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REVIEW

Polyhydroxyalkanoates: opening doors for a sustainable future

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Polyhydroxyalkanoates (PHAs) comprise a group of natural biodegradable polyesters that are synthesized by microorganisms. However, several disadvantages limit their competition with traditional synthetic plastics or their application as ideal biomaterials. These disadvantages include their poor mechanical properties, high production cost, limited functionalities, incompatibility with conventional thermal processing techniques and susceptibility to thermal degradation. To circumvent these drawbacks, PHAs need to be modified to ensure improved performance in specific applications. In this review, well-established modification methods of PHAs are summarized and discussed. The improved properties of PHA that blends with natural raw materials or other biodegradable polymers, including starch, cellulose derivatives, lignin, poly(lactic acid), polycaprolactone and different PHA-type blends, are summarized. The functionalization of PHAs by chemical modification is described with respect to two important synthesis approaches: block copolymerization and graft copolymerization. The expanded utilization of the modified PHAs as engineering materials and the biomedical significance in different areas are also addressed.
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INTRODUCTION

Polymers from renewable resources have gained significant attention in recent decades due to environmental issues and the realization of limited petroleum resources. Among the natural polymers, polyhydroxyalkanoates (PHAs) comprise a family of biodegradable polyesters that are produced by an extensive variety of microorganisms for intracellular carbon and energy storage purposes (Figure 1a).[1] PHA synthesis is promoted by unbalanced growth during the fermentation and accumulation of PHA granules as part of a survival mechanism of the microbes.[2] The general molecular structure of PHAs is presented in Figure 1b. Depending on the carbon numbers in the monomeric constituents, PHAs can be classified as short-chain-length PHAs (C_3–C_5), which consists of 3–5 carbon monomers, and medium-chain-length PHAs (MCL-PHA, C_6–C_{14}), which consists of 6–14 carbon monomers in the 3-hydroxyalkanoate units (Figure 1b).[3,4] For example, poly(3-hydroxybutyrate) (PHB), poly(3-hydroxyvalerate) (PHV) and their copolymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) are typical examples of short-chain-length PHAs, whereas poly(3-hydroxyoctanoate) (PHO) and poly(3-hydroxynonanoate) (PHN), which are primarily formed as copolymers with 3-hydroxyhexanoate (HHx), 3-hydroxyheptanoate (HH) and/or 3-hydroxydecanoate (HD), are typical examples of MCL-PHAs. More than 150 different PHA monomers have been identified, which renders them the largest group of natural polyesters.[5]

The applications of PHAs are limited due to several undesirable physical properties. The presence of large crystals in PHB causes poor mechanical properties, which renders PHB unsuitable for various uses, including packaging film and biomedical applications such as heart valves and other vascular applications or controlled drug delivery systems.[6] In addition, the melting temperature (T_m) of PHB is similar to its thermal decomposition temperature. Its poor thermal stability renders PHB incompatible to conventional thermal processing techniques and susceptible to thermal degradation. Although a few studies demonstrated that MCL-PHAs at certain side chain lengths have elastomeric properties at atmospheric temperatures, the corresponding low T_m of MCL-PHAs indicates that this behavior is only observed in a narrow temperature window. At temperatures above or close to T_m, the polymers are completely amorphous and sticky.[7] An additional increase in the side chain length of MCL-PHAs will produce even more viscous and tacky polymers, which demonstrates the limitations of modifying PHA properties by side chain adjustment.[8] The development of new MCL-PHA copolymers involves a complicated genetic engineering process. Biosynthetic approaches remain inaccessible to the majority of chemists and material scientists who seek to optimize the properties of PHAs. The high hydrophobicity, lack of advanced functionality and high cost have also restricted further expansion of its utilization. Regarding the biodegradability of PHAs, the slow degradation rate and highly hydrophobic nature of unmodified PHAs are not favored in many advanced biomedical applications. Therefore, the utilization of PHAs as direct substitutes for synthetic plastics remains a significant challenge. PHAs need to be tailored to achieve low cost and better performance, such as tunable

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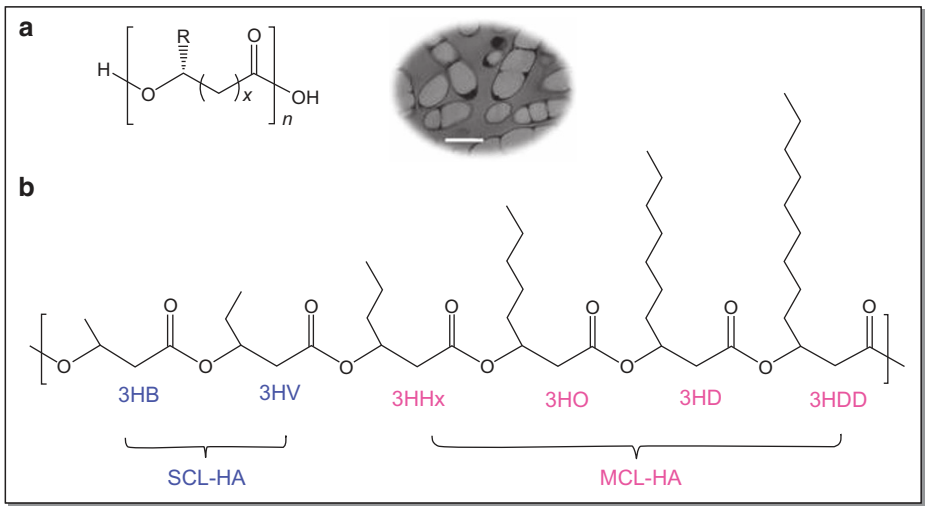

Figure 1 (**a**) General molecular formula of PHAs. Typically, x = 1–8, and n ranges from 100 to 1000 s. (**b**) Some commonly synthesized short-chain-length PHA monomers (SCL-HA) and middle-chain-length PHA monomers (MCL-HA). 3HB: 3-hydroxybutyrate, 3HV: 3-hydroxyvalerate, 3HHx: 3-hydroxyhexanoate, 3HO: 3-hydroxyoctanoate, 3HD: 3-hydroxydecanoate, 3HDD: 3-hydroxydodecanoate. Insert TEM image that shows thin sections of recombinant *R. eutropha* PHB – 4 cells that contain large amounts of P(3HB-*co*-5mol% 3HHx). The bar represents 0.5 μm. Adapted from ref 78 (2000 Elsevier).

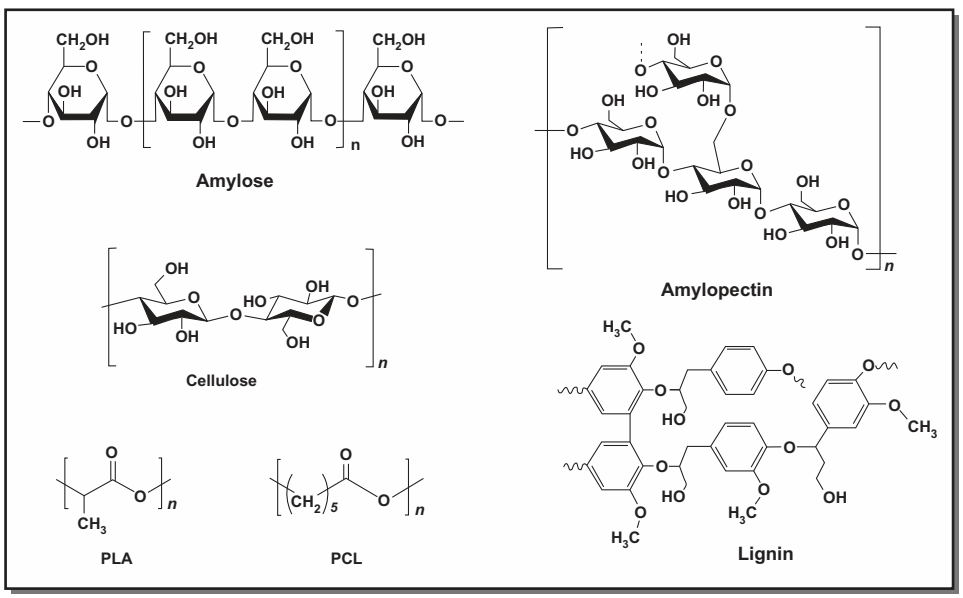

Figure 2 Molecular structures of the most common biodegradable polymers in PHA blends.

mechanical properties, crystallinity, surface features, amphiphilicity and degradation rates to fulfill specific applications.

In this review, the modifications of PHAs with improved properties are discussed with respect to two important strategies: physical blends and chemical modifications. Various functionalities that are not typically achieved by biosynthetic processes are introduced to PHAs. Microstructures at different levels and with new material features are elucidated to understand the relationship between the structures and properties of modified PHAs. With enhanced performance, the expanded utilization of the newly developed PHAs from renewable resources into value-added products is also discussed.

