time, temperature medium as well as surface-to-volume ratio. Chen et al. (2014) conducted research on the cytotoxicity including biosafety of PHB and [P(3HB-*co*-3HV)] films in vitro with 3T3 tissue of fibroblast cells and in vivo by subcutaneous attachment of film in rats. In vitro assay, it was noticed that PHB and [P(3HB-*co*-3HV)] matrices were supporting cell attachment together with growth. Furthermore, the film-soaked conditional media did not reveal significant cytotoxic/inhibitory impacts toward cell growth and development. The in vivo absorption assay revealed that both PHB and [P(3HB-*co*-3HV)] films undergo slow degradation, where PHB degraded at slower rate compared to [P(3HB-*co*-3HV)] films. This PHB film exploitation in hernia repair revealed favorable result, i.e., the biopolymeric matrix was capable to repair the abdominal ventral hernia through provoking tissue of connective cells as well as fat ingrowth with very sluggish disintegration rate. In addition, the PHB biopolymer depicted benefit of lower intestinal attachment to the ventral hernia location over [P(3HB-*co*-3HV)] and commercial PP films. The physical characteristics of PHA polymers could be modified through incorporation of some other functional groups, which might be beneficial to develop almost innocuous PHA scaffolds. All these studies and findings revealed that the cytotoxicity of PHA polymers could be minimized to an insignificant level or complete non-cytotoxicity focused toward certain kinds of animal cells could be acquired during medical implantations by suitable incorporation of monomeric units (Ali and Jamil 2016).

Hydrophobicity issues

PHAs depict hydrophobicity owing to long-chain hydroxy fatty acids with lesser functional groups. Because of their hydrophobic properties, the biocompatibility associated with PHAs is found to inadequate (Yang et al. 2002). Furthermore, as soon as biopolymeric PHAs are implanted in vivo, it is the surface, which undergoes interactions with the host surroundings. The surface chemistry, wettability as well as topography assist significantly to find out the associations operating among biomaterial and bio-environment together with water as well as ion diffusion, protein adsorption with impact on cellular adhesion as well as proliferation (Leal-Egaña et al. 2013; Raza et al. 2018). For instance, the associations among proteins as well as hydrophobic surfaces result in distortion of protein molecules as more hydrophobic portion of protein molecules will undergo interaction with the surface so as to diminish the contact of aqueous phase. Thus, the protein molecule undergoes distortions, even denaturation, because of PHA-directed rearrangements (Peptu and Kowalczuk 2018). Generally, hydrophilicity was found to greatly affect the cell attachment with a substance. The greater the hydrophilicity of a material surface, the stronger the cells attached (Yang et al. 2002). Thus, poor hydrophilicity of PHA profoundly diminished the cell attachment as well as led to low effectiveness of cell substitution (Yang et al. 2002; Shen et al. 2010). Thus, the inherent hydrophobicity and poor hydrophilicity of unmodified biopolymeric PHAs are inappropriate for biomedical uses as various biomedical uses need improved hydrophilicity (Chen and Wu 2005; Li and Loh 2015; Li et al. 2016b). In this context, Xue et al. (2018) carried out the most recent study where tachyplesin I (TPI) was associated to PHA granule linked protein (PGLP) followed by immobilization on [P(3HB-*co*-3HV)] co-polymer by hydrophobic linkage. In this investigation, TPI-PGLP covering resulted in the enhancement of surface hydrophilic property of co-polymeric [P(3HB-*co*-3HV)] toward increasing the fibroblast growth and development in vitro. Further, unmodified PHAs are not only devoid of chemical functionalities but also the polymers are often not found to be compatible when exploited with drugs. Though biologically decomposable,

unmodified PHAs with inherent hydrophobicity are very stable, therefore reducing their therapeutic applications in other areas. Hence, it is essential to enhance the hydrophilicity of PHAs so as to meet the demand for controlled drug delivery system and TE (Chen and Wu 2005; Li and Loh 2015; Li et al. 2016b).

The hydrophilicity of unmodified PHAs can be improved through surface or chemical modifications. Interestingly, chemical modifications developed as an effective strategy to improve the applicative range of PHAs as biomaterials through valuable incorporation of various functional groups, viz., carboxylic acid, hydroxyl groups, chlorination, epoxy, and double bonds into PHA polymer backbones (Raza et al. 2018). These chemical modifications accountable toward the fabrication of wide-range of new PHA polymers with improved material properties, which cannot be attained through biotechnological strategies (Kai and Loh 2014). For example, hydrophilicity of PHAs can be attained through the functionalization of unsaturated side chains to dihydroxyl, hydroxyl as well as carboxylic acid groups (Hazer and Steinbüchel 2007; Li and Loh 2015). However, chemical modification approaches are seldom antagonistic that cause decreased molecular weight of polymeric molecule and undesirable side reaction(s) including poisonous impurities. In some cases, a gentle surface alteration process is needed, lacking which the polymeric material become ineffective toward its promising uses. This is supported by the fact that neat polymeric material lacking appropriate alteration can lead to uncovering of adhesive bonds, poor attachment of cell, permanent staining of a fabric, or can affect protein membrane fouling, etc. Such reasons advocate the exploitation of surface modification techniques in order to enhance the hydrophilic property of biopolymeric PHAs. Surface modification approach is promising as well as efficient in order to enhance the hydrophilicity of PHAs. Various approaches together with grafting method (Ma et al. 2003), surface hydrolysis (Benavente and Vazquez 2004), ultraviolet (Shangguan et al. 2006) as well as plasma treatments (Ren et al. 2008) are exploited toward enhancing the hydrophilic property of polymers. Nevertheless, such approaches still depict several shortcomings. For instance, grafting approach cause the surface of substance extremely unsteady owing to extremely feeble association among bulk polymeric material and the biologically active substances (Ma et al. 2003). The straight ultraviolet light found to considerably decrease the mechanical characteristics of polymeric materials, thereby making them extremely fragile (Shangguan et al. 2006). Also, the treatment based on plasma approach can merely enhance the temporary surface hydrophilic property of PHAs, where momentarily after the treatment the surface have a tendency to return to previous unmodified condition (Ren et al. 2008). Treatment based upon alkali as well as lipase found to be extremely efficient for enhancing the wetting as well as surface interaction feature of polymeric material. It has been found that sodium hydroxide treatment lead to improvement in the surface hydrophilicity of PHAs along with their blood compatibility (Shen et al. 2010). Sodium hydroxide treatment also accountable for the improvement in the attachment of [P(3HB-*co*-3HHx)] polymer with the neural stem cells. However, lipase treatment was reported to be more efficient over sodium hydroxide for enhancing PHA biocompatibility as well as growth of L929 cells on PHA polymers (Yang et al. 2002). Overall, surface modifications temporally emerged as efficient approaches toward the enhancement of hydrophilicity of PHAs, thereby enabling potential biomedical uses as controlled drug delivery system and TE.

Crystallinity, mechanical properties, biocompatibility, and biodegradability issues

Most of the PHA polyesters are remarkably hydrophobic as well as depict brittle mechanical characteristics that hamper their uses as biomaterials. For example, PHAs should show more amphiphilic property instead of hydrophobicity for biomedical uses particularly drug delivery systems and tissue engineering (Yang et al. 2002). For instance, the SCL-PHAs together with PHB are found to be inflexible and stiff and thus limit their biomedical uses owing to higher content of crystallinity, resulting from the short carbon chain lengths (Zhao et al. 2007; Brzeska et al. 2014). Such crystalline nature of SCL-PHAs results in poor mechanical characteristics as well as biocompatibility. However, these issues can be resolved by PHB co-polymerization with PEG, which removed the undesirable rigidity as well as brittle property of PHB with significantly improved Young's modulus values of 21 MPa. In addition, the elongation to break values improved substantially up to 1912%. Such improvement is feasible as PHB depicts rigidity as well as hydrophobicity, whereas PEG exhibits flexibility together with hydrophilicity. The crystalline nature and water uptake capability of the co-polymer could be altered as a result of changing the length of PHB as well as PEG blocks so as to outfit the application prerequisites. The biologically decomposable amphiphilic PHB-PEG polymers also found to reveal good cell biocompatibility during investigations conducted on cytotoxicity (Loh et al. 2007). Moreover, the flexible nature of PHB can be further improved with little crystallinity through incorporation of long alkyl side chain like HB as well as HV that result in the production of [P(3HB-*co*-4HB)] and [P(3HB-*co*-3HV)] co-polymers, correspondingly.

The aforementioned limitations also give rise to new material class known as 'blends of PHAs." This new material class has evolved as important approach for overcoming the PHAs limitations toward biomedical applications by tuning their thermal and mechanical properties and is under intense research (Volova et al. 2014). The amalgamation of P(3HB) and PLA is comparatively most investigated blends with

mechanical properties, which are in between among the individual components. Zhao et al. (2013) studied the formation of P(3HB-*co*-3HV) and PLA blends in which the resulted blends depicted improved mechanical features. Zembouai et al. (2013) further established that the thermal stability of the P(3HB-*co*-3HV) and PLA blends might be enhanced through enhancing the PLA quantity.

