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Review

Poly(3-hydroxyalkanoate)s: Diversification and biomedical applications A state of the art review

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ABSTRACT

Biomaterials have played an important role in the treatment of disease and the improvement of health care. Synthetic and naturally occurring biodegradable and biocompatible polymers have been used as biomaterials. Polyhydroxyalkanoates (PHAs) are promising materials for biomedical applications because they are biodegradable, non-toxic and biocompatible. We will shortly summarize the modification reactions, which include functionalization and grafting reactions, to improve the mechanical, thermal and hydrophilic properties of PHAs. The use of the modified PHAs in numerous biomedical applications, such as sutures, cardiovascular patches, wound dressings, scaffolds in tissue engineering, tissue repair/regeneration devices, drug carriers will be discussed in this review.

Contents

Abbreviations: PHA, Polyhydroxyalkanoates; *Scl*PHA, short chain length PHA; *Mcl*PHA, medium chain length PHA; PHACOS, poly-3-hydroxy-6-acetylthiohexanoate-co-4-acetylthiobutanoate; PHBHx, poly(3-hydroxybutyrate-co-3-hydroxyhexanoate); PHOU, poly(3-hydroxy-octanoate-co-3-hydroxy 10-undecenoate); PHA-Sy, poly(3-hydroxy alkenoate) from soybean oil; PMMA, poly(methyl methacrylate); THF, tetrahydrofuran; PHA-g-PTHF, poly(3-hydroxy alkanoates-graft-poly tetra hydro furan); ATRP, atom transfer radical polymerization; P(3HB-co-4HB), poly(3-hydroxy butyrate-co-4-hydroxy butyrate); PHBV, poly(3-hydroxybutyrate-co-3-hydroxyvalerate); CRP, C-Reactive Protein; HA, hydroxy apatite; P(3HB-co-3HHx), poly(3-hydroxy butyrate-co-3-hydroxy hexanoate); HMSCs, human mesenchymal stem cells.

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1. Introduction

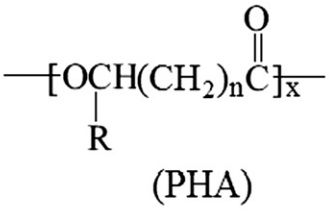
Synthetic and naturally occurring biodegradable and biocompatible polymers are referred to as "biomaterials". Any material, natural or man-made, that comprises the whole or part of a living structure or a biomedical device which performs, augments, or replaces a function that has been lost through disease or injury is known as a biomaterial. They have played an important role in the treatment of disease and the improvement of health care [1]. Among them, Polyhydroxyalkanoates (PHAs) are promising materials for biomedical applications because they are natural, renewable, biodegradable and biocompatible thermoplastics [2,3]. PHAs (Fig. 1) can be synthesized from renewable resources [4–11]. As the length of the side chain on the ß-carbon of the PHA increases, the physical property of polymer is changed from glassy state to more soft and sticky material.

There are three types of PHAs with respect to the length of the side chain: (1) short chain length hydroxyalkanoic acids such as *scl*PHA with an alkyl side chain that are produced by *Ralstonia eutropha* and many other bacteria. *scl*PHAs contain 3–5 carbon atoms, for example P3HB, P4HB. (2) Medium chain length hydroxyalkanoic acids such as *mcl*PHA with alkyl side chain that are produced by *Pseudomonas oleovorans* and other *pseudomonas sensu strictu* [12] *mcl*PHAs contain 6–14 carbon atoms, for example P3HHx, P3HO, P3HD, P3HDD, P3HTD and P3HHD. (3) *lcl*PHAs obtained from long chain fatty acids, which contain more than 14 carbon atoms [13].

Since these different types of PHAs have various structural and mechanical properties, they should be classified according to their properties and modified in order to be easily used for medical applications.

2. Why do PHAs need modification?

The physical and material properties of PHAs are significantly influenced by their monomer composition and chemical structure [14–16]. Some *scl*PHAs may be too rigid and brittle and may lack the superior mechanical properties required for biomedical and packaging film applications. In contrast, *mcl*PHAs may be elastomeric but have very low mechanical strength. Therefore, for packaging materials, tissue engineering, and other specific applications, the physical and mechanical properties of microbial polyesters need to be diversified and improved. In addition to this, highly hydrophobic PHAs need to have hydrophilic character for biomedical applications especially for drug delivery systems.



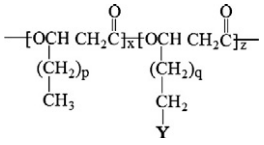
(PHA)

x = 100 - 30000

		PHA	
n = 1	R = H	Poly(3-hydroxypropionate)	(P3HP)
	R = methyl	Poly(3-hydroxybutyrate)	(P3HB)
	R = ethyl	Poly(3-hydroxyvalerate)	(P3HV)
	R = propyl	Poly(3-hydroxyhexanoate)	(P3HHx)
	R = pentyl	Poly(3-hydroxyoctanoate)	(P3HO)
	R = nonyl	Poly(3-hydroxydodecanoate)	(P3HDD)
n = 2	R = H	Poly(4-hydroxybutyrate)	(P4HB)
n = 3	R = H	Poly(5-hydroxyvalerate)	(P5HV)

Fig. 1. General structure of polyhydroxyalkanoates.

Table 1
Biosynthetic modification of PHAs.



PHA side chain containing	Functional side chain, Y	Reference
-Methyl branching	-CH-CH_3 (with CH_3)	[17,18]
-Aryl groups	$-C_6H_5$, $pCH_3-C_6H_5$	[19,20]
-Unsaturation	$-CH{=}CH_2$	[21–24]
-Halogen	−Br, −Cl, −F	[25,26]
-Cyano groups	−CN	[27]
-Alkyn group	-C≡CH	[28]
-Ester group	$-C(O)OCH_3$	[29]
-Epoxide group		[30]

2.1. Improving the mechanical properties

For these purposes, fermentation can be a useful tool by means of feeding microorganism with functionalized substrates. However, there is a limitation for microorganism growing on any substrate functionalized in order to diversify microbial polyesters. Only several functionalized alkanoic acids were used in growing *Pseudomonads* to diversify PHAs.

The *mcl* and *lcl*PHAs obtained from functionalized substrates can have the same functionalities. Among them, methyl branching [17,18], aryl [19,20], olefin [21–24], halogen [25,26], cyano [27], alkyn [28], ester [29], and epoxide groups [30] can be counted. Table 1 contains the diversified PHAs via biosynthetic modification.

Molecular biology strategies have been designed to increase the production of *mcl*PHA in many microorganisms. Many genes related to PHA biosynthesis from various bacteria have been cloned and were employed in metabolic engineering [31,32].

Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate), PHBHx, copolymers with low 3HHx fraction are known as one of the most useful polymers among PHAs because they have appropriate mechanical properties for use as flexible films, compared with PHB [33]. Escapa et al. recently reported that poly-3-hydroxy-6-acetylthiohexanoate-co-4-acetylthiobutanoate (PHACOS) was obtained when *P. putida KT2442* and its *fadB* mutant were grown on a mixture of 6-acetylthiohexanoic acid (6-ATH) [34]. PHACOS show good thermal stability up to 200 °C. This could result in a good processing ability for these materials and potentially interesting mechanical properties. This *mcl*PHA with low values of glass transition (T_g) are expected to display relatively high softness and marked elasticity which are very interesting properties for materials dedicated to biomedical applications. Fig. 2 shows the chemical structures of PHBHx (Fig. 2a) and PHACOS (Fig. 2b).

2.2. Improving the hydrophilic character of the PHAs

During biosynthetic production of PHAs, the PHA synthesis is very selective to the substrate and the bacteria produce only hydrophobic PHAs. Therefore, PHAs need much more versatile modification reactions in order to obtain new materials with improved mechanical, thermal and hydrophilic properties [35,36]. PHAs need to have a hydrophilic character, particularly for drug delivery systems via chemical synthesis other than biosynthetic modification.

In this manner, functionalized PHAs with terminal vinyl groups gained the most interest because terminal vinyl groups are highly reactive for further modification reactions. By using unsaturated substrates such as 10-undecenoic acid or soya bean fatty acids to grow *Pseudomonads*, PHAs containing terminal vinyl groups are obtained. These unsaturated polyesters are usually produced as a copolymer

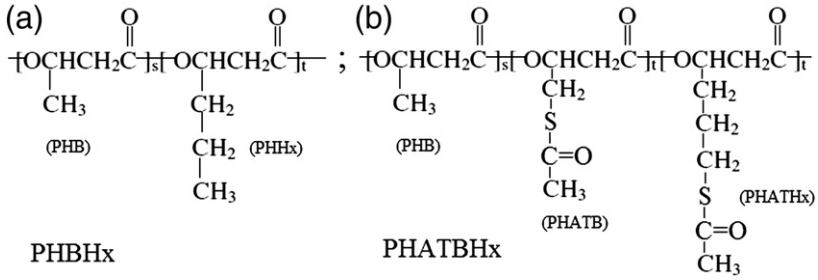


Fig. 2. The chemical structures of (a) poly-3-hydroxy-butyrate-co-hydroxy-hexanoate (PHBHx) and (b) poly-3-hydroxy-4-acetylthiobutanoate-co-6-hexanoate (PHATBHx).

of saturated polyester (e.g. PHO) to control the vinyl content. Fig. 3 shows some unsaturated PHAs: (a) poly(3-hydroxy-octanoate-co-10-undecenoate) (PHOU) and poly(3-hydroxy alkenoate) from soybean oil (PHA-Sy) [13,37].

The most chemical modification reactions have been reported on the PHOU among the microbial polyesters. For example, bromine can be added to the vinyl ends of PHOU in order to obtain brominated PHA [38]. Then the brominated PHA is reacted with the hydroxyl groups of polyethylene glycol (PEG) [39] for obtaining PHA amphiphilic graft copolymers. The double bonds of PHOU can also be coupled by free radical addition of 11-mercaptoundecanoic acid to obtain side chains with terminal carboxyl groups [40]. Epoxidation of the unsaturated side chains of PHOU can be carried out by using m-chloroperoxybenzoic acid. The glass transition temperatures increase with the epoxide content in the polymer [21]. Pendant vinyl groups of PHOU can be converted to the hydroxyl groups by using oxidant such as $KMnO_4$ in cold alkaline solution (pH 8–9) and 9-borobicyclononane without a severe reduction in molecular weight [41–43]. The hydroxylated polymers are completely soluble in polar solvents, indicating a considerably enhanced hydrophilicity of the modified PHAs. Carboxylation of the unsaturated bacterial polyesters can be carried out by the oxidation of the side chain double bonds at slightly higher temperature [44]. Carboxylation of the PHOU can also be done by the capping reaction of the double bond with mercapto undecanoic acid [45]. When epoxidized PHOU [21] is reacted with diethanol amine, the epoxide ring is opened generating poly(β-hydroxyoctanoate-co-(β-hydroxy-11-(bis(2-hydroxyethyl) amino)-10-hydroxyundecanoate)) [46].

The solubility of copolymer in water is further enhanced as the formation of each terminal amine group is accompanied by the formation of a hydroxyl group, a direct result of the ring-opening reaction. The whole summary of the modification reactions on PHOU has been given in Fig. 4.

Chlorination of saturated and unsaturated PHAs can be performed via chlorine addition to the double bonds and substitution reactions with saturated polyesters such as PHB and PHO [47,48]. Fig. 5 shows the chlorination reactions of PHO, PHB and PHA-Soya (PHA-Sy).

The chlorinated PHAs indicate higher glass transition (T_g) than that of the pristine. The thermal properties of the PHAs before and after chlorination are tabulated in Table 2.

PHB-Cl was blended with commercial atactic polymethyl metacrylate (PMMA) [50]. PHB-Cl acted like a plasticizer in the PMMA blend. The polar nature of the PHB-Cl molecule can also act to dissociate some of the dipolar ester associations in PMMA and bring the birefringence levels closer to zero. In addition, the stress optical behavior becomes positive at 20 wt.% PHB-Cl loading suggesting that there is a positive contribution of PHB-Cl to the intrinsic birefringence of the blend.