PHA MODIFICATION VIA BLENDING

Blending is a simple and effective approach for obtaining new polymeric materials with improved properties, in which the drawbacks of the parent components can be suppressed. Typically, the physical and mechanical properties of polymer blends can be tuned by choosing the proper starting materials and varying the compositions of the blend and preparation conditions. In addition, biodegradable blends add interesting properties to PHAs, such as full biodegradability and excellent biocompatibility. These blends have attracted increased interest because they employ conventional technology at low cost and have high potential applications, which range from environmentally degradable resins and biomedical implants to absorbable surgical sutures. A novel blend of PHA with other biodegradable polymers can significantly enhance the performance of PHAs. In this section, blend of PHAs with natural raw materials from sustainable resources or other synthetic biodegradable polymers are discussed. The molecular structures of biodegradable polymers that are commonly employed in PHA blends are presented in Figure 2.

Blending of PHA with natural raw materials

PHA blended with starch. Starch is one of the most promising natural polymers due to its inherent biodegradability, overwhelming abundance and annual renewal.[9] Starch consists of two types of molecules: the linear and helical amylose and the branched amylopectin (Figure 2). The blending of PHA, especially PHB with starch, has been extensively investigated. Godbole *et al.*[10] discussed the compatibility of PHB with starch to achieve improved properties and reduce cost. The results revealed that films had a single glass transition temperature (T_g) for all proportions of PHB: starch blends. The nature of all combinations was crystalline. The tensile strength of the blend at a ratio of 30:70% showed a significant increase compared with the virgin PHB. Because starch is abundant at a very low cost, the blending of starch with a maximum percentage of 30 in PHB would substantially reduce the cost of PHB, without compromising its physical properties. These blending materials extended the use of PHB as a coating material on paper or cardboard for food packaging applications.[10] In another study, Zhang *et al.*[11] analyzed the thermal behavior and phase morphology of PHB/starch acetate (SA) blends. The team demonstrated that PHB/SA blends were immiscible and the T_m of PHB in the blends shifted with an increase in SA concentration. The melting enthalpy of the PHB phase in the blend was similar to the value for pure PHB, whereas the T_g of the PHB in the blends remained constant at 9 °C. A differential scanning calorimetry (DSC) cycle test showed that PHB crystallization was affected by the presence of the SA component in the heating and cooling processes. For example, the temperature and enthalpy of the non-isothermal crystallization of the PHB in the blends were substantially lower than the temperature and enthalpy of the non-isothermal crystallization of pure PHB, and the cold crystallization peaks of the PHB in the blends shifted to higher temperatures compared with the cold crystallization peaks of pure PHB.[11]

Although blending of PHA with starch or SA showed some potential for reducing the production cost and improving the mechanical properties, these blends are brittle and do not form intact films due to the incompatibility between the starch/SA and the PHA matrix. To increase the compatibility and produce PHA blends with better mechanical properties, Willett *et al.*[12] employed grafted copolymer of starch and glycidyl methacrylate to improve the mechanical properties of PHBV, which has better mechanical properties than PHB. The tensile and flexural strengths of the blends increased with the use of starch and glycidyl methacrylate compared with the untreated starch; this effect is proportional to the increased graft concentrations. The scanning electron microscope of the cryogenic fracture surface showed improved adhesion between the starch-g-PGMA and the PHBV matrix, which indicates the increased compatibility of the blending components. A promising result of this study was the significant increase in the fracture toughness of these blends due to the incorporation of grafted starch.[12] Recently, poly (vinyl acetate) (PVAc)-modified corn starch (CSV) was exploited to improve the compatibility and flexibility of PHA/starch blends. With a PVAc-grafted ratio of 0.27, the starch-*g*-PVAc possessed a higher T_g at 44 °C compared with 35 °C of neat PVAc, probably due to the hindered molecular mobility that was imposed by starch on the grafted PVAc. After blending PHB with the CSV, only a single T_g was detected for all PHB/CSV blends, which increased from 2 to 37 °C with an increase in the CSV concentration, which indicates that the CSV was compatible with the PHB. The presence of PHB and PVAc components did not hinder the enzymatic degradation of the corn starch by α-amylase.[13]

PHA blended with cellulose derivatives. Cellulose derivatives such as ethyl cellulose (EC), cellulose propionate and cellulose acetate butyrate (CAB) are attractive biomaterials that are extensively employed as blood coagulant, a pharmaceutical tablet coating and as carriers for poorly soluble drugs.[14] Cellulose derivatives are susceptible to enzymatic digestion in the human body. Cellulose derivatives have attracted a significant amount of interest as blending components with PHA primarily due to their compatibility with PHA and their ability to enhance the degradation of PHA. Zhang *et al.*[15] investigated the miscibility, crystallization and melting behavior and phase morphology of PHB/EC blends. The results indicated that T_g was composition-dependent; an increase in T_g was observed with a decrease in the PHB concentration in the blends. Unlike the pure PHB component, the blends displayed no crystallization when cooled from a melt state during the DSC non-isothermal crystallization runs, and the growth of the PHB spherulites in the blends was significantly delayed by the EC concentration. Ei-Shafee *et al.*[16] investigated the properties of a blend of PHB and CAB in a film that was formed by solution casting. The single T_g indicated that CAB/PHB blends were miscible. A depression in the equilibrium melting point of PHB and a significant reduction in the spherulites growth rate of PHB in the blends were also observed in the blend films. Additional phase structure analyses showed that the presence of a homogeneous amorphous phase, which consisted of CAB and noncrystalline PHB chains, was situated in the interlamellar regions of the crystalline PHB. In addition, the dispersion of CAB in the PHB-crystallized phase also induced improved mechanical properties with an elongation at break that increased from 2.2 to 7.3%.

PHA blended with lignin. Lignin is an amorphous macromolecule that is composed of a phenylpropane repeat unit and possesses aliphatic and aromatic hydroxyl groups, as well as carboxylic acid groups.[17] These active functional groups and their amorphous nature render lignin a suitable candidate for blending with PHA. The amorphous nature of lignin can reduce the formation of large spherulites crystals and secondary nucleation, which has a significant impact on PHB brittleness. Previous studies showed that organosolv lignin and its butyrate derivative achieved higher miscibility with PHB and can significantly retard PHB crystallization.[18] The effect of lignin fine powder on the PHB crystallization process was investigated by Kai *et al.*[19] The results of the kinetic study of the melt for both pure PHB and PHB/lignin blends revealed that the crystallization half-time was reduced by the presence of lignin, which indicates a favorable isothermal crystallization of the PHB/lignin blend compared with pure PHB. With the addition of lignin, the rate of PHB spherulitic growth from the melt increased, the size of the spherulites decreased and the number of the spherulites increased compared with pure PHB.[19] These results indicated that lignin fine powder can be employed as a new type of nucleating agent to modulate PHB crystallization. Recently, Mousavioun *et al.*[20] investigated the thermo–physical properties and rheology of PHB/lignin blends. Soda lignin was employed as a blending component to improve the properties of PHB. The results indicated that soda lignin can improve the total thermal stability of PHB but may reduce the initial decomposition temperature of PHB. The thermogravimetric analysis, DSC and scanning electron microscope results of the PHB/soda lignin blends suggested that the intermolecular interaction between PHB and soda lignin was favored at a maximum soda lignin concentration of 40 wt%. These intermolecular interactions were attributed to the hydrogen bonding formation between the reactive functional groups of lignin and the carbonyl groups of PHB.[20] The degradation behaviors of the PHB/lignin blends were recently investigated by

burying the samples in garden soil.[21] The results indicated that PHB films disintegrated with 45 wt% of mass loss within 12 months. However, the mass loss comprised 12 wt% when 10 wt% of lignin was present, which suggests that the presence of lignin can reduce the rate of PHB degradation. This may be caused by the inhibited colonization of the microorganisms that increased the resistance of the blends to microbial attack. Further analysis suggested that lignin can form strong hydrogen bonds with PHB in the buried samples, which likely caused a reduced rate of breakdown of PHB and prevented rapid degradation of the blends.[21] In addition, an accumulation of biofilms was observed on the surface of the buried samples with increased surface roughness during the degradation study, which revealed a biodegradation mechanism.[21]