In contradiction of SCL-PHAs, MCL-PHAs exhibited lower T_m, T_g, and level of crystalline nature. It was found that the physical characteristics of MCL-PHAs may be adjusted from semi-crystalline elastomers to amorphous sticky polymeric materials, where the T_g reduces with the increase in pendant chain length. Also, the MCL-PHAs having particular side chain lengths are reported to be valuable biopolymeric elastomers that exhibit lower tensile strength with higher elongation to break. However, additional increment in the side chain length of MCL-PHAs will result in stickier biopolymeric materials (Caon et al. 2014). Such limitations can be efficiently overcome as a result of investigations based on blending or co-polymerization with other substances. A few of these substances involve natural and non-natural rubbers including other polymeric materials. For example, MCL-PHA poly(3-hydroxyundecenoate) [P(3HU)] could be co-polymerized with PEG so as to adjust the mechanical characteristics of the resulted co-polymer, i.e., [P(3HU)-*co*-PEG] (Chung et al. 2003). Interestingly, the elongation to break as well as tensile strength was improved to utmost values of 606 and 263 MPa, correspondingly by enhancing the PEG amount in co-polymer. Similarly, in association with PEG, the biocompatible nature of [P(3HU)-*co*-PEG] was also enhanced considerably. Overall, SCL-PHAs as well as MCL-PHAs are valuable polymeric materials, which may be made-up into elastomers toward more superior uses together with medical field (Ye et al. 2018). The mechanical features of PHB may also be enhanced once PHB is mingled with [P(3HB-*co*-3HHx)] co-polymer. The resulting blend, i.e., [P(3HB-*co*-3HHx)]/PHB blend polymers revealed improved elongation to break value from 15 to 106% as soon as content of [P(3HB-*co*-3HHx)] co-polymer enhanced from 40 to 60% in the blend (Zhao et al. 2003a). The elevated crystallization content along with rapid rate of crystallization of PHB produces minute openings including protuberance on the surface of PHB polymer, thereby giving rise to coral-shaped surface that might inhibit the attachment as well as growth of mammalian cells. The occurrence of [P(3HB-*co*-3HHx)] content in PHB not only drastically diminished the crystallization content together with crystallization rate of [P(3HB-*co*-3HHx)]/PHB films but also depicted reasonably consistent as well as smooth surface that facilitated cellular attachment and growth, hence, significantly improved the biocompatible nature of PHB. As the content of [P(3HB-*co*-3HHx)] co-polymer enhanced from 0 to 100%, the surface of [P(3HB-*co*-3HHx)]/PHB blend polymers modified from a coralloid one into a regular, minute opening-free surface that further assisted toward the cellular attachment and enhancement of cell number. Apart from this, treatment of PHB with lipase was also capable to modify the coral-shaped surface to a continuous one. Interestingly, the cells cultivated with lipase-treated PHB polymer enhanced 40-fold in number over untreated PHB film. In three-dimensional permeable scaffolds, the nutrient as well as waste transportation capability turns out to be very important. Hence, the scaffold composed of [P(3HB-*co*-3HHx)] and PHB blend polymers in the ratio of 2:1 respectively exhibited improved biocompatibility over merely [P(3HB-*co*-3HHx)] co-polymer. This study also revealed the viability of exploiting [P(3HB-*co*-3HHx)]/PHB blend polymers for scaffolds as matrix toward TE (Zhao et al. 2003b). Although many investigations were carried out concerning the role of PHAs on cell multiplication as well as biocompatibility, presently no obvious proof is available regarding complete non-carcinogenicity of each and every cell line (Ali and Jamil 2016). Therefore, further intensifying research is needed on every single cell line in order to arrive at a particular conclusion regarding the carcinogenicity/non-carcinogenicity impact of PHAs.

The biocompatible nature of PHAs like PHB, [P(3HB-*co*-3HHx)], or [P(3HB-*co*-3HHx)]/PHB blends was assessed in vitro employing mouse fibroblast cell line L929 (MFCLL929). Remarkably, the proliferation of L929 cells was not good on PHB and PLA polymers. However, this limitation was overcome by producing PHB and [P(3HB-*co*-3HHx)] blend films, where substantial enhancement in the proliferation of cells L929 was observed, but the degree of this improvement depends upon the quantity of [P(3HB-*co*-3HHx)] in the blend films. For instance, the surface characteristics of [P(3HB-*co*-3HHx)] co-polymer modified consequently with the increment in the quantity of 3HHx monomeric units. The occurrence of 20% HHx monomers in co-polymer resulted in smoothest surface, whereas the surface of PHB polymer depicted highest hydrophilicity among the assessed PHB as well as all the co-polymeric [P(3HB-*co*-3HHx)]. All co-polymeric [P(3HB-*co*-3HHx)] also exhibited high protein molecule affinity as well as biocompatible property (Wang et al. 2005). Furthermore, the biocompatible properties of these polymeric materials were also enhanced significantly by treatment of lipases and sodium hydroxide, correspondingly. Interestingly, the biocompatible characteristics of PLA and treated PHB polymers were found to be almost similar, whereas [P(3HB-*co*-3HHx)] co-polymers along with their dominant blends displayed increased biocompatible properties over PLA (Yang et al. 2002; Zhao et al. 2002). On the other hand, Wang et al. (2003) carried out an investigation on PHB as well as co-polymeric [P(3HB-*co*-3HHx)] three-dimensional scaffolds using MFCLL929, where it was established that surfaces of PHB as well as co-polymeric [P(3HB-*co*-3HHx)] undergo enhancement in hydrophilic characteristics once exposed to lipase treatment. There was

almost 2-fold enhancement in the growth of L929 cells on these lipase-treated scaffolds over control. Nevertheless, as compared with control, there was 40% reduction in the cell proliferation on PHA scaffolds covered with hyaluronan. In fact, hyaluronan covering resulted in decreased cell attachment as well as growth on PHAs even though the scaffolds associated with improved hydrophilic properties. Overall, lipase treatment stimulated the cell proliferation on PHA scaffolds; however, such treatment failed to provide improved hydrophilic properties over hyaluronan covering. It seemed that a suitable amalgamation of hydrophilic as well as hydrophobic characteristics were crucial toward the biocompatible property of [P(3HB-*co*-3HHx)], particularly toward the multiplication/proliferation of L929 cells on the surface of this polymer (Wang et al. 2003).

In comparison with PLA and PLGA that have been approved by FDA as implant substances, the PHA polymers found to depict very little immunoreactions with generally very slow in vivo biodegradation (Qu et al. 2006). Thus, PHA polymeric materials alone are inappropriate toward uses demanding fast in vivo disintegration. Nevertheless, amalgamation of higher and smaller molecular mass PHAs might be helpful in maintaining a desirable mechanical strength toward fast biodegradation (Shangguan et al. 2006). Unquestionably, it is anticipated that either random or block co-polymerization or even amalgamation of PHA and PLA/PLGA might accelerate/improve the rate of PHAs disintegration in vitro as well as in vivo (Chen and Zhang 2017). In addition, biodegradability is also a crucial parameter toward the TE. The disintegration rates of PHAs must match with that of new tissue production. Though disintegration rates of PHB as well as [P(3HB-*co*-3HHx)] scaffolds could be regulated by adjusting the ratio of substances having diverse molecular masses, the decomposition of these scaffolds were reported to be always slower over the speediness of new tissue production (Young et al. 2002). Thus, further intensifying investigations must be carried out for developing PHA polymers, which support tissue regeneration along with suitable disintegration rate that matches with the tissue regeneration speediness.

Recent technological strategies for economical PHA production

The enhancing accessibility of raw renewable substances as well as intensifying demand and utilization of biodegradable polymeric materials toward biomedical, packaging uses, etc., together with favorable green procurement policies are anticipated to promote significantly the global growth of the PHA market. This is supported by the fact that the global PHA market is likely to attain US$93.5 million in 2021, from an appraised US$73.6 million in 2016 with compound annual growth rate of 4.88% (M and M 2018). The commercialization/industrialization of PHA polymeric materials are still struggling because price of the marketable PHAs is 15 to 17 times higher compared to traditional plastics as well as four to six times higher over marketable polylactic acid (Castilho et al. 2009; Singh et al. 2017; Chandel et al. 2018). However, metabolic engineering and improved fermentation environments with greater production volumes were capable to decrease the price in the range of US$2.25–2.75 lb^{-1} that was still three to four times higher over price (US$0.60–0.87 lb^{-1}) of conventional plastics like PP and PE (DiGregorio 2009; Plastics Technology 2017). Thus, PHAs still have a restricted market, despite their prospective to substitute 33% of marketable polymeric materials (Castilho et al. 2009). Thus, it is clear that the production price of PHAs is yet not decreased considerably in the current scenario despite the use of many strategies/approaches together with the exploitation of cost-effective substrates like cellulose, glycerol, waste materials, etc. (Li et al. 2017). Several parameters that influence the production price of biopolymeric PHA materials involve PHA productivity, content and yield, price of the organic carbon substrate as well as the use of recovery technique (Choi and Lee 1999). However, apart from these parameters, there is an urgent need to address other innovative technological strategies so as to minimize the overall production cost of PHAs, which are as follows:

PHAs co-production with other value-added products

Remarkably, PHAs co-productions along with other chemicals like succinate (Kang et al. 2011), exo-polysaccharide (Devi et al. 2012), hydrogen (Singh et al. 2013a, b), L-tryptophan (Gu et al. 2013), α-amylase (Sreekanth et al. 2013), L-arginine (Xu et al. 2016), etc., have been carried out to decrease the PHA production price. The viability of formation of intracellular PHAs together with extracellular products has been exhibited in which the economic co-production process can be further enhanced by optimization (Liang and Qi 2014).