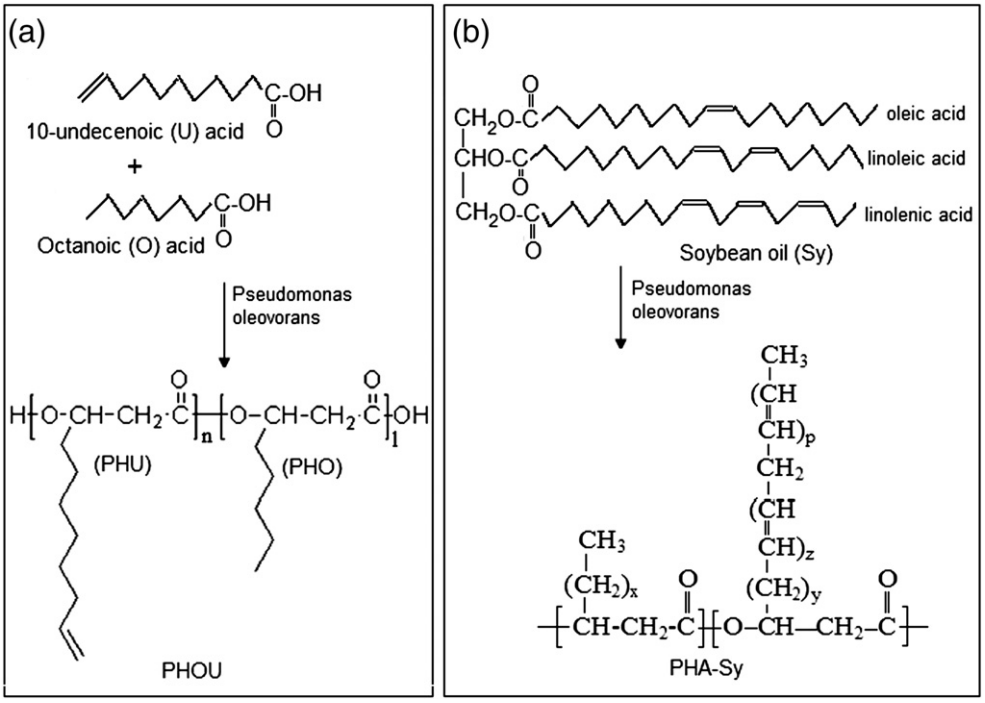


Fig. 3. The formation of the unsaturated PHAs: (a) poly(3-hydroxy-octanoate-co-10-undecenoate) (PHOU) and poly(3-hydroxy alkenoate) from soybean oil (PHA-Sy).

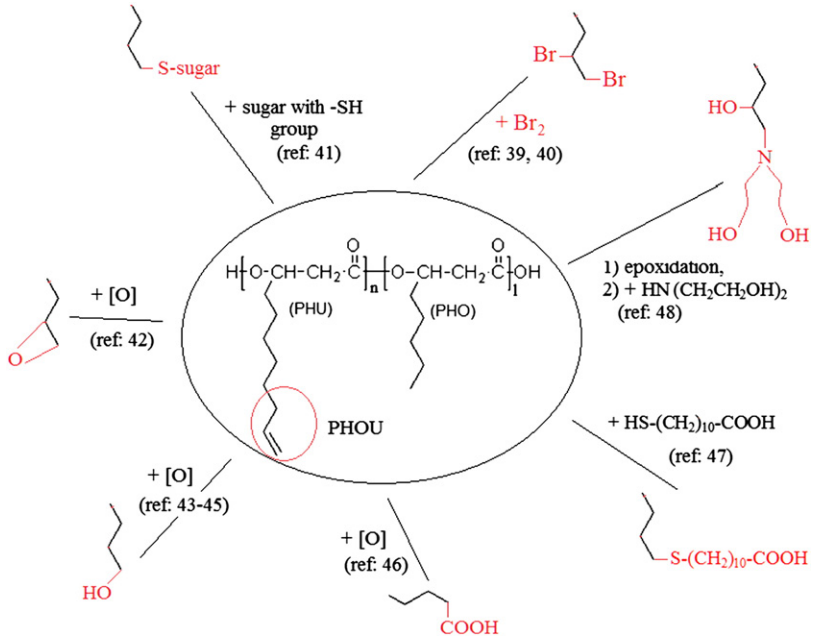


Fig. 4. The summary of the chemical modification reactions of PHOU.

Another type of modification is with graft copolymerization. Graft copolymerization of the PHAs combines the advantages of natural and synthetic polymers to obtain new diversified PHAs.

Atom transfer radical polymerization of methyl methacrylate can be performed by using halogenated PHAs in order to obtain brush type of graft copolymers [50]. Cationic polymerization of tetrahydrofuran (THF) with the chlorinated PHAs in the presence of silver salt was carried out to prepare PHA-g-PTHF brush type graft copolymer [51]. The hydroxyl ends of pendant PTHF segments mixed with cerium salt can initiate the polymerization of methyl methacrylate leading to multiblock brush type graft copolymer. Fig. 6 shows the brush type graft copolymer synthesis by using ATRP and cationic polymerization.

Free radical polymerization of methyl methacrylate can also be carried out by using gamma-ray irradiation, macro peroxy initiators and PHB macroazo initiators [52–58].

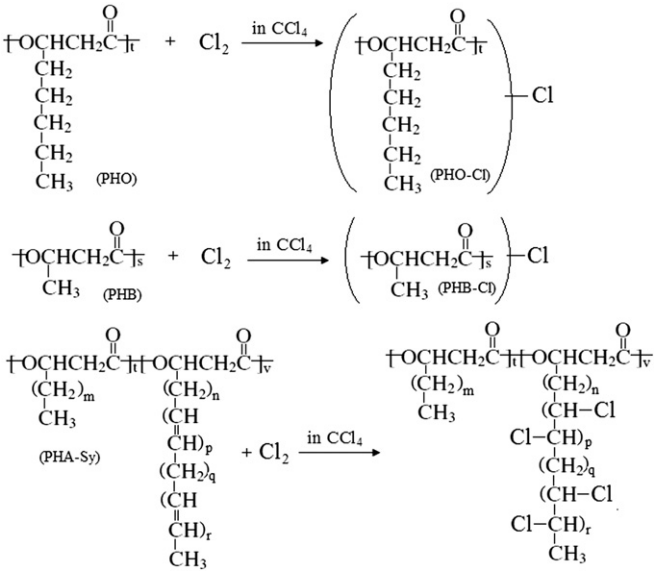


Fig. 5. The chlorination reactions of PHO, PHB and PHA-Soya with chlorine gas in CCl_4 solution.

Amphiphilic PHAs from swellable to soluble in water are desirable in drug delivery systems and tissue engineering. In most attempts to synthesize amphiphilic PHAs, polyethylene glycol was used [59]. Polyethylene glycol azo initiators can be attached onto the double bonds via free radical mechanism [60]. In the same manner, PEG was introduced into the unsaturated PHA obtained from unsaturated plant oils [61].

Crosslinking has been studied to overcome the weakness of *mcl*PHAs [62–65]. When an unsaturated polyester is exposed to air under sunlight, polymerization occurs which leads to crosslinking (autoxidation). Autooxidation of the PHOU and PHA-Soya gives highly flexible elastomers, crosslinked polyesters [64]. The mechanical properties of the oxidized PHA copolyesters are collected in Table 3 [64].

Gold nanoparticles were formed in PHOU films from auric acid reduced to metal by free radicals arising by the reaction with oxygen in air. It was found that the gold nano particles accelerated crosslinking reaction collapsing the metal nano particles inside [65]. Fig. 7 shows the crosslinked PHOU with gold nanoparticles in toluene.

3. Medical applications of PHAs

PHAs are emerging as a class of biodegradable polymers for applications in tissue engineering [66]. A member of PHA family, *mcl*PHAs has interesting potential applications in coatings and in medical temporary implants such as scaffolding for the regeneration of arteries and nerve axons [67]. On the other hand amphiphilic PHA copolymers which were prepared via chemical modification have significant applications in drug delivery and tissue engineering. For example

Table 2
The thermal properties of the PHAs before and after chlorination [49,50]. T_m: melting transition, T_g: glass transition.