Blending of different types of PHAs. The first member of PHA which was discovered in 1926 is PHB. Previously, more than a hundred different varieties of PHAs have been reported. PHB, PHBV, poly(3-hydroxybutyrate-co-4-hydroxybutyrate) and PHBHHx copolymers with various monomer molar ratios are produced on a large scale by commercial enterprises.[2] As previously mentioned, the properties of PHAs significantly vary due to their monomer structure and composition. For example, PHB is a brittle polymer that forms large crystal spherulitic morphology and has secondary crystallization characteristics, whereas its copolymerization with other MCL-HA monomers, such as 3HHx, 3HO, 3HD and 3HDD, render the resultant SCL-MCL-PHA copolymers more attractive in terms of polyolefin-like thermo/mechanical behaviors, polyester-like physico-chemical properties and unique biological characteristics. Another example is P3HB/4HB, which has been shown to exhibit an extensive range of physical properties and morphologies that range from highly crystalline to elastomeric properties, depending on the molar fraction of 4HB. In contrast to other monomers, including 3HB, 3HV, 3HHx and 3HP, 4HB monomer has one more carbon atom in the backbone, which produces unique crystallization behaviors of P3HB/4HB. Consequently, the blending of different types of PHAs offer the potential for alternating the properties of the PHAs, which facilitates expanded applications. For instance, Chen and co-workers systematically investigated PHB/PHBHHx blends and their biomedical applications.[22] The results revealed poor L929 cell growth on PHB films and a significant improvement in the growth on PHB/PHBHHx blending films, which indicated that blending is also an effective method for modifying the bioactive behaviors of the biomaterials. The different cellular behaviors were explained by the crystallization behaviors of the blending films.[22] The following detailed studies have shown that the high degree of crystallization and rapid crystallization rate of PHB generates pores and protrusions on the pure PHB film surface. This coralloid surface may prohibit the attachment and growth of mammalian cells. However, the presence of PHBHHx in PHB substantially reduced both the degree of crystallization and the crystallization rate of PHB. Consequently, the blending films exhibited a fairly regular and smooth surface, which facilitated cell attachment and growth and improved the biocompatibility of PHB.[23]

In addition, blends of different types of PHA members are usually compatible and can be employed as an effective approach for enhancing co-crystallization. For example, Yoshie *et al.* investigated solid-state structures and the crystallization kinetics of PHBV and PHB/PHBV blends.[24] The results indicated that observable phase structures include the co-crystallization of PHB and PHBV, the coexistence of two or more crystalline phases formed by phase segregation upon crystallization and immiscible phase separation, which changes with an increase in the HV content of PHBV. As the HV content increased, phase segregation proceeded to a higher degree prior to co-crystallization; as a result, the copolymer content in the co-crystalline phase decreased and/or the crystalline phases of the component polymers were formed. The final phase structure of the blends was determined by the competition between the co-crystallization and the phase segregation.[24] In another study, Saad analyzed the miscibility, melting and crystallization of PHB and oligo (R,S)-3HB-diol blends using DSC.[25] The thermograms of blends that contained 6 wt% oligo (R,S)-3HB-diol showed behaviors that are characteristic of a single-phase amorphous glass state with a composition-dependent glass transition and depression in the equilibrium T_m of PHB. The negative value of the interaction parameter that was determined from the equilibrium melting depression confirmed the miscibility of the blend components.[25]

PHA blended with synthetic biodegradable polymers

PHA blended with PLA. Poly(lactic acid) (PLA) is a chemically synthesized biodegradable polymer from lactic acid, which can also be produced by the fermentation of simple sugars, such as glucose and maltose from corn or potato, sucrose from cane or beet sugar and lactose from cheese. PLA is a linear aliphatic polyester thermoplastic that has been extensively employed as environmentally friendly package materials and biocompatible medical devices. However, low-impact toughness and low end-use temperature are important factors in the large-scale commercial adoption of PLA. Plasticizers, mineral fillers and traditional impact modifiers have been added to overcome the problems associated with PLA.[26] Blending PLA with other biodegradable polymers, such as PHA and PCL, was also shown to be an effective method for altering the properties of the polymer.[27,28] Several studies have focused on the study of PHA/PLA blends. For example, Gerard and Budtova demonstrated that PHA and PLA are immiscible polymers, and nodular and co-continuous morphologies were observed.[29] From the rheology analysis, the thermal stability of PHBV was extremely low, and the PLA/PHBV blends served a significant role in improving this property. Although PLA and PHBV are brittle polymers, blends that contain a small amount of PHBV in a PLA matrix exhibited a significant ductile plastic deformation.[29]

PHAs that consist of 3HB and a small amount of MCL-HA units have lower crystallinities and T_ms and more ductile properties than short-chain-length PHAs. Noda *et al.*[30] reported that blends of PLA with this type of PHA can form an excellent pair of complementary materials, which can effectively balance the shortcomings of these materials when combined. For example, the results indicated that the toughness and elongation at break of the blends significantly improved when 10% of PHA was added to PLA, which was attributed to an increase in the amorphous phase of the blends and caused unusually slow crystallization kinetics of finely dispersed PHA particles. In addition to short-chain-length PHAs, MCL-PHAs and chemically modified PHAs were also reported to blend with PLA to optimize their physical properties. For instance, Takagi *et al.*[31] blended a series of MCL-PHAs and epoxy-group-modified PHA (e-PHA) with PLA using solution casting methods. In contrast with PHB, the MCL-PHA or e-PHA are amorphous rubbery polymers. Although the PLA/MCL-PHA and the PLA/e-PHA blends were immiscible in the amorphous state, the impact toughness of the PLA increased after blending with these PHAs. The e-PHA with an inserted epoxy group side chain was more effective in improving the morphology and physical properties of the blend than MCL-PHA. The e-PHA exhibited better compatibility with PLA than MCL-PHA. A possible explanation is that the epoxy group is not stable and tends to combine with the hydroxyl

group of PLA in the end group, which may increase the interactions between phases.[31]

In addition to the PHA components, which affect their compatibility with PLA, the method of blending will also impact the miscibility of PLA. Zhang *et al.*[32] reported that PHB/PLA blends that were prepared by the solvent casting method were immiscible over the range of the analyzed compositions, whereas the melt-blended sample that was prepared at high temperatures exhibited evidence of greater miscibility. The crystallization of PHB in the blends was affected by the amount of added PLA. The thermal history caused a depression of the melting point and a decrease in the crystallinity of the PHB in the blends.[32] Ohkoshi *et al.*[27] investigated the miscibility and phase structure of the PLA/atactic PHB (a-PHB) of different molecular weights using DSC and optical microscopy. The molecular weight of a-PHB has a substantial impact on the miscibility with PLA. For example, low-molecular-weight a-PHB ($M_w = 9400$) is miscible in the melt at 200 °C with a maximum a-PHB concentration of 50 wt%. Conversely, high-molecular-weight a-PHB ($M_w = 140\ 000$) revealed two T_g in the melt, which indicates the immiscibility of these two phases.[27] Recently, Furukawa *et al.*[33] compared the properties between blends of PHB/PLA and PHBHHx/PLA. The results revealed that both the PHB/PLLA blends and PHBHHx/PLA blends were immiscible, but the PHBHHx/PLA blends were more compatible. The lattice parameters of each component in the blends were almost constant, which suggested that the crystalline structures in the blends were maintained.[33] The T_g for the PHBHHx/PLA components in the blends and the PHB and PLLA components in the PHB/PLLA blends did not significantly change. The observation of the crystallization temperature of PLA in both blend systems suggested that each component was not only the PHB/PLA but also the PHBHHx/PLA blends that formed the mixed semicrystalline structures. The crystallization temperature of PLA in the PHB/PLLA blend and PHBHHx/PLA blend shifted to a lower temperature and higher temperature, respectively, with an increased PHB or PHBHHx component in the blends. These results illustrated that the PHB/Poly(L-Lactic Acid) blends with decreasing crystallization temperature were completely immiscible, whereas the PHBHHx/PLA blends with increasing crystallization temperature were slightly compatible.[33] Recently, blends of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) and stereocomplexed polylactide with improved rheological and mechanical properties were also reported.[34]

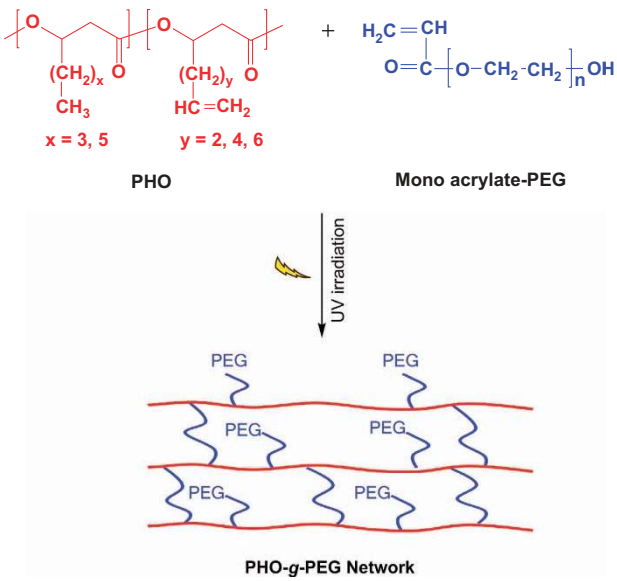

Figure 3 Schematic of the formation of PHO-*g*-PEG copolymers. Adapted from reference 41 (2003 Elsevier).