In the aforementioned context, economical co-production of alginate along with PHAs in *Pseudomonas mediterranea* was carried out using glycerol as inexpensive substrate, where cell biomass boosted up to 2.89 g l^{-1} on dry cell weight (DCW) basis with 0.52 g l^{-1} of crude MCL-PHA at 48 h of growth period. However, the partly purified alginate attained the value of 6.93 g l^{-1}. Alginate found to depicts physical features that are appropriate for stabilizer as well as gelling agent in cosmetics and foods including medical fields (Licciardello et al. 2016). On the other hand, resolving the issue of crude glycerol disposal is a great challenge; therefore, effective strategies are required toward its re-utilization. Considering this issue, Bhattacharya et al. (2016) conducted research concerning co-production of amino acids along with PHAs successfully from crude glycerol. A co-production strategy was devised using *Bacillus licheniformis* PL26 toward extracellular production of 0.2 g l^{-1} Ɛ-polylysine with

intracellular [P(3HB-*co*-3HV)] co-polymer accumulation of 64.6% on DCW basis from *Jatropha* biodiesel waste substances. Ɛ-Polylysine is an essential biopolymeric material that has a different application together with anticancer agents, drug carriers, etc. This approach was found to efficiently transform the biodiesel waste substances into two value-added products that could be further subjected to optimization study for improved production. On the other hand, biosurfactants synthesized from renewable feedstocks failed to compete with the surfactants produced chemically. Thus, there is an urgent need to develop innovative approaches toward improving biosurfactants commercial competitiveness for substituting environmentally unfriendly surfactants produced chemically. In this context, Rashid et al. (2015) conducted a study using recombinant *Pseudomonas aeruginosa* for the co-production of rhamnolipids as well as PHB in which the rhamnolipids and PHB production reached up to 0.05 g l^{-1} and 24% on DCW, respectively. On the other hand, Li et al. (2016c) carried out research on the co-production of 5-aminolevulinic acid and PHB polymer successfully from *E. coli* as well as *Halomonas* TD01. The 5-aminolevulinic acid depicts various promising uses toward cancer therapy, growth regulation of plant, etc. (Liu et al. 2014). The co-production of extracellular 5-aminolevulinic acid and intracellular PHB using recombinant *E. coli* was carried out, where 5-aminolevulinic acid boosted up to 1.6 g l^{-1} and PHB content accumulated up to 43% on DCW basis. This strategy resulted in further decrease of production price through co-production of two value-added materials. The price of the fabrication strategy was further decreased through exploiting *Halomonas* sp. TD01 in which 5-aminolevulinic acid and PHB fabrication reached up to 0.6 g l^{-1} and 22% on DCW basis, respectively, under continuous conditions as well as eliminating the step of sterilization (Li et al. 2016c).

Design of appropriate bioreactor system

The fabrication of bacterial and cyanobacterial biomass and PHAs differs considerably and typically reliant on the cultivation environments of microorganisms. Analogous to bacterial system, there is a high feasibility of co-production of PHAs and protein including other value-added pigments, if cyanobacterial strains/species are cultivated in optimized environments. As a prerequisite, the factors are to be constant all over the cultivation period if otherwise manipulation is required and has to be modified accordingly. These information are very essential for developing appropriate photo-bioreactor systems, which facilitates cyanobacterial strains/species toward higher performance (Koller and Maršálek 2015). This type of systems must permit adequate mingling of the culture, entry and exit of gas, maintaining process parameters as well as optimum light together with light penetration. Photo-bioreactor systems must also be flexible as such flexibility will facilitate the cultivation of various types of cyanobacterial species/strains including other phototrophic organisms. Considering this background, photon system instrument photo-bioreactor having flat panel system is a type of cutting-edge cultivation system toward large-scale growth of photoautotrophic organisms together with microalgae and cyanobacteria (Czech Republic 2015). This photo-bioreactor is developed as flexible system with capability to undergo adaption as per need of cultivation process for specific purposes like products, strains/species, etc. Furthermore, cultivation can be conducted within 15–60 °C together with thermophilic organism like *Synechococcus* sp. MA19. In this case, mixing process is facilitated through the bubbles from the import of CO_2-supplemented air. Such flexible photo-bioreactors can offer a good solution toward the phototrophic cultivation process including algal and cyanobacterial strains/species for the production of value-added product of interest (Koller and Maršálek 2015).

PHA production using synthetic biology

Synthetic biology as tool can help in developing economical bioprocess system through engineering biocatalyst that have the possibility of being exploited at commercial scale, fabricating huge quantities of cost-effective PHAs. Industrial biotechnology needs non-pathogenic and rapid-growing microorganisms including bacteria/cyanobacteria, which are not only incapable to form toxins but also their genome undergo manipulation without any difficulty. Apart from this, it is considered advantageous concerning the exploitation of cellulosic material with rapid growth in a wide range of temperatures as well as pH (Wang et al. 2014). The struggle for diminishing PHA fabrication price primarily concentrates upon developing species/strains, which depict high PHA fabrication effectiveness using raw waste substance, need little energy utilization throughout PHA fabrication process, simplify downstream strategies as well as yield tailored functional polymeric materials toward high value-added uses. So as to attain higher PHA volumetric productivity, there is a requirement of higher cellular density up to 200 g l^{-1}, characterized through higher cellular PHA content, more than 90% g PHA/g DCW. Manipulation of genes linked to the uptake of oxygen, quorum sensing as well as PHA biosynthetic mechanisms can improve the PHA fabrication process (Wang et al. 2014). For instance, inadequacy of oxygen can arise after achieving higher cellular density so as to begin/stimulate PHB fabrication process. In a related research, anaerobic metabolic routes were introduced in *E. coli* that were found to overexpress hydrogenase 3 as well as acetyl-CoA synthatase in order to enable fabrication process of both hydrogen as well as PHB. In this strategy, the production of toxic substances like formate as well as acetate was circumvented through allowing carbon fluxes for PHB fabrication. This engineered organism displayed enhanced

hydrogen as well as PHB formation. Furthermore, PHB route optimization has been also studied in *E. coli* through regulating expression level of the three genes *phbC*, *phbA* as well as *phbB* (Li et al. 2016d). Cloning of phbCAB operon was carried out from the natural PHA fabricating bacterial strain *Cupriavidus necator*. Rational designed ribosomal binding sites (RBS) libraries were created based on high or low copy number plasmids in a one-pot reaction by an Oligo-Linker Mediated Assembly method (OLMA). Bacterial strains producing cellular contents of 0–92% g PHB/g DCW were developed and various molecular masses in the range of 2.7 to 6.8×10^6 was attained. The investigation established that this semirational strategy combining library design, construction as well as appropriate screening is an effective tool so as to improve PHB fabrication.

Synthetic biology is also implemented in case of halophilic bacteria that facilitate toward PHA fabrication in continuous mode as well as unsterile environments. These improve the cost-effective fabrication of PHAs at industrial scale. Moreover, halophilic bacterial strains/species were found to undergo genetic manipulation without any difficulty, therefore enabling toward the development of a hyperfabricating strain/species (Wang et al. 2014; Fu et al. 2014). For instance, both recombinant as well as wild-type *Halomonas campaniensis* LS21 were capable to depict growth on wide-ranging substrates, i.e., kitchen wastes, in the presence of sodium chloride (26.7 g l^{-1}), pH 10, temperature 37 °C with incubation period of 65 days lacking contamination. Recombinant *H. campaniensis* fabricated nearly 70% PHB on DCW basis over wild-type strain, where PHA accumulated up to 26% on DCW basis (Yue et al. 2014).

Production of PHAs within microbial cells requires various extraction as well as purification processes. Synthetic biology strategy has been devised so as to regulate as well as enable the liberation of PHA polymers into the medium. For instance, the programmed self-disruptive strain *Pseudomonas putida* BXHL has been developed, deriving from the prototype *Pseudomonas putida* KT2440 that is a famous fabricator of MCL-PHAs (Martínez et al. 2011). It was established that the engineered lytic system of *Pseudomonas putida* BXHL offered innovative strategy for stimulating regulated cell disruption in PHA fabricating environments. This investigation established a novel perception on engineered cells enabling PHA extraction in an eco-friendlier as well as cost-effective manner.