PHA	Cl, mol%	T_g, °C	T_m, °C
PHA-Sy	0	−40	–
PHA-Sy-Cl	18	2	109
PHB	0	5	177
PHB-Cl	25	0	130
PHO	0	−40	59
PHO-Cl	22	6	–

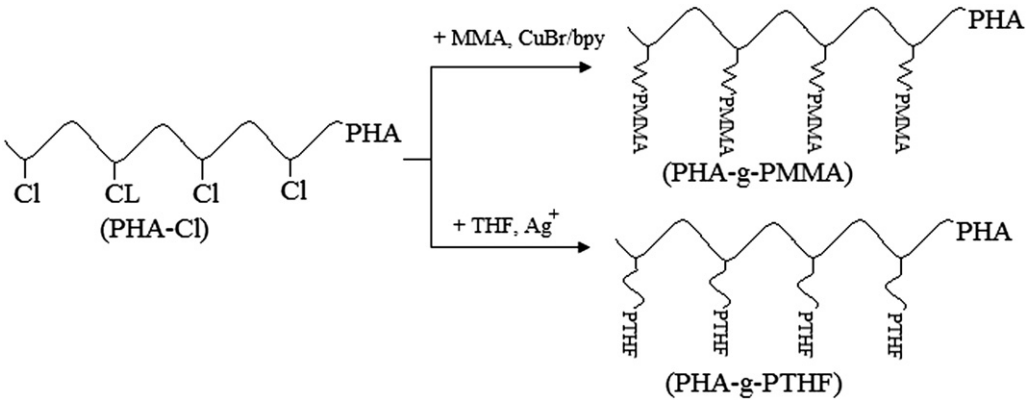


Fig. 6. Synthesis of PHA-g-PMMA and PHA-g-PTHF brush type graft copolymer via ATRP and cationic polymerization.

amphiphilic graft copolymers can be used as blood contacting devices due to their excellent blood compatibility. Another member of the PHA family, the sodium salt of 4-hydroxybutyrate (4HB) monomer was used as an intravenous anesthetic agent because it can cross the blood barrier rapidly [68–71]. P(3HB-co-4HB) is a biodegradable and biocompatible copolymer, and it has potential application in the controlled release of 4HB for therapeutic aim [72].

A living organism is composed of not only one type of cell, but also different cells in different character forming the tissues, followed by different organs [73]. Each organ has unique vascular network and therefore responds to implanted foreign bodies in a different pattern. Therefore, each system has different design of inflammatory reaction. Also many different microenvironments are present in different organ systems; for instance: hydrophilic media such as blood and cerebrospinal fluid and hydrophobic media such as central nervous system and some solid organs. Nowadays many types of different polyesters are used as substitutes for arterial tissues, vascular system, and scaffold materials in combination with ceramic materials; wound management (sutures, dusting powders, dressing); maxillofacial treatment (guiding tissue and bone regeneration) [73]; dental, urology; material for cardiac tissue engineering; orthopedy; computer supported tomography and ultrasound imaging; and drug delivery (tablets, implants, micro-carriers).

3.1. Subcutaneous tissue

The fastest and easiest form of in vivo experimental designs of newly formed PHAs is implantation of the substrate into the subcutaneous tissue. Some of the earliest investigations of in vivo tissue responses to PHA polymers were made by Baptist et al. in 1965. In this study, strips of P(3HB) films were implanted subcutaneously and intramuscularly in rabbits and harvested after 8 weeks. Examination of the implant sites revealed granulomatous foreign body reactions [74,75]. Later on many studies have been made on this issue. In 1993 Gogolewski et al. investigated P3(HB) in vivo and compared it with PHB-VA [76]. In another study, P3(HB) was used as a suture material in rat muscle and skin tissue and it was compared with catgut and silk sutures [77]. All parameters of inflammation had the maximum value at the 4th week of implantation and afterwards cell count, macrophage count and the capsule thickness subsided. Inflammatory reaction was found to be less in P3(HB) than silk and catgut [77].

PHO, another member of the PHA family with high mechanical strength and long duration of biodegradation was studied in vivo by Marious et al. in 1999 [78]. According to their data the acute inflammatory reaction with fast migration of polymorphonuclear cells begin as early as day 2 and it reached to its max level by day 7. This finding was also supported by recent studies. When the soft tissue response of both PHB grafts and PHO grafts were compared, PHO samples had the fastest reaction of acute inflammation [78,79]. Following these studies, our research group has investigated the biocompatibility properties of PHO and newly formed PHO-Sy implants in vivo [79].

Table 3
Mechanical properties of the oxidized PHA copolyesters [66].

PHA samples	E, Young's Modulus[a], N/mm^2	Strain @ Break, %	Plastic deformation[b], %
PHA-Sy-ox	0.52	100	0 (elastomer)
PHO-Sy-2O8O-ox	0.30	203	0 (elastomer)
PHO-Sy-2872-ox	0.34	233	0 (e1astomer)
PHU-Sy-5O5O-ox	0.34	170	0 (elastomer)
(PHU + PHA-Sy)-ox	080	81	4 (e1astoplastic)
PHO-Sy-5O5O-ox	2.36	299	18 (elastoplastic)
PHO-Sy-5O5O	186	596	132 (elastoplastic)
PHO (contro1)	0.89	843	220 (elastoplastic)
PHOU-5050-ox	077	347	20 (elastophislic)
PHU-ox	837	41	2 (brittle)

[a] Young's modulus E is defined as E = [stress]/[strain] which is equal to E = (Lo/ΔL)(F/A) where Lo is the equilibrium length, ΔL is the length change under the applied stres, F is the force applied, and A is the area over which the force applied.
[b] Plastic deformation % = (Final length of the PHA film after elongated / Initial length of the PHA film) × 100.

Fig. 7. Crosslinked PHOU with gold nano particles (3.1 wt.% for #210, 14 wt.% for #240) in toluene. Size of the gold nano particles are from 90 to 333 nm [65].
Obtained from reference [65].

We have also investigated the soft tissue response of Au nanoparticle embedded PHOU polymer samples and it has been shown that PHOU with gold clusters indicated that the biocompatibility was increased by the gold cluster inclusion of the PHOU sample [65].