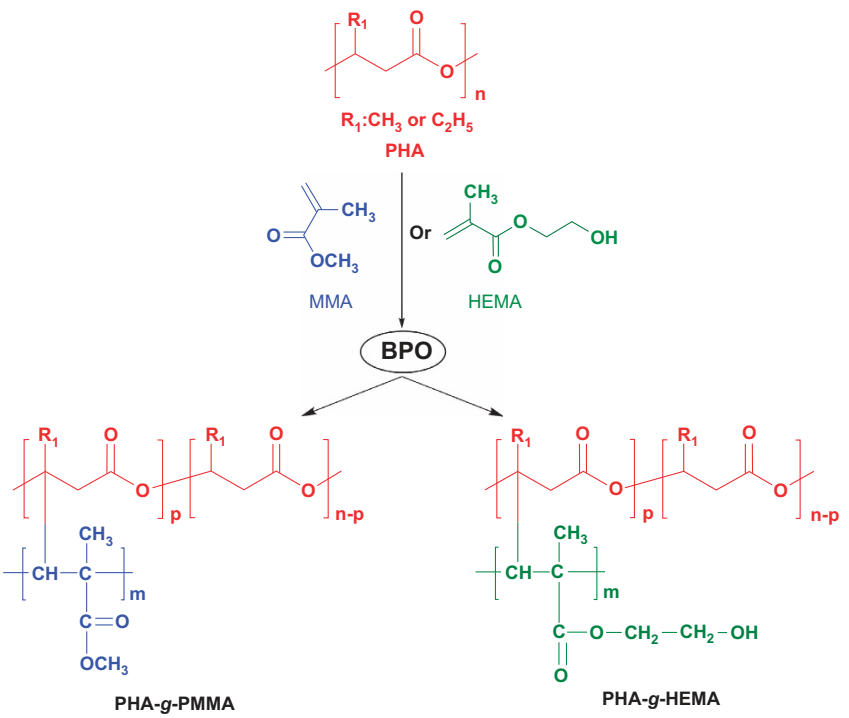

Figure 4 Free radical grafting of MMA and HEMA on PHA using BPO as an initiator.

PHA blended with PCL. Polycaprolactone (PCL) is a biodegradable semicrystalline aliphatic polyester that has been employed for long-term biomedical applications. PCL possesses superior mechanical strength with high strength and ductility. Consequently, PCL has been extensively explored as a biomaterial in various forms. For example, Lim *et al.*[35] blended PCL with PHBHHx and employed the blends as a substrate for musculoskeletal tissue engineering. PHBHHx possesses a low degree of crystallinity and nontoxic degradation products. In addition, PHBHHx is a soft and flexible polymer with a low tensile strength and Young's modulus.[36] Despite these advantages, PHBHHx exhibits inadequate mechanical strength and toughness for tissue engineering applications. However, the addition of PCL via polymer blends may cause PHBHHx to exhibit enhanced toughness with substantial elasticity. These improvements provided adequate matching of properties with human bone. At an optimal blend ratio of 30:70, the blends exhibited enhanced yield strength, yield strain and Young's modulus, as well as predictable degradation profile. PHBHHx can support human fetal mesenchymal stem cell adhesion and proliferation, which demonstrates significant potential as a bone scaffold material.[35] PHBV and PCL blends have been reported to be miscible. Chiono *et al.*[37] prepared hollow fibers for use as guides for tissue engineering by dry-jet-wet spinning of PHBV and PCL solutions in chloroform. The fibers obtained from these blends exhibited a low degree of surface and bulk porosity depending on the composition. The mechanical properties of PHBV/PCL blends showed increasing ductility of the blend samples with an increasing amount of PCL.[37] Del Gaudio *et al.*[38] developed PCL/PHBV blends using vascular grafts and compared the biological performance with the parent polymers. In their study, rat cerebral endothelial cells were used to assess the biological performance of these grafts. PCL and PCL/PHBV supported adhesion, viability, migration and proliferation of rat cerebral endothelial cells during the 14-day observation period. However, pure PHBV was less suitable for these cells, which may be related to the different mechanical features.[38]

CHEMICAL MODIFICATION OF PHAS

In addition to blending, chemical modification of PHAs enables the easy and precise modulation of the polymer structure with predictable functionalities. Controlled and/or living polymerization techniques that are combined with other new synthetic methodologies enable the synthesis of a large array of new functional PHA-based materials with various architectures and many new accessible materials features for specific applications. In the following sections, the chemically modified PHA-based materials will be discussed with respect to graft and block architectures, with a special focus on the significant impact of the expanded applications that are derived from the enhanced material properties.

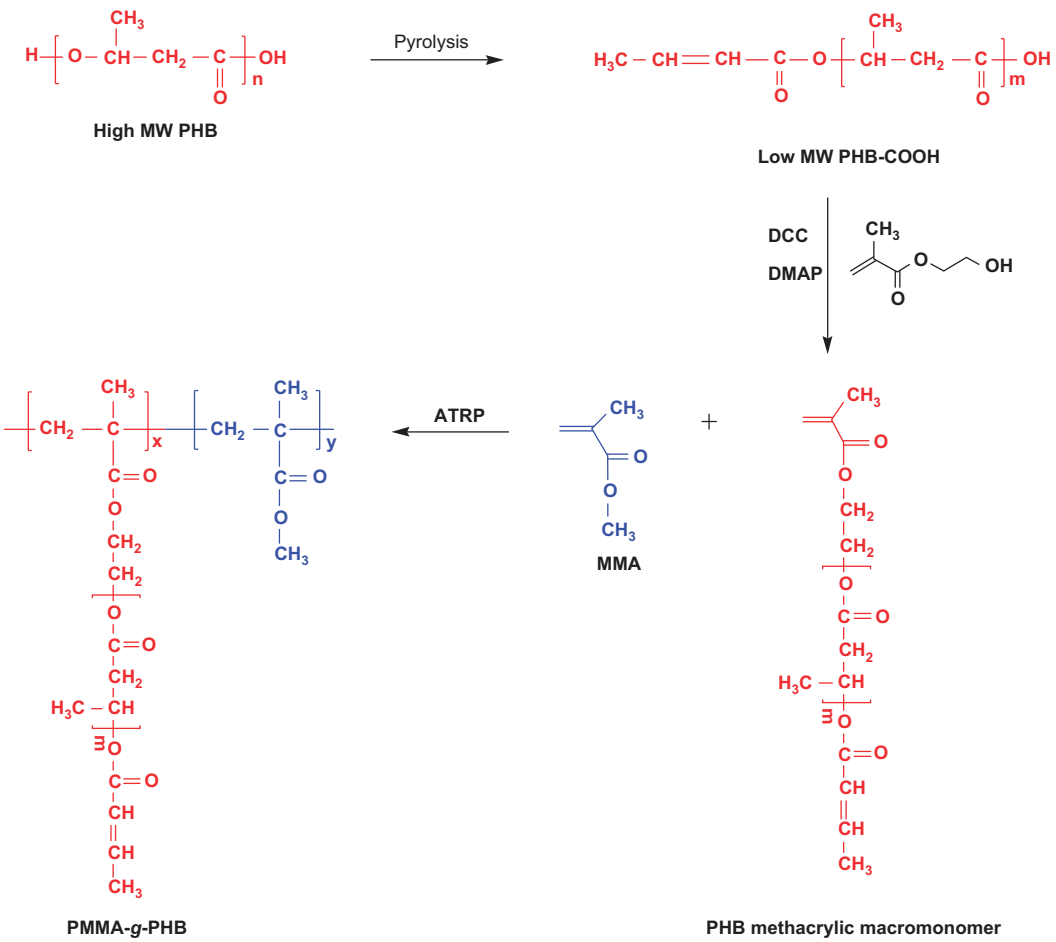

Figure 5 Synthesis route of PMMA-*g*-PHB copolymer by the combination of high-molecular-weight PHB pyrolysis, and the esterification and copolymerization of PHA macromonomers with MMA using ATRP.