Conclusion and outlook

In conclusion, the growing awareness of caring the environment and the feasibility of exploiting biopolymeric PHAs for various commercial applications similar to non-biodegradable conventional plastics enable the PHAs as promising polymeric substances of the twenty-first century. In recent years, these PHA polymers have attracted great attention in research/commercial venture because of their potential wide-range applicability in biomedical field as biopolymeric biomaterials. The inherent biocompatibility, non-toxic degradation products, modifiable mechanical properties, desired surface modifications, cellular growth support, attachment without carcinogenic effects, capability to function as nanoparticles as well as manageable biodegradability are the unique properties associated with these biopolymeric PHAs, which allow them to be exploited as attractive biomaterials over other biopolyesters. PHAs are exploited for the development of biomaterials such as sutures, heart valves, nerve repair and regeneration, drug delivery systems, etc. A perusal of the literature revealed that relatively little research is presently being conducted to study the potential of PHAs as biomedical materials. Therefore, advancing and intensifying research work is a foremost compulsion to explore completely their use as biopolymeric biomaterials. Such advanced research certainly results in prolific utilization of PHA biopolymer as a unique and wonderful biomaterial in the near future. Furthermore, no medical standard is developed to date toward PHA medical uses. For exploitation of PHAs as biomedical materials, purification challenges will have to be considered because polymer with close to 100% purity is required. Constant dissolution followed by re-precipitation of polymer is suitable at laboratory scale; however, industrial processes must be framed, which are economical. There is also a need to increase investigation on animal trial of PHAs along with associated products to make them successful as biomedical materials. Presently, the potential uses of PHAs are limited by their high fabrication price. Various factors that affect the overall fabrication price of biopolymeric PHAs are PHA productivity, PHA content and yield, and price of the carbon substrate including the use of recovery technique, which need to be optimized. Among these factors, the PHA content is very important as it has multiple impacts on the PHA yield and recovery efficiency. Furthermore, there is an urgent need to focus on other innovative technological strategies like PHAs co-productions with other value-added products, design of appropriate bioreactor system, and synthetic biology so as to diminish the total fabrication price of PHAs. Formulations of an effective recovery technique that will differ for each bacterium/cyanobacterium used are also important to the total economics of PHA production. Such assemblies certainly help in decreasing the overall PHA production cost in the near future, which not only results in prolific substitution of non-degradable conventional plastics but also improves the potential for wide-range applicability of PHAs as biopolymeric biomaterials.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

Abdelwahab MA, El-Barbary AA, El-Said KS, El Naggar SA, ElKholy HM (2019) Evaluation of antibacterial and anticancer properties of poly(3-hydroxybutyrate) functionalized with different amino compounds. Int J Biol Macromol 122:793–805

Ali I, Jamil N (2016) Polyhydroxyalkanoates: current applications in the medical field. Front Biol 11:19–27

Alves LP, Teixeira CS, Tirapelle EF, Donatti L, Tadra-Sfeir MZ, Steffens MB, de Souza EM, de Oliveira PF, Chubatsu LS, Müller-Santos M (2016) Backup expression of the PhaP2 Phasin compensates for phaP1 deletion in *Herbaspirillum seropedicae*, maintaining fitness and PHB accumulation. Front Microbiol 7:739

Anjum A, Zuber M, Zia KM, Noreen A, Anjum MN, Tabasum S (2016) Microbial production of polyhydroxyalkanoates (PHAs) and its copolymers: a review of recent advancements. Int J Biol Macromol 89: 161–174

Ansari NF, Annuar MSM (2018) Functionalization of medium-chain-length poly (3-hydroxyalkanoates) as amphiphilic material by graft copolymerization with glycerol 1, 3-diglycerolate diacrylate and its mechanism. J Macromol Sci A 55:66–74

Basnett P, Ching KY, Stolz M, Knowles JC, Boccaccini AR, Smith C, Locke IC, Keshavarz T, Roy I (2013) Novel poly (3-hydroxyoctanoate)/poly (3-hydroxybutyrate) blends for medical applications. React Funct Polym 73:1340–1348

Benavente J, Vazquez MI (2004) Effect of age and chemical treatments on characteristic parameters for active and porous sublayers of polymeric composite membranes. J Colloid Interface Sci 273:547–555

Bhatia SK, Wadhwa P, Hong JW, Hong YG, Jeon JM, Lee ES, Yang YH (2019) Lipase mediated functionalization of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) with ascorbic acid into an antioxidant active biomaterial. Int J Biol Macromol 123:117–123

Bhattacharya S, Dubey S, Singh P, Shrivastava A, Mishra S (2016) Biodegradable polymeric substances produced by a marine bacterium from a surplus stream of the biodiesel industry. Bioengineering 3:34–44

Bissery MC, Valeriote F, Thies C (1985) Therapeutic efficacy of CCNU-loaded microspheres prepared from poly(D,L)lactide (PLA) or poly-b-hydroxybutyrate (PHB) against Lewis lung (LL) carcinoma. Proc Am Assoc Cancer Res 26:355–355

Bonthrone KM, Clauss J, Horowitz DM, Hunter BK, Sanders JKM (1992) The biological and physical chemistry of polyhydroxyalkanoates as seen by NMR spectroscopy. FEMS Microbiol Rev 10:269–278

Bowald SF, Johansson EG (1990) A novel surgical material. European Patent No. 0349505A2

Brandl H, Gross RA, Lenz RW, Fuller RC (1990) Plastics from bacteria and for bacteria: poly(β-hydroxyalkanoates) as natural, biocompatible, and biodegradable polyesters. Adv Biochem Eng Biotechnol 41:77–93

Brigham CJ, Sinskey AJ (2012) Applications of polyhydroxyalkanoates in the medical industry. Int J Biotechnol Wellness Ind 1:53–60

Brzeska J, Heimowska A, Janeczek H, Kowalczuk M, Rutkowska M (2014) Polyurethanes based on atactic poly[(*R,S*)-3-hydroxybutyrate]: preliminary degradation studies in simulated body fluids. J Polym Environ 22:176–182

Bugnicourt E, Cinelli P, Lazzeri A, Alvarez V (2014) Polyhydroxyalkanoate (PHA): review of synthesis, characteristics, processing and potential applications in packaging. Express Polym Lett 8:791–808

Byrom D (1987) Polymer synthesis by microorganisms: technology and economics. Trends Biotechnol 5:246–250

Cao Q, Zhang J, Liu H, Wu Q, Chen J, Chen GQ (2014) The mechanism of anti-osteoporosis effects of 3-hydroxybutyrate and derivatives under simulated microgravity. Biomaterials 35:8273–8283

Caon T, Berezina N, Lin CSK, Fakhouri FM, Martelli SM (2014) Polyhydroxyalkanoates and their potential in controlled-release drug delivery systems: biomedical applications and factors affecting the drug release. In: Linping W (ed) Polyhydroxyalkanoates (PHAs): biosynthesis, industrial production and applications in medicine. Nova Science Publishers, Inc., Hauppauge, p 219–236

Capulli AK, Emmert MY, Pasqualini FS, Kehl D, Caliskan E, Lind JU, Sheehy SP, Park SJ, Ahn S, Weber B, Goss JA, Hoerstrup SP, Parker KK (2017) JetValve: rapid manufacturing of biohybrid scaffolds for biomimetic heart valve replacement. Biomaterials 133:229–241

Carr NG (1966) The occurrence of poly-*β*-hydroxybutyrate in the blue-green alga, *Chlorogloea fritschii*. Biochem Biophys Acta 120:308–310

Castilho LR, Mitchell DA, Freire DMG (2009) Production of polyhydroxyalkanoates (PHAs) from waste materials and by-products by submerged and solid-state fermentation. Bioresour Technol 100:5996–6009

Chandel AK, Garlapati VK, Singh AK, Antunes FAF, da Silva SS (2018) The path forward for lignocellulose biorefineries: bottlenecks, solutions, and perspective on commercialization. Bioresour Technol 264:370–381

Chaput C, Yahia LH, Selmani A, Rivard C-H (1995) Natural poly(hydroxybutyrate-hydroxyvalerate) polymers as degradable biomaterials. Mat Res Soc Symp Proc 385:49–54

Chen W, Tong YW (2012) PHBV microspheres as neural tissue engineering scaffold support neuronal cell growth and axon–dendrite polarization. Acta Biomater 8:540–548

Chen G-Q, Wu Q (2005) The application of polyhydroxyalkanoates as tissue engineering materials. Biomaterials 26:6565–6578

Chen G-Q, Zhang J (2017) Microbial polyhydroxyalkanoates as medical implant biomaterials. Artif Cells Nanomed Biotechnol 46:1–18

Chen Y, Tsai Y, Chou IN, Tseng SH, Wu HS (2014) Application of biodegradable polyhydroxyalkanoates as surgical films for ventral hernia repair in mice. Int J Polym Sci 2014:789681

Choi J, Lee SY (1999) Factors affecting the economics of polyhydroxyalkanoate production by bacterial fermentation. Appl Microbiol Biotechnol 51:13–21

Chuah JA, Yamada M, Taguchi S, Sudesh K, Doi Y, Numata K (2013) Biosynthesis and characterization of polyhydroxyalkanoate containing 5-hydroxyvalerate units: effects of 5HV units on biodegradability, cytotoxicity, mechanical and thermal properties. Polym Degrad Stab 98:331–338

Chung CW, Kim HW, Kim YB, Rhee YH (2003) Poly(ethylene glycol)-grafted poly(3-hydroxyundecenoate) networks for enhanced blood compatibility. Int J Biol Macromol 32:17–22

Czech Republic (2015) http://www.psi.cz/products/photobioreactors. Accessed 14 Dec 2018

Del Gaudio C, Fioravanzo L, Folin M, Marchi F, Ercolani E, Bianco A (2012) Electrospun tubular scaffolds: on the effectiveness of blending poly(e-caprolactone) with poly(3-hydroxybutyrate-co-3-hydroxyvalerate). J Biomed Mater Res B Appl Biomater 100: 1883–1898

Devi ES, Vijayendra SVN, Shamala TR (2012) Exploration of rice bran, an agro-industry residue, for the production of intra-and extra-cellular polymers by *Sinorhizobium meliloti* MTCC 100. Biocatal Agric Biotechnol 1:80–84