3.2. Cardiovascular system

PHAs have been widely used in the cardiovascular area such as artery augments, cardiologic stents, vascular grafts, heart valves, pericardial patches, implants, dressing tablets and microparticulate carriers [80]. Vascular grafting is a frequently used technique in cardiovascular pathologies. One of the most important factors in the considerable recurrence rates in vascular stent is vascular smooth muscle cell proliferation, which might be triggered by the stent-induced trauma to the vessel wall. Another important factor is the insufficient re-endothelization of the graft. With the application of biodegradable polymer grafts these factors are tried to be eliminated. The ideal cardiovascular patch material should have resistance to degradation and infection, where as these patches should exhibit long durability, feasibility to various size to suit cardiac and peripheral vascular reconstructions, and there should be a lack of immunogenicity and non-toxicity [81]. There are different types of PHAs which are studied experimentally for this purpose.

P4HB is an elastic polymer to be used in cardiovascular tissue engineering. However it is degraded quickly in vivo [82,83], and therefore is not a proper type of PHA in cardiovascular system applications.

In order to replace an infracted tissue of myocardium, one should create a thick tissue patch on which myocardial cell can stick and proliferate. Mixtures of natural and synthetic polymers were used to obtain cell carriers like sponges for this purpose. Kenar et al. reported the design of a 3D microfibrous material which is formed by the blend and electrospun into fiber materials of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), poly(L-D,L-lactic acid) (P(L-D,L) LA) and poly(glycerol sebacate) (PGS) (Fig. 8). It was shown that biodegradable macroporous tubing was possible to create a thick myocardial patch to replace myocardial infarctions [84].

In order to apply PHA as a vascular prosthesis, or vascular stents, PHA should cause minor inflammatory reaction to prevent narrowing of the lumen of the stent. On the other hand they should trigger minor surface endothelial cell lining in order to ease the blood flow [84,85]. Therefore PHAs should be modified as adding hydrophilic block copolymers to cause minor soft tissue response, or having porous surface to allow cell proliferation and endothelial cell lining [85,86].

When the P3(HB) were implanted as stent for iliac arteries of rabbit for up to 30 weeks, it was found out that P3(HB) instigated intense inflammatory reactions with an increase in collagen, thrombosis and intense lumen narrowing via inflammatory cell accumulation especially on the first 1–10 weeks of implantation. Therefore this product is forbidden in the use of vascular stent applications [85–88].

Later on, Stock et al. designed an experiment with a PHO polymer patch implanted in the pulmonary artery of a sheep [78]. The constructs were seeded with autologous ovine cells, incubated, and implanted in sheep to replace both the native pulmonary valve and main pulmonary artery at time points ranging from 1 to 24 wk. They have found out that when the graft is seeded with autologous cells, at the end of 24 weeks there was an organized viable cell accumulation on the graft containing an endothelial cell lining. There was neither thrombosis nor stenosis. Although PGA was completely degraded by 24 week, the conduits continued to demonstrate PHO at the end of this period. Sodian et al. in 2000 constructed a biodegradable and biocompatible trileaflet heart valve scaffold that was fabricated from a porous PHO with ovine endothelial cells seeded onto the heart valve scaffold [89] (Fig. 9).

In order to slow down the degradation of the polymer grafts and therefore to decrease the risk of thrombosis, the surface of the polymer graft should be modified with viable cell lining onto the inner surface of the graft [90,91] (Fig. 10). Another factor needed to facilitate the hemocompatibility of these grafts is to use PHO which has a long duration of biodegradation especially for the replacement of the leaflets.

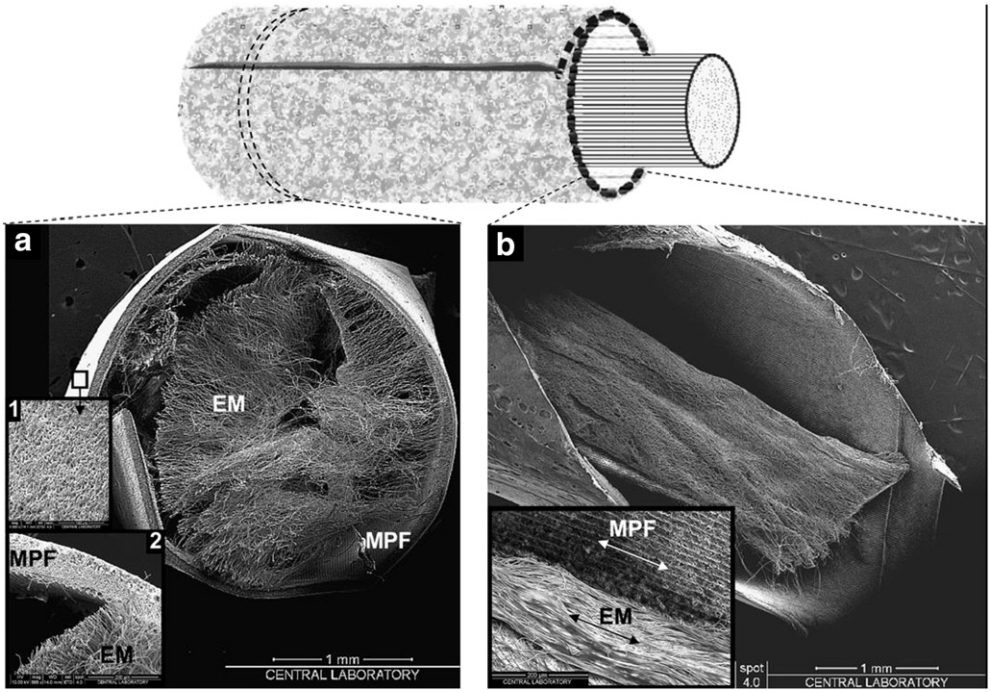


Fig. 8. Formation of 3D construct and various cross sectional views of the construct by SEM (EM: electrospun mat, MPF: porous micropatterned film). (a) Inset 1: SEM image of the exterior surface of the tubular construct. Inset 2: SEM images of certain parts of the constructs at higher magnifications (b): inset; SEM images of certain parts of the constructs at higher magnifications [86].
Obtained from reference [86].

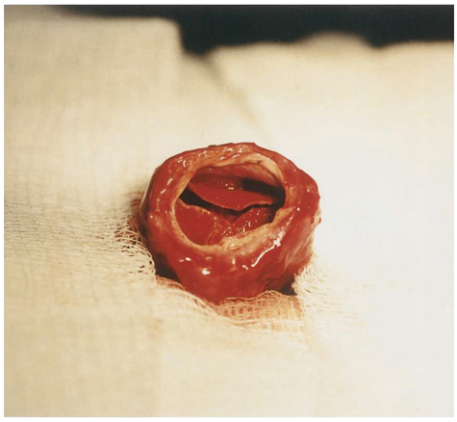


Fig. 9. Explanted tissue-engineered valved conduit after 5 weeks in vivo (seen from arterial side). It was found to be hemocompatible up to 120 days with no trombosis and mild stenosis [91].
Obtained from reference [91].