PHAs modification via graft copolymerization

PHA grafted on natural polymers. PHA-grafted derivatives were synthesized by conjugation with natural polymers. The amine groups of chitosan react with the carboxyl group-terminated PHB to yield PHB-*g*-chitosan graft copolymers, whereas the esterification between the carboxyl group-terminated PHB and hydroxyl functions in cellulose generates PHB-*g*-cellulose graft copolymers.[39] In a similar approach, PHBV and PHO oligomers have also been reported to graft onto chitosan to yield either PHBV-*g*-chitosan or PHO-*g*-chitosan copolymers.[40] The presence of hydrophilic chitosan caused the PHA-grafted derivatives to exhibit an amphiphilic behavior, in which the solubility of the final product was dependent on the degree of grafting percentage. PHA-*g*-chitosan copolymers with controllable solubility in 2 wt% acetic acid and water can be controlled by changing the grafting percentage. These new grafted materials exhibited high potential in medical applications, such as tissue engineering and drug delivery systems, due to their antimicrobial activity and biocompatibility.[40]

PHA/vinyl- and (meth)acrylate-grafted copolymers. PHA-grafted copolymer can also be produced by radical polymerization of monomers/oligomers that contain vinyl or (meth)acrylate groups. The grafting chains that include Polyethylene glycol (PEG), 2-hydroxyethylmethacrylate (HEMA) and poly(methylmethacrylate) (PMMA) have been previously investigated.[7] For example, PEG that contains acrylate groups were employed to prepare PHA-grafted copolymers by irradiation (Figure 3).[41] The presence of PEG chains in the polymer networks increased the hydrophilicity of the final product. The significant concentrations of water within the PHO-*g*-PEG polymers provided a low interfacial tension with blood and reduced the protein adsorption and platelet adhesion, which has been recognized as an essential requisite for materials that are employed in blood contacting devices. The blood compatibility test confirmed the increasing trend due to an increased fraction of grafted PEG chains, which indicates the potential use of PHO-*g*-PEG polymer networks in blood-compatible biomedical applications.[41] In addition, the mechanical strength was optimized by the PEG concentration. In a typical example, the optimal tensile strength of the PHO-*g*-PEG (50/50, w/w) is 219 kPa, and the elongation at break is 379%, which indicated a significant improvement compared with the pure PHU polymers.[41]

Active radical sites along the polyhydroxyundecenoate (PHA) main chain can be created by abstracting the proton at the C-3 carbon in PHA to graft vinyl chains by monomer polymerization.[42] For example, when methyl mechacrylate (MMA) was polymerized in the presence of aqueous PHB suspension using benzoyl peroxide (BPO) as an

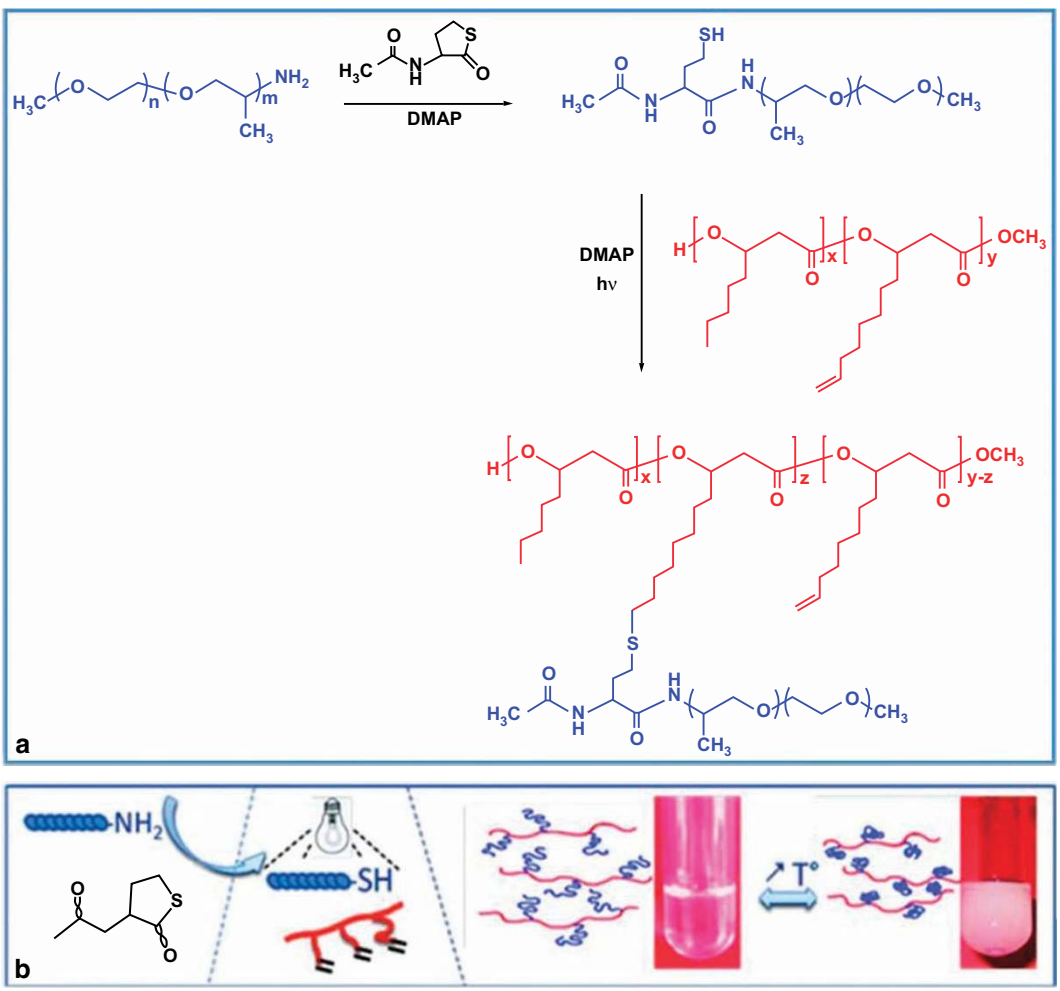

Figure 6 (**a**) Synthesis route of PHOU-*g*-Jeffamine copolymers and (**b**) thermoresponsive illustration of the copolymers in aqueous solution. Adapted from reference 50 (2012 WILEYVCH Verlag GmbH & Co. KGaA, Weinheim).

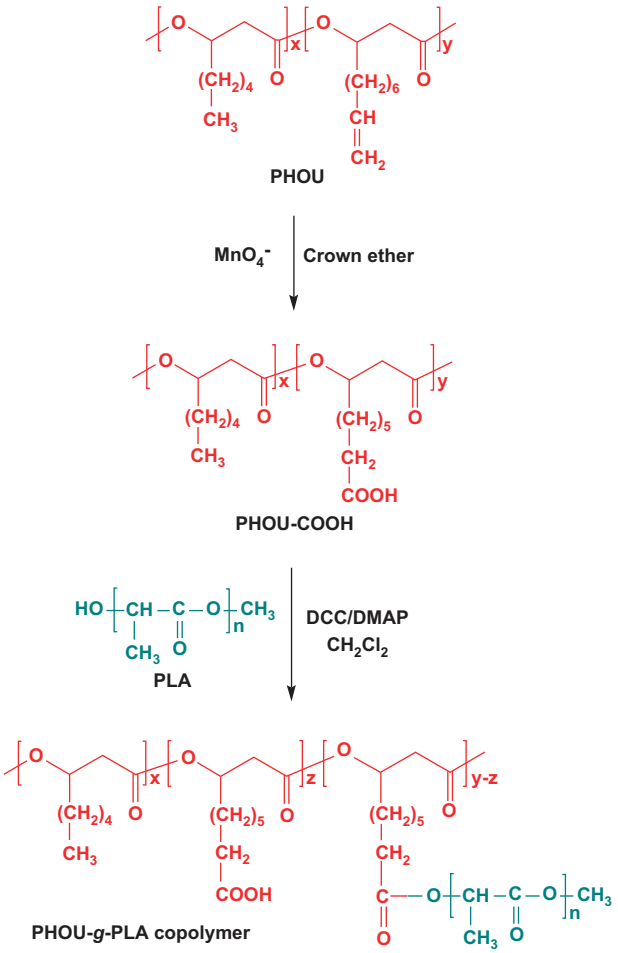

Figure 7 Synthesis route of PHOU-COOH by oxidation and its subsequent preparation of PHOU-*g*-PLA copolymers from esterification with hydroxyl groups of PLA.

initiator, PMMA branches were able to graft on PHB by the covalent bonding between C-3 carbon of PHB and the vinyl CH of MMA repeating units (Figure 4).[43] However, both PHB-*g*-PMMA and PMMA homopolymer were detected under this grafting mechanism. In another study, PHA-*g*-PMMA grafting copolymers were prepared by irradiation-induced radical polymerization[44] to determine the irradiation-dose-dependent grafting efficiency of MMA onto both PHB and PHBV. However, the higher crystallinity of the PHB compared with PHBV caused a lower degree of grafting for PHB-*g*-PMMA than PHBV-*g*-PMMA. According to a previous study, the degree of grafting was dependent on the differences in regularity in the crystalline regions or the crystallinity and rate of radical decay. In this study, the crystalline regions of PHB remained almost unchanged after grafting, whereas the crystalline regions of PHBV in the PHBV-*g*-PMMA samples were partially destroyed by the introduction of grafting.[44] In addition, the irradiation method was also employed to prepare PHBV-*g*-poly(acrylic acid) (PAA) copolymers.[45] The glucosamine was covalently linked to the functionalized PHBV-*g*-PAA surface by carbodiimide chemistry, which reveals that the modified substrates are suitable for the additional conjugation of bioactive molecules.[45]

Recently, 2-hydroxyethylmethacrylate (HEMA)-grafted PHBV films (HEMA-*g*-PHBV) were also prepared using BPO as an initiator (Figure 4).[46] With an increase in HEMA concentration, the graft copolymerization occurred in the bulk of the film and was more prominent at the PHBV surface. Compared with the untreated PHBV, the HEMA-*g*-PHBV showed a significant decrease in crystallinity and improvement of wettability due to the introduction of hydroxyl groups in HEMA. The presence of many hydroxyl groups on the PHBV surface provides a new opportunity to modify the instinct PHAs with bioactive molecules.