DiGregorio BE (2009) Biobased performance bioplastic: Mirel. Chem Biol 16:1–2

Dinjaski N, Fernández-Gutiérrez M, Selvam S, Parra-Ruiz FJ, Lehman SM, San Román J, García E, García JL, García AJ, Prieto MA (2014) PHACOS, a functionalized bacterial polyester with bactericidal activity against methicillin-resistant *Staphylococcus aureus*. Biomaterials 35:14–24

Dumaz N, Drougard C, Sarasin A, Daya-Grosjean L (1993) Specific UV-induced mutation spectrum in the p53 gene of skin tumors from DNA-repair-deficient xeroderma pigmentosum patients. Proc Natl Acad Sci U S A 90:10529–10533

Durai P, Batool M, Choi S (2015) Structure and effects of cyanobacterial lipopolysaccharides. Mar Drugs 13:4217–4230

Emmert MY, Weber B, Behr L, Sammut S, Frauenfelder T, Wolint P, Scherman J, Bettex D, Grünenfelder J, Falk V, Hoerstrup SP (2014) Transcatheter aortic valve implantation using anatomically oriented, marrow stromal cell-based, stented, tissue-engineered heart valves: technical considerations and implications for translational cell-based heart valve concepts. Eur J Cardiothorac Surg 45:61–68

Fava F, Totaro G, Gavrilescu M (2015) Material & energy recovery and sustainable development, ECOMONDO 2014. Environ Eng Manag J 14:1475–1471

Fu X-Z, Tan D, Aibaidula G, Wu Q, Chen J-C, Chen G-Q (2014) Development of *Halomonas* TD01 as a host for open production of chemicals. Metab Eng 23:78–91

Furrer P, Panke S, Zinn M (2007) Efficient recovery of low endotoxin medium-chain-length poly([R]-3-hydroxyalkanoate) from bacterial biomass. J Microbiol Methods 69:206–213

Furrer P, Zinn M, Panke S (2008) Polyhydroxyalkanoate and its potential for biomedical applications. In: Reis RL, Neves NM, Mano JF, Gomes ME, Marques AP, Azevedo HS (eds) Natural-based polymers for biomedical applications. Elsevier, Amsterdam, pp 416–445

Gardel M, Schwarz U (2010) Cell–substrate interactions. J Phys Condens Matter 22:190301

Geiger B, Spatz JP, Bershadsky AD (2009) Environmental sensing through focal adhesions. Nat Rev Mol Cell Biol 10:21–33

Gould PL, Holland SJ, Tighe BJ (1987) Polymers for biodegradable medical devices. IV. Hydroxybutyrate-valerate copolymers as non-disintegrating matrices for controlled release oral dosage forms. Int J Pharm 38:231–237

Gu P, Kang J, Yang F, Wang Q, Liang Q, Qi Q (2013) The improved L-tryptophan production in recombinant *Escherichia coli* by expressing the polyhydroxybutyrate synthesis pathway. Appl Microbiol Biotechnol 97:4121–4127

Gursel I, Korkusuz F, Turesin F, Gurdal Alaeddinoglu N, Hasirci V (2001) *In vivo* application of biodegradable controlled antibiotic release systems for the treatment of implant-related osteomyelitis. Biomaterials 22:73–80

Hazari A, Johansson-Ruden G, Bostrom KJ, Ljungberg C, Terenghi G, Green C, Wiberg M (1999a) A new resorbable wrap around implant as an alternative nerve repair technique. J Hand Surg Br 24B:291–295

Hazari A, Wiberg M, Johansson-Ruden G, Green C, Terenghi G (1999b) A resorbable nerve conduit as an alternative to nerve autograft in nerve gap repair. Br J Plast Surg 52:653–657

Hazer B, Steinbüchel A (2007) Increased diversification of polyhydroxyalkanoates by modification reactions for industrial and medical applications. Appl Microbiol Biotechnol 74:1–12

Hazer DB, Kiliçay E, Hazer B (2012) Poly(3-hydroxyalkanoate)s: diversification and biomedical applications: a state of the art review. Mater Sci Eng C 32:637–647

He Y, Hu Z, Ren M, Ding C, Chen P, Gu Q, Wu Q (2014) Evaluation of PHBHHx and PHBV/PLA fibers used as medical sutures. J Mater Sci Mater Med 25:561–571

Hollstein M, Sidransky D, Vogelstein B, Harris C (1991) p53 mutations in human cancers. Science 253:49–53

Hu YJ, Wei X, Zhao W, Liu YS, Chen GQ (2009) Biocompatibility of poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyhexanoate) with bone marrow mesenchymal stem cells. Acta Biomater 5:1115–1125

Inan K, Sal FA, Rahman A, Putman RJ, Agblevor FA, Miller CD (2016) Microbubble assisted polyhydroxybutyrate production in *Escherichia coli*. BMC Res Notes 9:338

Insomphun C, Chuah JA, Kobayashi S, Fujiki T, Numata K (2017) Influence of hydroxyl groups on the cell viability of polyhydroxyalkanoate (PHA) scaffolds for tissue engineering. ACS Biomater Sci Eng 3:3064–3075

Janousek P, Kabelka Z, Rygl M, Lesný P, Grabec P, Fajstavr J, Jurovcík M, Snajdauf J (2006) Corrosive injury of the oesophagus in children. Int J Pediatr Otorhinolaryngol 70:1103–1107

Jendrossek D, Pfeiffer D (2014) New insights in the formation of polyhydroxyalkanoate granules (carbonosomes) and novel functions of poly(3-hydroxybutyrate). Environ Microbiol 16:2357–2373

Ji Y, Li XT, Chen GQ (2008) Interactions between a poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyhexanoate) terpolyester and human keratinocytes. Biomaterials 29:3807–3814

Jiang X, Ramsay JA, Ramsay BA (2006) Acetone extraction of mcl-PHA from *Pseudomonas putida* KT2440. J Microbiol Methods 67:212–219

Kai D, Loh XJ (2014) Polyhydroxyalkanoates: chemical modifications toward biomedical applications. ACS Sustain Chem Eng 2:106–119

Kang Z, Du L, Kang J, Wang Y, Wang Q, Liang Q, Qi Q (2011) Production of succinate and polyhydroxyalkanoate from substrate mixture by metabolically engineered *Escherichia coli*. Bioresour Technol 102:6600–6604

Koller M, Maršálek L (2015) Cyanobacterial polyhydroxyalkanoate production: status quo and quo Vadis? Curr Biotechnol 4:464–480

Kumar A, Srivastava JK, Mallick N, Singh AK (2015) Commercialization of bacterial cell factories for the sustainable production of polyhydroxyalkanoate thermoplastics: progress and prospects. Recent Pat Biotechnol 9:4–21

Kuroda K, Caputo GA (2013) Antimicrobial polymers as synthetic mimics of host-defense peptides. Interdiscip Rev Nanomed Nanobiotechnol 5:49–66

Leal-Egaña, Díaz-Cuenca A, Boccaccini AR (2013) Tuning of cell-biomaterial anchorage for tissue regeneration. Adv Mater 25:4049–4057

Lee SY, Choi JI, Han K, Song JY (1999) Removal of endotoxin during purification of poly(3–hydroxybutyrate) from gram-negative bacteria. Appl Environ Microbiol 65:2762–2764

Lee J, Jung SG, Park CS, Kim HY, Batt CA, Kim YR (2011) Tumor-specific hybrid polyhydroxybutyrate nanoparticle: surface modification of nanoparticle by enzymatically synthesized functional block copolymer. Bioorg Med Chem Lett 21:2941–2944

Lenz RW, Marchessault RH (2005) Bacterial polyesters: biosynthesis, biodegradable plastics and biotechnology. Biomacromolecules 6:1–8

Levine AC, Sparano A, Twigg FF, Numata K, Nomura CT (2015) Influence of cross-linking on the physical properties and cytotoxicity of polyhydroxyalkanoate (PHA) scaffolds for tissue engineering. ACS Biomater Sci Eng 1:567–576

Li Z, Loh XJ (2015) Water soluble polyhydroxyalkanoates: future materials for therapeutic applications. Chem Soc Rev 44:2865–2879

Li J, Yun H, Gong Y, Zhao N, Zhang X (2005) Effects of surface modification of poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) on physicochemical properties and on interactions with MC3T3-E1 cells. J Biomed Mater Res A 75:985–998

Li X, Chang H, Luo H, Wang Z, Zheng G, Lu X, He X, Chen F, Wang T, Liang J, Xu M (2015) Poly (3-hydroxybutyrate-*co*-3-hydroxyhexanoate) scaffolds coated with PhaP-RGD fusion protein promotes the proliferation and chondrogenic differentiation of human umbilical cord mesenchymal stem cells *in vitro*. J Biomed Mater Res A 103:1169–1175

Li X, Li X, Chen D, Guo J-L, Feng D-F, Sun M-Z, Lu Y, Chen D-Y, Zhao X, Feng X-Z (2016a) Evaluating the biological impact of polyhydroxyalkanoates (PHAs) on developmental and exploratory profile of zebrafish larvae. RSC Adv 6:37018–37030

Li Z, Yang J, Loh XJ (2016b) Polyhydroxyalkanoates: opening doors for a sustainable future. NPG Asia Mater 8:e265

Li T, Guo YY, Qiao GQ, Chen GQ (2016c) Microbial synthesis of 5-aminolevulinic acid and its coproduction with polyhydroxybutyrate. ACS Synth Biol 5:1264–1274