PHBHHx is a newly developed member of the PHA family, with improved hemocompatibility and cytocompatibility; it holds promise as a blood-contact material with less platelet adhesion, reduced erythrocyte contact and hemolysis reactivity compared with PHB and PHBV films in vitro [86]. Following studies were made to enhance the hemocompatibility of the PHBHHx via covering the inner surface with smooth muscle cell lines, or with plasma or fibronectin, and showed good results [86,87].

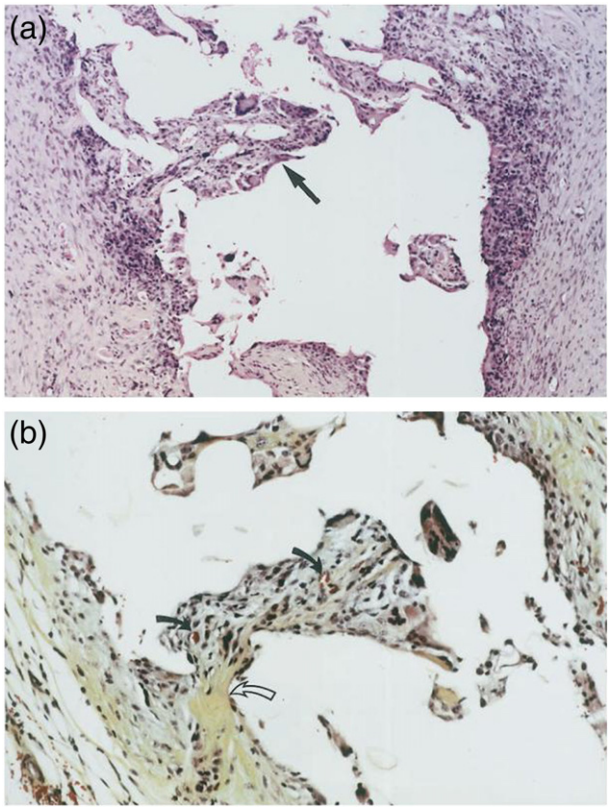


Fig. 10. A, Histological examination (hematoxylin–eosin stain) of conduit wall. Filled arrow shows ingrowth of vascularized tissue islands and destruction of polymer. B, Histological examination (Movat pentachrome stain), which stains elastin black, collagen yellow, and glycosaminoglycans blue-green, demonstrates a significant amount of collagen (open arrow) and glycosaminoglycans. Filled arrow indicates capillaries in ingrown neotissue [91].
Obtained from reference [91].

3.3. Nerve tissue engineering

The synthetic nerve conduits have been developed for the repair of peripheral nerve faults. In order to have proper neural prosthesis, PHAs are modified in different aspects. In neural regeneration, communication between microenvironments outside and inside of the prosthesis is a must, and therefore porous type of polymer prosthesis will be a better choice for neural application [93,94]. Fiber structured polymer tubes may enhance regeneration via providing microfibers for Schwann cell to line up and facilitate axonal regeneration. Also neural growth factors embedded polymer prosthesis improves the neuroregeneration.

Hazari et al. used P3(HB) as a neuronal conduit in different techniques and found out that the rate and amount of regeneration in the P3(HB) conduit did not fully match the autologous nerve graft but it demonstrated good axonal regeneration with low level of inflammatory infiltration [92,93]. Chen et al. in 2009 used PHBHHx as a nerve conduit and investigated the difference between porous and nonporous conduits [94]. After 3 months of implantation, PHBHHx had been found to be highly compatible in the nervous system and in all test subjects it was found to have been bridged by the regenerated nervous tissue 1 month after the implantation. Rodriguez et al. reported applying the PCL tube for a 6 mm gap in the rat sciatic nerve. Kiyotani et el. investigated the collagen covered PGA tube and achieved the regeneration of a 25 mm gap in the feline sciatic nerve [95]. Novikov and coworkers searched the fibronectin and alginate covered PHB-fiber and after spinal cord injury, this PHB fiber improved the repair process [96].

There are also recent studies on spinal cord injuries with tissue engineered nerve tubes carrying aligned nerve stem cells and astrocytes for neuroregeneration in the spinal cord [97]. Yucel et al. reported a biodegradable nerve conduit which was constructed via turning a porous micropatterned film (PHBV-P(L-D,L)LA-PLGA) into a tube wrapping aligned electrospun fibers (PHBV-PLGA). This film had good mechanical properties to function as a nerve guide [98]. We had also previously investigated the neuroregeneration in histology and electrophysiology with PHO nerve conduits and found out that PHO conduits are more favorable then control autografts in aspect of inflammation and neuroregeneration.

3.4. Gastrointestinal system

In gastrointestinal system, PHAs are used as organ wall patches, and therefore they should have strong structural properties such as hydrophobic, thick and not brittle [99].

To test the applicability of biodegradable patches in the gastrointestinal tract, asymmetric patches made from PHB were sutured onto the stomach wall and systemic and local immune reactions were discussed [99]. It has been shown that C-Reactive Protein (CRP) level, which is a strong systemic marker of acute inflammation is synthesized in great amount in acute inflammatory reaction locally around the implantation side on the 14th day of implantation.

3.5. Bone tissue

Polymers are used in bone tissue engineering for the purpose of bone tissue repair and reinforcement, regeneration of cartilage, help in partial replacement of bones via metallic parts and used as carriers of antibiotics to the infected bone tissues [100]. When a high mechanical strength is required for a hard tissue like bone tissues, researchers mainly focus on *scl*PHA like PHB and PHBV. These bone substitutes should also have rough surface area or microporous structure for the attachment of osteoblasts. Meanwhile, hydroxyl apatite (HA), which contributes 65–70% to the bone matrix, is commonly used as an additional blending material. Some in-vitro studies [101,102] showed that the best parameters of growth and differentiation of

murine marrow osteoblasts were found on PHB/HA blends containing 10% and 20% HA [103]. P(3HB-co-3HHx) scaffolds could sustain osteoblast phenotypes; high alkaline phosphatase activity, round cell shape, fibril collagen synthesis and strong calcium deposition. The best proliferation was observed on the P(3HB-co-3HHx) scaffolds. It was seen from SEM that P(3HB-co-3HHx) scaffolds had suitable roughness for osteoblast attachment and proliferation than that of P(3HB) and PLA ones. P(3HB-co-3HHx) was the best biomaterial for osteoblast attachment, proliferation and differentiation for bone marrow cells. Cool et al. in 2008 [102] studied the in vitro biological response of pure PHBV and mineral-reinforced PHBV composites were assessed, using cultured osteoblasts, osteoclasts, and macrophages. PHBHHx had better performances on attachment, proliferation, and differentiation of osteoblasts compared with PHB.