Saad *et al.*[47] investigated the grafting behavior of N-vinylpyrrolidone (VP) onto PHBV. The polyvinylpyrrolidone (PVP) grafting content may increase to 45% if the reaction is conducted in a solution using 2,2′-azobisisobutyronitrile as an initiator. Compared with the ungrafted PHBV, the obtained PHBV-*g*-PVP grafting copolymer possessed improved thermal stability. The hydrophilic PVP grafting promoted the degradation of the polymer. In addition, the hydrophilic nature of PVP with a positive charge also enhanced the swelling properties and active antibacterial effect of the grafted PHBV-*g*-PVP copolymer.[47] The graft copolymers that contain PHAs as the branches can also be constructed via the copolymerization of PHA macromonomers with other (meth)acrylic family members. Nguyen *et al.*[48] reported PMMA-*g*-PHB graft copolymers with acrylic-type backbone and side chains that are composed of segments of PHB as branches (Figure 5). The reactivity of PHB macromonomers that were prepared from the esterification of PHB-COOH and HEMA was higher than the macromonomers that were prepared using MMA at a low molar feed ratio but decreased at higher macromonomers proportions. This finding was due to the incompatibility between the macromonomer chains and the propagating comonomer chains, which have a significant influence on the degree of interpenetration and the excluded volume effects around the terminal polymerizable group of the growing chains.[48] PMMA-*g*-PHB-grafted copolymers were evaluated as possible constituents in acrylic bone cements.[49] A formulation with a concentration of PMMA-*g*-PHB copolymer that exceeded 20 wt% produced cement doughs. The ultimate compressive strength test demonstrated that the value exceeded the minimum level and was similar to the value of Antibiotic Simplex or AKZ (Stryker Canada LP, Howmedica Osteonics, Guelph, ON, Canada).[49]

Other functional PHA-grafted copolymers. The unsaturated groups in the pendant chain of PHAs can be employed to conjugate with different oligomers to prepare other types of functional PHA-grafted copolymers. For example, Renard and colleagues reported the synthesis of amphiphilic graft copolymers that consist of hydrophobic PHAs backbone and hydrophilic α-amino-ω-methoxy poly (oxyethylene-*co*-oxypropylene) (Jeffamine) branches via a thiol-ene reaction (Figure 6a).[50] Poly(3-hydroxyoctanoate-co-3-hydroxyundec-10-enoate) (PHOU)-*g*-Jeffamine-grafted copolymers with >13 mol% of Jeffamine were determined to be water soluble and exhibited thermoresponsive behaviors (Figure 6b). In addition, the thermoresponsive properties were tunable with different Jeffamine compositions, which encompassed a range of cloud-point temperatures from 17 to 29 °C. In a similar manner, PHOU-*g*-PEG-grafted copolymers were synthesized and formed well-defined nanoscale vesicles in water.[51] In contrast to these strategies, the unsaturated groups in PHAs were also converted to carboxyl groups via oxidation and employed to prepare PHA-graft copolymers by direct esterification with hydroxyl groups of PEG or PLA oligomers (Figure 7).[52]

Zhou *et al.* reported the synthesis of PHA-grafted poly (ethyleneimine) (PEI) copolymers (PHA-*g*-bPEI) by Michael addition between acrylated-PHA and branched PEI (Figure 8a). The hydrophilic nature of PEI rendered P3/4HB-*g*-bPEI copolymer soluble in buffer solution; the surface charge was effectively employed to bind synthetic RNA (siRNA) to prevent its degradation by nucleases.

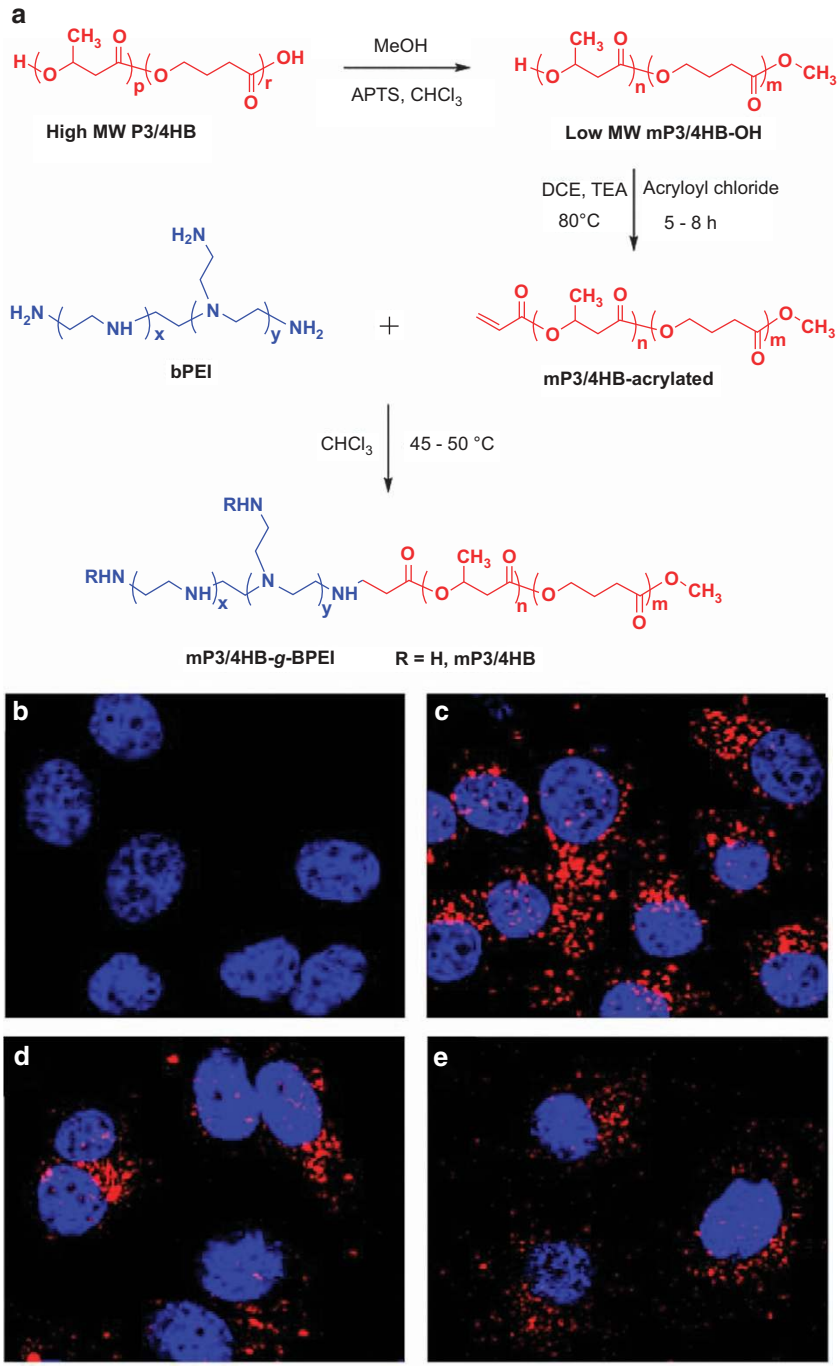

Figure 8 (**a**) Synthesis route of mP3/4HB-acrylate and its preparation of mP3/4HB-*g*-bPEI copolymer by Michael addition. Confocal microscopy images of the A549 cell uptake of (**b**) free siRNA and (**c**) P3/4HB-*g*-bPEI/siRNA complexes. (**d**) Lipofectamine and (**e**) bPEI were employed as the control. Cy3-labeled siRNA appeared red fluorescence and the cell nuclei were stained blue using DAPI. Adapted with permission from reference 53 (2012 Elsevier).