Li T, Ye J, Shen R, Zong Y, Zhao X, Lou C, Chen G-Q (2016d) Semirational approach for ultrahigh poly(3-hydroxybutyrate) accumulation in *Escherichia coli* by combining one-step library construction and high-throughput screening. ACS Synth Biol 5:1308–1317

Li T, Elhadi D, Chen GQ (2017) Co-production of microbial polyhydroxyalkanoates with other chemicals. Metab Eng 43:29–36

Liang Q, Qi Q (2014) From a co-production design to an integrated single-cell biorefinery. Biotechnol Adv 32:1328–1335

Licciardello G, Ferraro R, Russo M, Strozzi F, Catara AF, Bella P, Catara V (2016) Transcriptome analysis of *Pseudomonas mediterranea* and *P. corrugata* plant pathogens during accumulation of medium-chain-length PHAs by glycerol bioconversion. New Biotechnol 37:39–47

Liu S, Zhang G, Li X, Zhang J (2014) Microbial production and applications of 5-aminolevulinic acid. Appl Microbiol Biotechnol 98:7349–7357

Lizarraga-Valderrama LR, Nigmatullin R, Taylor C, Haycock JW, Claeyssens F, Knowles JC, Roy I (2015) Nerve tissue engineering using blends of poly (3-hydroxyalkanoates) for peripheral nerve regeneration. Eng Life Sci 15:612–621

Ljungberg C, Johansson RG, Bostrom KJ, Novikov L, Weiberg M (1999) Neuronal survival using a resorbable synthetic conduit as an alternative to primary nerve repair. Microsurgery 19:250–264

Löbler M, Saß M, Schmitz KP, Hopt UT (2002) Biomaterial implants induce the inflammation marker CRP at the site of implantation. J Biomed Mater Res 61:165–167

Loh XJ, Wang X, Li H, Li X, Li J (2007) Compositional study and cytotoxicity of biodegradable poly(ester urethane)s consisting of poly[(R)-3-Hydroxybutyrate] and poly(ethylene glycol). Mater Sci Eng C 27:267–273

Lomas AJ, Webb WR, Han J, Chen GQ, Sun X, Zhang Z, El Haj AJ, Forsyth NR (2013) Poly (3-hydroxybutyrate-*co*-3-hydroxyhexanoate)/collagen hybrid scaffolds for tissue engineering applications. Tissue Eng Part C Methods 19:577–585

Lu X-Y, Ciraolo E, Stefenia R, Chen G-Q, Zhang Y, Hirsch E (2011) Sustained release of PI3K inhibitor from PHA nanoparticles and in vitro growth inhibition of cancer cell lines. Appl Microbiol Biotechnol 89:1423–1433

Lu X, Wang L, Yang Z, Lu H (2013) Strategies of polyhydroxyalkanoates modification for the medical application in neural regeneration/nerve tissue engineering. Adv Biosci Biotechnol 4:731–740

Lu HX, Yang ZQ, Jiao Q, Wang YY, Wang L, Yang PB, Chen XL, Zhang PB, Wang P, Chen MX, Lu XY, Liu Y (2014) Low concentration of serum helps to maintain the characteristics of NSCs/NPCs on alkali-treated PHBHHx film *in vitro*. Neurol Res 36:207–214

M & M (2018) Markets and Markets Polyhydroxyalkanoate (PHA) Market by Type (Monomers, Co-Polymers, Terpolymers), Manufacturing Technology (Bacterial Fermentation, Biosynthesis, Enzymatic Catalysis), Application (Packaging, Bio Medical, Food Services, Agriculture)—Global Forecast to 2021 (Available online: http://www.marketsandmarkets.com/Market-Reports/pha-market-395.html (accessed on 10 December, 2018)

Ma Z, Gao C, Gong Y, Shen J (2003) Chondrocyte behaviors on poly-L-lactic acid (PLLA) membranes containing hydroxyl, amide or carboxyl groups. Biomaterials 24:3725–3730

Mallick N, Sharma L, Singh AK (2007) Poly-β-hydroxybutyrate accumulation in *Nostoc muscorum*: effects of metabolic inhibitors. J Plant Physiol 164:312–317

Martínez V, García P, García JL, Prieto MA (2011) Controlled autolysis facilitates the polyhydroxyalkanoate recovery in *Pseudomonas putida* KT2440. Microb Biotechnol 4:533–547

Masood F, Chen P, Yasin T, Fatima N, Hasan F, Hameed A (2013) Encapsulation of ellipticine in poly-(3-hydroxybutyrate-co-3-hydroxyvalerate) based nanoparticles and its *in vitro* application. Mater Sci Eng C Mater 33:1054–1060

Masood F, Yasin T, Hameed A (2015) Polyhydroxyalkanoates—what are the uses? Current challenges and perspectives. Crit Rev Biotechnol 35:514–521

Mergaert J, Anderson C, Wouters A, Swings J, Kersters K (1992) Biodegradation of polyhydroxyalkanoates. FEMS Microbiol Rev 10:317–322

Montazeri M, Karbasi S, Foroughi M, Monshi A, Ebrahimi-Kahrizsangi R (2015) Evaluation of mechanical property and bioactivity of nanobioglass 45S5 scaffold coated with poly-3-hydroxybutyrate. J Mater Sci Mater Med 26:62

Nair LS, Laurencin CT (2006) Polymers as biomaterials for tissue engineering and controlled drug delivery. Adv Biochem Eng Biotechnol 102:47–90

Nigmatullin R, Thomas P, Lukasiewicz B, Puthussery H, Roy I (2015) Polyhydroxyalkanoates, a family of natural polymers, and their applications in drug delivery. J Chem Technol Biotechnol 90:1209–1221

Noble JR, Zhong ZH, Neumann AA, Melki JR, Clark SJ, Reddel RR (2004) Alterations in the p16INK4a and p53 tumor suppressor genes of hTERT-immortalized human fibroblasts. Oncogene 23:3116–3121

Obruca S, Sedlacek P, Koller M, Kucera D, Pernicova I (2018) Involvement of polyhydroxyalkanoates in stress resistance of microbial cells: biotechnological consequences and applications. Biotechnol Adv 36:856–870

Opitz F, Schenke-Layland K, Richter W, Martin DP, Degenkolbe I, Wahlers T, Stock UA (2004) Tissue engineering of ovine aortic blood vessel substitutes using applied shear stress and enzymatically derived vascular smooth muscle cells. Ann Biomed Eng 32:212–222

Pelham RJ Jr, Wang Y (1997) Cell locomotion and focal adhesions are regulated by substrate flexibility. Proc Natl Acad Sci USA 94:13661–13665

Peng SW, Guo XY, Shang GG, Li J, Xu XY, You ML, Li P, Chen GQ (2011) An assessment of the risks of carcinogenicity associated with polyhydroxyalkanoates through an analysis of DNA aneuploid and telomerase activity. Biomaterials 32:2546–2555

Peptu C, Kowalczuk M (2018) Biomass-derived polyhydroxyalkanoates: biomedical applications. In: Popa V, Volf I (eds) Biomass as renewable raw material to obtain bioproducts of high-tech value. Elsevier, Peptu, p 271–313

Plastics Technology (2017) http://www.ptonline.com/articles/prices-bottom-out-forpolyolefins-pet-ps-pvc-move-up (accessed on 10 December, 2018)

Pramanik N, Bhattacharya S, Rath T, De J, Adhikary A, Basu RK, Kundu PP (2019) Polyhydroxybutyrate-co-hydroxyvalerate copolymer modified graphite oxide based 3D scaffold for tissue engineering application. Mater Sci Eng C 94:534–546

Pramual S, Assavanig A, Bergkvist M, Batt CA, Sunintaboon P, Lirdprapamongkol K, Svasti J, Niamsiri N (2016) Development and characterization of bio-derived polyhydroxyalkanoate

nanoparticles as a delivery system for hydrophobic photodynamic therapy agents. J Mater Sci Mater Med 27:40

Qu XH, Wu Q, Liang J, Qu X, Wang SG, Chen GQ (2005) Enhanced vascular-related cellular affinity on surface modified copolyesters of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx). Biomaterials 26:6991–7001

Qu XH, Wu Q, Zhang KY, Chen GQ (2006) *In vivo* studies of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) based polymers: biodegradation and tissue reactions. Biomaterials 27:3540–3548

Rai R, Yunos DM, Boccaccini AR, Knowles JC, Barker IA, Howdle SM, Tredwell GD, Keshavarz T, Roy I (2011) Poly-3hydroxyoctanoate P(3HO): a medium chain length polyhydroxyalkanoate homopolymer from *Pseudomonas mendocina*. Biomacromolecules 12:2126–2136

Rajaratanam DD, Ariffin H, Hassan MA, Nik Abd Rahman NMA, Nishida H (2018) *In vitro* cytotoxicity of superheated steam hydrolyzed oligo((*R*)-3-hydroxybutyrate-*co*-(*R*)-3-hydroxyhexanoate) and characteristics of its blend with poly(L-lactic acid) for biomaterial applications. PLoS ONE 13:e0199742

Rashid NFM, Azemi MAFM, Amiru AAA, Wahid MEA, Bhubalan K (2015) Simultaneous production of biopolymer and biosurfactant by genetically modified *Pseudomonas aeruginosa* UMTKB-5. Conference: international proceedings of chemical, biological and environmental engineering, Auckland, New Zealand, 90:3–8