Many researches experiments were designed using *scl*PHA such as PHB and PHBV since these polymer types maintain the high mechanical strength to get a hard tissue like bone. P(HB-co-8%HV)/HA (30% w/w) was found to have approximately the same behavior with several human bones. PHB/HA which contains 10% and 20% HA had the best growth parameters and differentiation of murine marrow osteoblasts [104,105]. PHBHHx scaffolds which contain HA particles did not improve the mechanical properties or osteoblast responses [106]. For in vivo study, PHB and PHB reinforced with particulate HA were demonstrated to produce a consistent favorable bone tissue adaptation response with no evidence of an undesirable chronic inflammatory response after an implantation period up to 12 months [105]. Highly organized new bone was rapidly formed close to the implant material independent of the implant material's chemical composition [107,108]. Osteoblasts and osteocytes were identified throughout the interface region in a rabbit tibias implantation study using PHBV/HA [103,108]. Lamellar bone formed at the interface took the shape of the implant, and it was shown that HA incorporation to the polymer not only increases the osteoblastic activity and the bone integrity but also aggravates the anti-inflammatory action of the polymer [109].

3.6. Cartilage tissue-tendon and ligament tissue engineering

Articular cartilage tissue with chondrocytes coats the ends of bones in diarthrodial joints for distribution of applied loads. It has the lowest volumetric cellular density of any tissue in the human body. When cartilage tissue is damaged, it becomes difficult to heal it. It causes osteoarthritis and functional loss of the joints. Therefore healing process of cartilage tissue is vital.

Similar to bone tissue, PHA substitutes for cartilage tissue should be chosen as strong in structure such as *scl*PHA, PHB and its derivatives. It should also cause minimal foreign body reaction, and neocartilage formation could be enhanced via Ca–P or stem cell embedding process. Porous three dimensional scaffolds made from PGA [110], PLA [111], PLGA [112,113], chitosan [114,115], collagen [116], silk fibroin [117] and PHA were used in order to regenerate long lasting hyaline cartilage in the defects. Similar to bone tissue studies cartilage tissue responded well to PHBV implants. In vivo tissue repair was investigated using PHBV matrices implanted into full thickness cartilage defects compared with collagen containing calcium phosphate at 8th and 20th weeks of implant (CaP-Gelfix) [103]. PHBV matrices presented early cartilage formation resembling normal articular cartilage with minimal foreign body reaction with fibrous tissue formation and neovascularization on 8th week and this foreign body reaction subsided on 20th week of implantation [108]. Very recently, three-dimensional PHBHHx scaffolds with or without seeded chondrocytes were evaluated in a rabbit articular cartilage defect model and the defects were found to be filled with white cartilaginous tissue throughout 16 weeks of implantation in vivo [106]. In vitro chondrocytes proliferated better on the PHBHHx/PHB scaffolds than on PHB scaffolds [118,119]. Köse and coworkers [120,121] studied collagen matrices containing calcium phosphate (CaP-Gelfix) and poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) (PHBV). They were fabricated for creating novel cartilage by tissue engineering. Cell seeded PHBV matrices showed early cartilage formation that seemed like normal articular cartilage and observed minimal foreign body reaction. PHBV revealed a better healing response compare to CaP-Gelfix [120,121]. Later on, PHB/PHBHHx scaffolds were used to produce neocartilage with human adipose derived stem cells for 6 months time and it was found out that PHB/PHBHHx is a suitable material for cartilage tissue engineering [122].

3.7. PHAs as carrier systems

3.7.1. PHAs as drug carrier system

Conventional drug therapy, managed intravenously or by an extravascular route (sublingual, oral, nasal, rectal, etc.) does not sustain drug concentrations within the desired frame at the target region for extended periods of time. Controlled drug delivery systems can deal with releasing therapeutic device at desired rate for desired duration so that the drug level in the body within the therapeutic frame can be sustained [123–128]. The drug delivery system was developed for releasing, targeting, uptaking, retaining, activating, bringing and localizing the drugs at the right time, place, dose and period.

The drug release kinetics can be controlled via conveniently engineering the PHA matrix parameters to reach desired degradation rates. *scl*PHAs are degraded by surface erosion that makes them an attractive polymer for using drug carriers. Since *scl*PHAs are crystalline and hydrophobic, many pores are formed on the surface. This brings the release of drugs too quickly without any polymer degradation. On the other hand, *mcl*PHAs copolymers have low melting point and low crystallinity, therefore they are more suitable for drug delivery. The

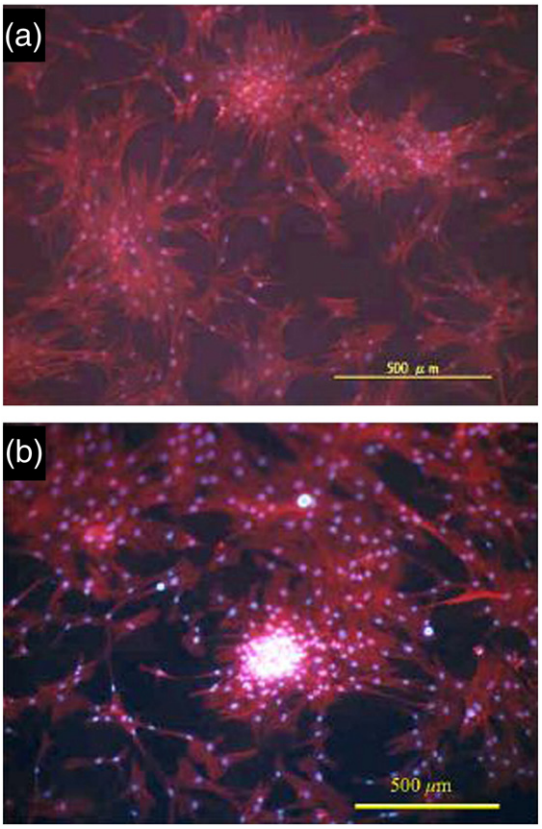


Fig. 11. HMSCs cultured on (a) cast PHBHHx film and (b) HA-coated cast PHBHHx film. The cells were fixed after culturing for 3 days and stained with phalloidin (actin filaments, red) and DAPI (nuclei, blue) before visualization by fluorescent microscopy. This figure is published in color in the online of this journal that can be accessed *via* http://www.brill.nl/jbs [134].
Obtained from reference [134].

drug delivery systems can be prepared with different shapes such as gels, films, microcapsules, microspheres, nanoparticles, porous matrices, polymeric micelles and polymer linked drugs. The physical interactions are generally preferred in order to bind the drug to the polymer and not damage the molecular structure of the drug [125].