In a typical example, the high intensity of intracellular Cy3-siRNA fluorescence was observed in cells that were transfected with P3/4HB-*g*-bPEI copolymer compared with cells that were incubated with commercial Lipofectamine and PEI, which indicates improved cellular uptake and subcellular distribution (Figures 8b–e).[53] The stability of the P3/4HB-g-bPEI/siRNA complexes was disrupted by the presence of PHA, and the degradation of the ester bonds in P3/4HB simplified the release of siRNA into cytoplasm for efficient gene silencing. In the tested A549-Luc cells and MCF-7-Luc cells, a significant breakdown of luciferase expression from 70 to 90% was observed when the cells were transfected with P3/4HB-g-bPEI copolymers/siRNA complexes, which demonstrated their potential as safe and efficient siRNA carriers for human gene therapy.[53]

PHAs modification via block copolymerization

Block copolymerization of PHAs with different functional polymers enables the modification of their properties. Many advanced biomedical applications were achieved using the newly developed

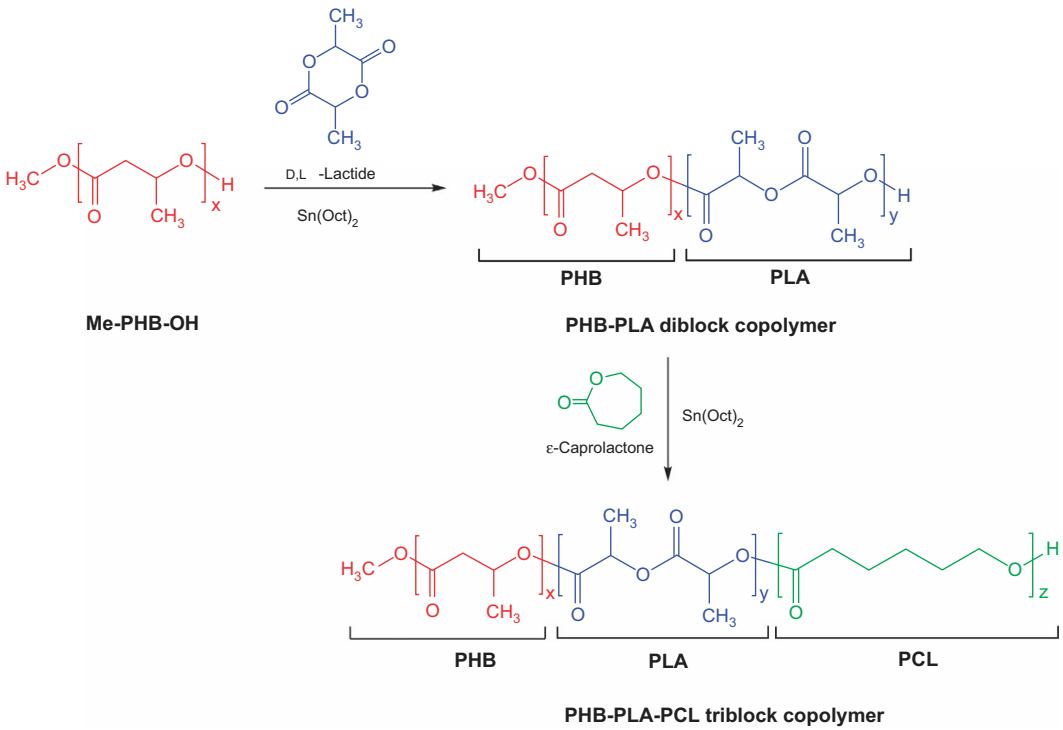

Figure 9 Synthesis of the PHB-PLA-PCL triblock copolymer by consecutive ring opening polymerization.

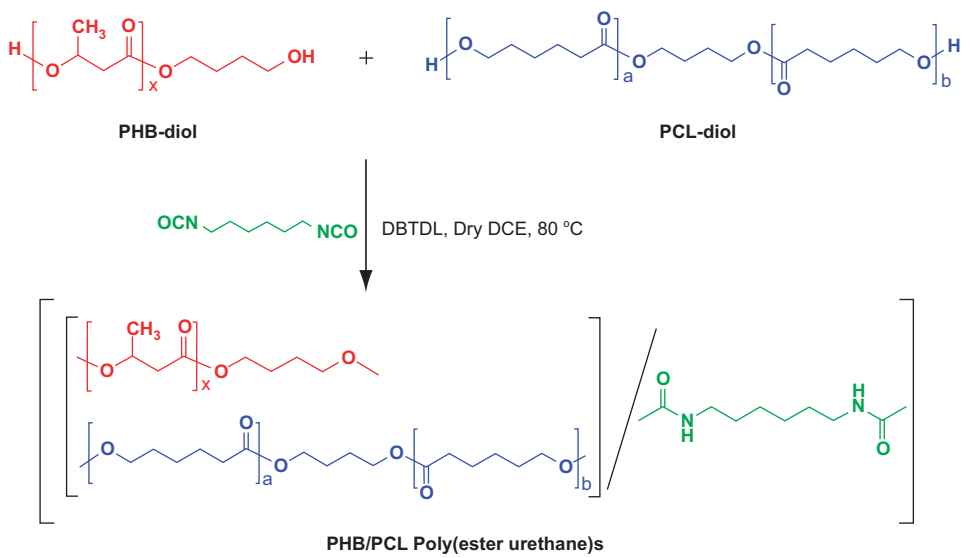

Figure 10 Synthesis of PHB/PCL poly(ester urethane)s block copolymers that use HDI as a coupling agent.

PHA block copolymers. In this section, PHAs-based block copolymers that contain various types of building blocks will be reviewed.

PHA/polyester block copolymers. Active PHB-O-$AlEt_2$ macroinitiator species were previously prepared from the reaction between mono-hydroxyl-terminated PHB macromonomers and $AlEt_3$. This macro-initiator was employed to conduct the ring opening polymerization of ε-caprolactone (CL) or lactide monomers to obtain PHB-PCL and PHB-PLA diblock copolymers.[54] Similarly, elastmeric soft segments of PHO and PCL were combined to prepare semicrystalline PHO-PCL diblock copolymers, in which the crystallinity of the copolymers were also controlled by the lengths of the two different blocks.[55] Consecutive ROP of lactide followed by CL using PHB as a macroinitiator afforded PHB-PLA-PCL triblock copolymers (Figure 9). The A-B-C type of triblock copolymers are composed of a PHB-PLA hard segment and a PCL soft segment that induced microphase separation in the polymer film. The PHB-PLA hard segment domains serve as physical crosslinkers for the PCL soft segment matrix.[56]

Another interesting approach to prepare the PHA-polyester block copolymer is to link PHAs and telechelic polyester oligomers with a proper coupling agent, such as diisocyanate and terephthaloyl

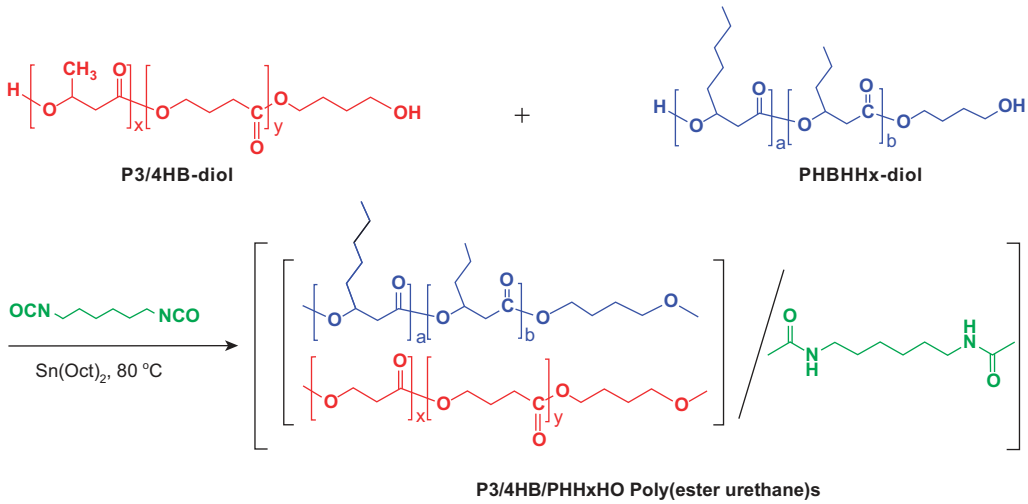

Figure 11 Synthesis of PHA-based poly(ester urethane)s that contain different PHAs as building blocks. Adapted from reference 60 (2009 Elsevier).