Rathbone S, Furrer P, Lübben J, Zinn M, Cartmell SJ (2010) Biocompatibility of polyhydroxyalkanoate as a potential material for ligament and tendon scaffold material. J Biomed Mater Res A 93:1391–1403

Raza ZA, Riaz S, Banat IM (2018) Polyhydroxyalkanoates: properties and chemical modification approaches for their functionalization. Biotechnol Prog 34:29–41

Ren Y, Wang C, Qiu Y (2008) Aging of surface properties of ultra high modulus polyethylene fibers treated with He/O atmospheric pressure plasma jet. Surf Coat Technol 202:2670–2676

Ren Q, Ruth K, Thöny-Meyer L, Zinn M (2010) Enatiomerically pure hydroxycarboxylic acids: current approaches and future perspectives. Appl Microbiol Biotechnol 87:41–52

Ricotti L, Polini A, Genchi GG, Ciofani G, Iandolo D, Vazão H, Mattoli V, Ferreira L, Menciassi A, Pisignano D (2012) Proliferation and skeletal myotube formation capability of C2C12 and H9c2 cells on isotropic and anisotropic electrospun nanofibrous PHB scaffolds. Biomed Mater 7:035010

Saito T, Tomita K, Juni K, Ooba K (1991) *In vivo* and *in vitro* degradation of poly(3-hydroxybutyrate) in rat. Biomaterials 12:309–312

Samantaray S, Mallick N (2014) Production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) co-polymer by the diazotrophic cyanobacterium *Aulosira fertilissima* CCC 444. J Appl Phycol 26:237–245

Samantaray S, Mallick N (2015) Impact of various stress conditions on poly-β-hydroxybutyrate (PHB) accumulation in *Aulosira fertilissima* CCC 444. Curr Biotechnol 4:366–372

Sankhla SS, Bhati R, Singh AK, Mallick N (2010) Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer production from a local isolate, Brevibacillus invocatus MTCC 9039. Biores Technol 101:1947–1953

Schmidt CE, Leach JB (2003) Neural tissue engineering: strategies for repair and regeneration. Ann Rev Biomed Eng 5:293–347

Sevastianov VI, Perova NV, Shishatskaya EI, Kalacheva GS, Volova TG (2003) Production of purified polyhydroxyalkanoates (PHAs) for applications in contact with blood. J Biomater Sci Polym Ed 14:1029–1042

Shah M, Ullah N, Choi MH, Kim MO, Yoon SC (2012) Amorphous amphiphilic P(3HV-*co*-4HB)-*b*-mPEG block copolymer synthesized from bacterial copolyester via melt transesterification: nanoparticle preparation, cisplatin loading for cancer therapy and *in vitro* evaluation. Eur J Pharm Biopharm 80:518–527

Shah AA, Kato S, Shintani N, Kamini NR, Nakajima-Kambe T (2014) Microbial degradation of aliphatic and aliphatic-aromatic co-polyesters. Appl Microbiol Biotechnol 98:3437–3447

Shangguan YY, Wang YW, Wu Q, Chen GQ (2006) The mechanical properties and *in vitro* biodegradation and biocompatibility of UV-treated poly(3-hydroxybutyrate-co-3-hydroxyhexanoate). Biomaterials 27:2349–2357

Sharma L, Panda B, Singh AK, Mallick N (2006) Studies on poly-β-hydroxybutyrate synthase activity of *Nostoc muscorum*. J Gen Appl Microbiol 52:209–214

Sharma L, Singh AK, Panda B, Mallick N (2007) Process optimization for poly-β-hydroxybutyrate production in a nitrogen fixing cyanobacterium, *Nostoc muscorum* using response surface methodology. Bioresour Technol 98:987–993

Sharma L, Srivastava JK, Singh AK (2016) Biodegradable polyhydroxyalkanoate thermoplastics substituting xenobiotic plastics: a way forward for sustainable environment. In: Singh A, Prasad SM, Singh RP (eds) Plant responses to xenobiotics. Springer-Verlag, New York, pp 317–346

Shen F, Zhang E, Wei Z (2010) *In vitro* blood compatibility of poly (hydroxybutyrate-co-hydroxyhexanoate) and the influence of surface modification by alkali treatment. Mater Sci Eng C 30:369–375

Shishatskaya EI, Volova TG, Gitelson II (2002) *In vivo* toxicological evaluation of polyhydroxyalkanoates. Dokl Biol Sci 383:109–111

Shishatskaya EI, Volova TG, Puzyr AP, Mogilnaya OA, Efremov SN (2004) Tissue response to the implantation of biodegradable polyhydroxyalkanoate sutures. J Mater Sci Mater Med 15:719–728

Shishatskaya EI, Volova TG, Gordeev SA, Puzyr AP (2005) Degradation of P(3HB) and P(3HB-*co*-3HV) in biological media. J Biomater Sci Polymer Edn 16:643–657

Shrivastav A, Kim HY, Kim YR (2013) Advances in the applications of polyhydroxyalkanoate nanoparticles for novel drug delivery system. Biomed Res Int 2013:581684

Singh AK, Mallick N (2008) Enhanced production of SCL-LCL-PHA co-polymer by sludge-isolated *Pseudomonas aeruginosa* MTCC 7925. Lett Appl Microbiol 46:350–357

Singh AK, Mallick N (2009a) Exploitation of inexpensive substrates for production of a novel SCL-LCL-PHA co-polymer by *Pseudomonas aeruginosa* MTCC 7925. J Ind Microbiol Biotechnol 36:347–354

Singh AK, Mallick N (2009b) SCL-LCL-PHA copolymer production by a local isolate, *Pseudomonas aeruginosa* MTCC 7925. Biotechnol J 4:703–711

Singh AK, Mallick N (2016) Biological system as reactor for production of biodegradable thermoplastics, polyhydroxyalkanoates. In: Thangadurai D, Sangeetha J (eds) Industrial biotechnology: sustainable production and bioresource utilization. CRC Press Taylor and Francis, USA, pp 281–323

Singh AK, Mallick N (2017) Advances in cyanobacterial polyhydroxyalkanoates production. FEMS Microbiol Lett 364(20). https://doi.org/10.1093/femsle/fnx189

Singh AK, Bhati R, Samantaray S, Mallick N (2013a) *Pseudomonas aeruginosa* MTCC 7925: producer of a novel SCL-LCL-PHA co-polymer. Curr Biotechnol 2:81–88

Singh M, Kumar P, Patel SKS, Kalia VC (2013b) Production of polyhydroxyalkanoate co-polymer by *Bacillus thuringiensis*. Indian J Microbiol 53:77–83

Singh AK, Ranjana B, Mallick N (2015) *Pseudomonas aeruginosa* MTCC 7925 as a biofactory for production of the novel SCL-LCL-PHA thermoplastic from non-edible oils. Curr Botechnol 4:65–74

Singh AK, Sharma L, Mallick N, Mala J (2017) Progress and challenges in producing polyhydroxyalkanoate biopolymers from cyanobacteria. J Appl Phycol 29:1213–1232

Singh AK, Sharma L, Srivastava JK, Mallick N, Ansari MI (2018) Microbially originated polyhydroxyalkanoate (PHA) biopolymers: an insight into the molecular mechanism and biogenesis of PHA

granules. In: Singh OV, Chandel AK (eds) Sustainable biotechnology-enzymatic resources of renewable energy. Springer, Cham, pp 355–398

Sodian R, Hoerstrup SP, Sperling JS, Daebritz S, Martin DP, Moran AM, Kim BS, Schoen FJ, Vacanti JP, Mayer JE Jr (2000) Early *in vivo* experience with tissue-engineered trileaflet heart valves. Circulation 102:III22–III29

Sreekanth MS, Vijayendra SV, Joshi GJ, Shamala TR (2013) Effect of carbon and nitrogen sources on simultaneous production of a-amylase and green food packaging polymer by *Bacillus* sp. CFR 67. J Food Sci Technol 50:404–408

Steinbüchel A (1991) Polyhydroxyalkanoic acids. In: Byrom D (ed) Biomaterials: novel materials from biological sources. Stockton, New York, pp 124–213

Stock UA, Degenkolbe I, Attmann T, Schenke-Layland K, Freitag S, Lutter G (2006) Prevention of device-related tissue damage during percutaneous deployment of tissue-engineered heart valves. J Thorac Cardiovasc Surg 131:1323–1330

Tang S, Ai Y, Dong Z, Yang Q (1999) Tissue response to subcutaneous implanting poly-3-hydroxybutyrate in rats. Disi Junyi Daxue Xuebao 20:87–89

Tezcaner A, Bugra K, Hasirci V (2003) Retinal pigment epithelium cell culture on surface modified poly(hydroxybutyrate-*co*-hydroxyvalerate) thin films. Biomaterials 24:4573–4583

Tokiwa Y, Calabia BP (2004) Degradation of microbial polyesters. Biotechnol Lett 26:1181–1189

Turesin F, Gursel I, Hasirci V (2001) Biodegradable polyhydroxyalkanoate implants for osteomyelitis therapy: *in vitro* antibiotic release. J Biomater Sci Polym Edn 12:195–207

Ueda H, Tabata Y (2003) Polyhydroxyalkanonate derivatives in current clinical applications and trials. Adv Drug Deliv Rev 55:501–518