Rifampicin loaded PHB microspheres were studied for use as a chemoembolizing agent [127,128]. The microspheres were prepared via a solvent evaporation technique. The amount of drug released from the microspheres was found approximately 90% in 24 h. The encapsulation (52–65% w/w) was more efficient in the neutral form, but the drug was quickly released without any particle degradation in both the acidic and neutral forms. In another study, PHB, PHBV and P(3HB-4HB) implantable rods were used for the local delivery of antibiotics [129]. Poulton and Aktar [130] have also reviewed the potential of PHAs in this area. They have postulated that PHB homopolymers were unable to entrap the drug because of its high melting temperature and rapid crystallization rate. PHBV copolymers are semicrystalline.

3.7.2. PHAs as stem cell carrier system such as scaffold material

PHAs are usually used as scaffolds on which cells can be seeded in order to induce new tissue growth. The PHA scaffold has to supply an assist surface for the cells to adhere, support cell growth without cell release and sustain diffusion of nutrients and passage of waste. It must be able to guide and organize the cells in a demand condition. When the new tissue is inserted, the scaffold must be able to degrade and the degradation products must be non toxic and well tolerated [131]. Yu et al. [132] described that the hyaluronic acid (HA) coating on PHA membranes could improve the metabolic activity and reduce the death rate of human mesenchymal stem cells (hMSCs). Aggregates and spherical clusters of hMSCs were found on the surface of cast poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) membranes (Fig. 11).

Similarly, terpolyesters of 3-polyhydroxyalkanoates were used as scaffolds for human bone marrow stem cells to neurons [123]. Also PHB/PHBHHx scaffolds carrying human adipose-derived stem cells were used for the cartilage tissue engineering [133]. Growth factors and cytokine have an ability to promote proliferation, recruitment and differentiation of cells. Therefore scaffold matrix can combine with a suitable growth factor and cytokine. Most of the growth factors spontaneously bind to the regulatory molecules that exist in the extracellular matrix and are released via enzymatic degradation of the molecules. Tissue regeneration based on the fundamental combination of growth factors with their carriers [134–136].

There have been many studies for PHA application in different tissues. When biomedical applications of PHAs are considered, interfacial interactions, the details of molecular organization and interactions with biologically relevant compounds play a crucial role [137,138]. In order to improve the medical application of PHAs, one should be aware of the tissue micro environment and also different modifications of PHAs. For the use of PHAs as vessel prosthesis or

Table 4
PHAs used in biomedical applications.

Reference	Modified/unmodified PHA[a]	Physical properties	Medical application	Ref. #
Baptist et al. (1965)	P3(HB)		Subcutanous patches	[75]
Gogolewski et al. (1993)	PHB/VA		Subcutanous patches	[76]
Volova et al. (2003)	PHB/PHV		Suture	[77]
Kenar et al.	PHB/VA	Design of a 3D microfibrous material-formed by the blend and electrospun into fiber materials	Myocardial patch	[84]
Hazari et al. (1992)	P3(HB)		Peripheral nerve guide	[92]
Moshahebi et al. (2002)	P3(HB)		Peripheral nerve guide	[93]
Qu et al. (2006)	PHBHHx	Flexible	Vessel stent, hemocompatibility and cytocompatibility; it holds promise as a blood-contact material with less platelet adhesion, reduced erythrocyte contact	[86,87]
Chen et al. (2009)	PHBHHx	Porous tube form, flexible	Nerve conduit	[94]
Navikov et al. (2002)	Fibronectin and alginate covered PHB-fiber		Spinal cord injury	[96]
Yücel et al. (2010)	PHBV-PLGA	Turning a porous micropatterned film into a tube wrapping aligned electrospun fibers	Nerve guide	[97,98]
Löbler et al. (2002)	PHB	Asymetric patches	Stomach wall patch	[99]
Wang et al. (2004)	PHBHHx	Scaffold, flexible	Bone regeneration	[101]
Cool et al. (2008)	PHB/VA		Bone regeneration, better performances on attachment, proliferation, and differentiation of osteoblasts	[102]
Shishatskaya et al. (2006)	HA reinforced PHB		Bone regeneration	[103,105]
Luklinska et al. (2003)	HA reinforced PHB/VA		Bone regeneration	[108]
Wang et al. (2005)	PHBHHx	Porous three dimensional scaffolds, flexible	Cartilage proliferation	[106]
Kose et al. (2005)	PHB/VA	Chondrocyte seeded	Cartilage proliferation	[120,121]
Ye et al. (2009)	PHB/PHBHHx	Human adipose tissue embedded scaffolds	Cartilage proliferation	[122]
Kassab et al. (1999)	PHB microspheres	Solvent evaporation technique	Drug delivery, chemoembolization	[127,128]
Turesin et al. (2001)	PHBV and P(3HB-4HB)	Semicrystalline	Drug delivery	[129]
Poulton and Aktar (1996)	PHB homopolymers	Unable to entrap the drug because of its high melting temperature and rapid crystallization rate	Not available for drug delivery	[130]
Stock et al. (2000)	PHO	Autologous ovine endothelial cell seeded	Pulmonary valve	[78]
Sodian et al. (2000)	PHO	Autologous ovine endothelial cell seeded porous PHO patches	Pulmonary heart valve	[89]
Hazer et al. (2009)	PHO-Sy		Subcutaneous patches	[79]
Hazer et al. (2011)	PHOU-Au		Subcutaneous patches	[95]
Escapa et al. (2011)	PHACOS	Biosynthetic good thermal stability up to 200 °C		[34]

[a] Please see the text for the abbreviations.

drug carrier systems in blood circulation, amphiphilic properties of PHAs can be improved. To increase cell to cell communication within the prosthesis, microporous and fibril structure can be modified especially for the use in peripheral nervous system. The most important disadvantage of PHA prosthesis in medical application in vivo is the foreign body reaction. To decrease this inflammatory reaction and to add antibacterial effect to the newly formed PHA prosthesis, nanoparticles such as gold or silver can be attached. Further prospects should include collaboration of the physical chemists, polymer chemists and medical researchers together with to overcome to the PHAs modifications and related medical applications. There are many kinds of diversified PHAs by using chemical modification reactions mentioned above. However, as we see in Table 4, the biomedical applications of the modified PHAs are still open for researchers.

4. Conclusion

Tissue engineering is a multi disciplinary area combining material science, biology and surgical reconstruction for supplying living tissue products that improve tissue and restore and maintain function. Microbial polyesters are biocompatible and biodegradable hydrophobic polyesters. They have unique properties and are good candidates for biomedical applications. They play an important role in the development of second and third generation biomaterials, especially biomedical areas. Various modifications techniques were developed to improve their thermomechanical and surface properties. This review is focused on new designed biomaterials which contain bacterial polyesters in use for many medical applications. In recent studies, biocompatible PHAs are chemically modified to become appropriate scaffold materials for various types of stem cells for regeneration of different tissue samples. Future studies may focus on the improvement of amphiphilic and antimicrobial characteristics of PHAs in order to achieve better compatibility in vivo.

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