Urtuvia V, Villegas P, González M, Seeger M (2014) Bacterial production of the biodegradable plastics polyhydroxyalkanoates. Int J Biol Macromol 70:208–213

US Department of Health and Human Services, FDA (1997) Guidance for industry. Rockville, p 54

Valappil SP, Misra SK, Boccaccini AR, Roy I (2006) Biomedical applications of polyhydroxyalkanoates: an overview of animal testing and in vivo responses. Expert Rev Med Devices 3:853–868

Vieyra H, Juárez E, López UF, Morales AG, Torres M (2018) Cytotoxicity and biocompatibility of biomaterials based in polyhydroxybutyrate reinforced with cellulose nanowhiskers determined in human peripheral leukocytes. Biomed Mater 13:045011

Volova T, Goncharov D, Sukovatyi A, Shabanov A, Nikolaeva E, Shishatskaya E (2013) Electrospinning of polyhydroxyalkanoate fibrous scaffolds: effects on electrospinning parameters on structure and properties. J Biomater Sci Polym Edn 25:370–393

Volova TG, Shishatskaya EI, Nikolaeva ED, Sinskey A (2014) *In vivo* study of 2D PHA matrices of different chemical compositions: tissue reactions and biodegradations. J Mater Sci Technol 30:549–557

Wang YW, Wu Q, Chen GQ (2003) Reduced mouse fibroblast cell growth by increased hydrophilicity of microbial polyhydroxyalkanoates via hyaluronan coating. Biomaterials 24:4621–4629

Wang YW, Wu Q, Chen GQ (2004) Attachment, proliferation and differentiation of osteoblasts on random biopolyester poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) scaffolds. Biomaterials 25:669–675

Wang YW, Yang F, Wu Q, Cheng YC, Yu PHF, Chen J, Chen GQ (2005) Effect of composition of poly(3-hydroxybutyrate-*co*-3hydroxyhexanoate) on growth of fibroblast and osteoblast. Biomaterials 26:755–761

Wang B, Pugh S, Nielsen DR, Zhang W, Meldrum DR (2013a) Engineering cyanobacteria for photosynthetic production of 3-hydroxybutyrate directly from CO_2. Metab Eng 16:68–77

Wang Y, Jiang XL, Peng SW, Guo XY, Shang GG, Chen JC, Wu Q, Chen GQ (2013b) Induced apoptosis of osteoblasts proliferating on polyhydroxyalkanoates. Biomaterials 34:3737–3746

Wang Y, Yin J, Chen GQ (2014) Polyhydroxyalkanoates, challenges and opportunities. Curr Opin Biotechnol 30:59–65

Weber B, Scherman J, Emmert MY, Gruenenfelder J, Verbeek R, Bracher M, Black M, Kortsmit J, Franz T, Schoenauer R, Baumgartner L, Brokopp C, Agarkova I, Wolint P, Zund G, Falk V, Zilla P, Hoerstrup SP (2011) Injectable living marrow stromal cell-based autologous tissue engineered heart valves: first experiences with a one-step intervention in primates. Eur Heart J 32:2830–2840

Wei X, Hu YJ, Xie WP, Lin RL, Chen GQ (2009) Influence of poly(3hydroxybutyrate-co-4-hydroxybutyrate-co-3-hydroxyhexanoate) on growth and osteogenic differentiation of human bone marrow derived mesenchymal stem cells. J Biomed Mater Res A 90:894–905

Wei DX, Dao JW, Chen GQ (2018) A micro-ark for cells: highly open porous polyhydroxyalkanoate microspheres as injectable scaffolds for tissue regeneration. Adv Mater 30:e1802273

Williams SF, Martin DP, Horowitz DM, Peoples OP (1999) PHA applications: addressing the price performance issue: I. Tissue engineering. Int J Biol Macromol 25:111–121

Williams SF, Martin DP, Gerngross T, Horowitz DM (2001) Removing endotoxin with an oxidizing agent from polyhydroxyalkanoates produced by fermentation. US Patent No 6245537

Winnacker M, Rieger B (2017) Copolymers of polyhydroxyalkanoates and polyethylene glycols: recent advancements with biological and medical significance. Polym Int 66:497–503

Wu Q, Wang Y, Chen GQ (2009) Medical application of microbial biopolyesters polyhydroxyalkanoates. Artif Cells Blood Substit Immobil Biotechnol 37:1–12

Xu XY, Li XT, Peng SW, Xiao JF, Liu C, Fang G, Chen KC, Chen GQ (2010) The behaviour of neural stem cells on polyhydroxyalkanoate nanofiber scaffolds. Biomaterials 31:3967–3975

Xu M, Qin J, Rao Z, You H, Zhang X, Yang T, Wang X, Xu Z (2016) Effect of Polyhydroxybutyrate (PHB) storage on L-arginine production in recombinant *Corynebacterium crenatum* using coenzyme regulation. Microb Cell Factories 15:15–26

Xue Q, Liu XB, Lao YH, Wu LP, Wang D, Zuo ZQ, Chen JY, Hou J, Bei YY, Wu XF, Leong KW, Xiang H, Han J (2018) Anti-infective biomaterials with surface-decorated tachyplesin I. Biomaterials 178:351–362

Yan C, Wang Y, Shen XY, Yang G, Jian J, Wang HS, Chen GQ, Wu Q (2011) MicroRNA regulation associated chondrogenesis of mouse MSCs grown on polyhydroxyalkanoates. Biomaterials 32:6435–6444

Yang X, Zhao K, Chen GQ (2002) Effect of surface treatment on the biocompatibility of microbial polyhydroxyalkanoates. Biomaterials 23:1391–1397

Yang Y, De Laporte L, Rives CB, Jang J-H, Lin W-C, Shull KR, Shea LD (2005) Neurotrophin releasing single and multiple lumen nerve conduits. J Control Release 104:433–446

Yang XD, Li HM, Chen M, Zou XH, Zhu LY, Wei CJ, Chen GQ (2010) Enhanced insulin production from murine islet beta cells incubated on poly(3-hydroxybutyrate-co-3-hydroxyhexanoate). J Biomed Mater Res A 92:548–555

Ye H, Zhang K, Kai D, Li Z, Loh XJ (2018) Polyester elastomers for soft tissue engineering. Chem Soc Rev 47:4545–4580

Young RC, Wiberg M, Terenghi G (2002) Poly-3-hydroxybutyrate (PHB): a resorbable conduit for long-gap repair in peripheral nerves. Br J Plast Surg 55:235–240

Yue H, Ling C, Yang T, Chen X, Chen Y, Deng H, Wu Q, Chen J, Chen G-Q (2014) A seawater-based open and continuous process for polyhydroxyalkanoates production by recombinant *Halomonas campaniensis* LS21 grown in mixed substrates. Biotechnol Biofuels 7:108

Zembouai I, Kaci M, Bruzaud S, Benhamida A, Corre YM, Grohens Y (2013) A study of morphological, thermal, rheological and barrier properties of poly (3-hydroxybutyrate-co-3Hydroxyvalerate)/polylactide blends prepared by melt mixing. Polym Test 32:842–851

Zhang J, Cao Q, Li S, Lu X, Zhao Y, Guan JS, Chen JC, Wu Q, Chen GQ (2013) 3-Hydroxybutyrate methyl ester as a potential drug against Alzheimer's disease via mitochondria protection mechanism. Biomaterials 34:7552–7562

Zhang J, Shishatskaya EI, Volova TG, da Silva LF, Chen GQ (2018) Polyhydroxyalkanoates (PHA) for therapeutic applications. Mater Sci Eng C Mater Biol Appl 86:144–150

Zhao K, Yang X, Chen GQ, Chen JC (2002) Effect of lipase treatment on the biocompatibility of microbial polyhydroxyalkanoates. J Mater Sci-Mater M 13:849–854

Zhao K, Deng Y, Chen JC, Chen GQ (2003a) Polyhydroxyalkanoate (PHA) scaffolds with good mechanical properties and biocompatibility. Biomaterials 24:1041–1045

Zhao K, Deng Y, Chen GQ (2003b) Effects of surface morphology on the biocompatibility of polyhydroxyalkanoates. Biochem Eng J 16:115–123

Zhao Y, Zou B, Shi Z, Wu Q, Chen G-Q (2007) The effect of 3-hydroxybutyrate on the in vitro differentiation of murine osteoblast MC3T3-E1 and in vivo bone formation in ovariectomized rats. Biomaterials 28:3063–3073

Zhao H, Cui Z, Sun X, Turng HL, Peng X (2013) Morphology and properties of injection molded solid and microcellular polylactic acid/polyhydroxybutyrate-valerate (PLA/PHBV) blends. Ind Eng Chem Res 52:2569–2581

Zhou L, Chen Z, Chi W, Yang X, Wang W, Zhang B (2012) Monomethoxy-poly(3-hydroxybutyrate-co-4-hydroxybutyrate)-graft-hyper-branched polyethylenimine copolymers for siRNA delivery. Biomaterials 33:2334–2344

Zinn M, Witholt B, Egli T (2001) Occurrence, synthesis and medical application of bacterial polyhydroxyalkanoate. Adv Drug Deliv Rev 53:5–21

Zubairi SI, Mantalaris A, Bismarck A, Aizad S (2016) Polyhydroxyalkanoates (PHAs) for tissue engineering applications: biotransformation of palm oil mill effluent (POME) to value-added polymers. J Teknol 78:13–29