chloride. Saad *et al.*[57] reported a one-pot solution synthesis of PHB/PCL/1,6-hexamethylene diisocyanate (HDI)-segmented poly (ester urethane)s with different compositions (Figure 10). The results indicated that the thermal and mechanical properties of the PHB/PCL poly(ester urethanes) were successfully manipulated by changing the block lengths of the hard and soft components. In another study, P3/4HB/PCL poly(ester urethane)s that consisted of P3/4HB and PCL were coupled by HDI in a melt state.[58] The P3/4HB/PCL poly(ester urethane)s with a concentration of 4 HB possessed richer platelet adhesion and rapid blood coagulation, as well as lower cytotoxicity toward rat aortic smooth muscle cells, which indicates that P3/4HB/PCL poly(ester urethane)s can be employed as a potential candidate for wound healing applications.[58]

The coupling of different types of PHA segments have diversified a large group of new PHAs-based block copolymers with enhanced properties, such as adjustable mechanical strength, surface features and programmed degradation rate. For example, the physical properties of P3/4HB, which range from soft elastomers to high crystallinity plastics, were produced by altering the 3HB rate in the copolymer to a 4HB rate. Recently, Xu and coworkers reported a coupling synthesis of different types of P3/4HB-diols with a 4HB concentration that varied from 9.3 to 32.3%.[59] The crystallinity, hydrophobicity and surface free energy were tailored by the 4HB content, the P3/4HB segmental length and the urethane linkage concentration in P3/4HB poly(ester urethane)s. From the improved properties of lactate dehydrogenase activity, strong platelet adhesion and cell compatibility, the authors demostrated the use of P3/4HB poly(ester urethane)s as potential hemostatic materials.[59] Similarly, tailored poly(ester urethane)s based on P3/4HB and poly(3-hydroxyhexanoate-*co*-3-hydroxyoctanoate) (PHHxHO) were synthesized (Figure 11).[60] The obtained P3/4HB/PHHxHO poly(ester urethane)s were hydrophobic with a minimum water contact angle of 100°, which is more hydrophobic than the corresponding raw polymers and PLA. Hydrophobicity is an important factor that affects the platelet activation and produces strong platelet adhesion for blood coagulation. The incorporation of PHAs with different side chain lengths can tune the hydrophobicity and platelet adhesion behaviors of P3/4HB/PHHxHO poly(ester urethane) s toward specific requirements as wound healing materials.[60] Andrade *et al.*[61] reported the preparation of novel block copolymers that contain telechelic PHO-diol and telechelic PHB-diol by different coupling methods. The newly chemically synthesized polymer that contained both soft segments and hard segments, that were derived from PHAs showed excellent thermoplastic properties and mechanical properties, which is a potentially useful material for medical applications.

PHA-polyether block copolymers. The intrinsic hydrophobicity of natural PHAs and their slow degradation rate have raised many concerns in biomedical applications. The presence of desired hydrophilic components in PHA block copolymers enabled the manipulation of the amphiphilicity and chemicophysical properties. Diblock copolymers that contain high-molecular bacterial PHB with monomethoxy-PEG (mPEG) were synthesized in a melt reaction using *bis*(2-ethylehexanote) tin as a catalyst. This single-step synthesis of mPEGylated PHB proceeded with a concomitant depolymerization of PHB molecular weight to a few thousands.[62] Once formed from oil-in-water emulsion, PHB-PEG diblock copolymer can self-assemble into nanoparticles, which can be potentially employed as drug carriers or binders. Using the same approach, nanometer-sized core-shell micelles were prepared from amorphous amphiphilic block copolymer P(3HV-*co*-4HB)-mPEG and were further explored in cancer therapy. The *in vitro* release profile of cisplatin from P(3HV-*co*-4HB)-mPEG micelles showed a sustained release of the drug, and the drug accumulation became more distinct compared with the free drug in solution.[63] Li *et al.*[64] reported the synthesis of amphiphilic triblock copolymers PEG-PHB-PEG by the coupling reaction between methoxy-PEG-monocarboxylic acid and PHB-diol, which was catalyzed by dicyclohexylcarbodiimide (DCC) and DMAP (Figures 12a and b). Prior to threading with α-CD, PEO–PHB–PEO aqueous solution is a flowable solution; after threading with α-CD, it forms a self-assembled hydrogel (Figures 12c and d). The inner structure α-CD–PEO–PHB–PEO hydrogel formation is also illustrated (Figure 12e). The water-soluble PEG-PHB-PEG copolymer self-assembled into micelles, which are useful for potential drug delivery applications.

The region-selective channel structure of the solution with α-cyclodextrins can trigger the phase transformation from sol to hydrogel formation, which provided a well-controlled long-term release profile of the encapsulated molecules without burst release.[65] PHA-polyether block copolymers were prepared via a diisocyanate chain extension reaction with hydroxyl-capped polyethers such as PEG and Poly(propylene glycol) (PPG). A series of PHB/PEG poly(ether

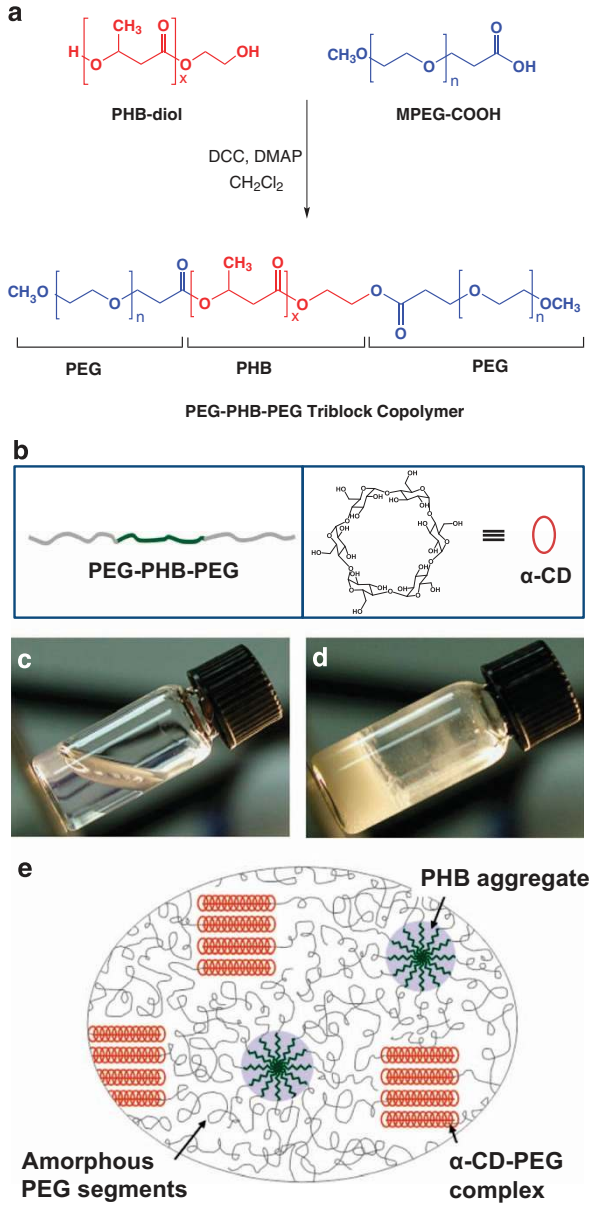

Figure 12 (**a**) Preparation of PEG-PHB-PEG triblock copolymers and (**b**) Schematic of PEG-PHB-PEG triblock copolymer and α-CD; (**c**) Optical photographs of the PEO–PHB–PEO aqueous solution and (**d**) its self-assembled hydrogel with α-CD; (**e**) Illustration of the inner structure of α-CD–PEO–PHB–PEO hydrogel formation. Adapted with permission from reference 65 (2006 Elsevier).

ester urethane)s block copolymers with various PHB and PEG segment lengths were synthesized.[66] The hydrophilicity and water swelling analysis was significantly improved via the incorporation of PEG segments. The degradation studies revealed a random chain scission of the ester backbone bonds of the PHB segments, and the surface roughness of the films increased as the degradation proceeded.[67] The degradation components were water-soluble products, including PEG blocks, 3-hydroxybutyric acid and crotonic acid. The synthesis of alternative block polyurethane copolymers P3/4HB-*alt*-PEG based on the coupling reaction between P3/4HB-diol and PEG-diisocyanate with different segment lengths was reported (Figure 13).[68] The synthetic methodology for P3/4HB-*alt*-PEG poly (ether ester urethane)s provided a method for controlling the exact structure of the biomaterials and tailoring the properties to subtle requirements. The hydrophilic surface and regular surface microstructure endowed the P3/4HB-*alt*-PEG poly(ether ester urethane)s with better hemocompatibility. The cell culture assay revealed that both fibroblasts and rat glial cells were more favorable for the attachment on P3/4HB-*alt*-PEG poly(ether ester urethane)s films, which indicates that the arrangement of PHA/polyether polyurethanes in an alternative block architecture can easily realize the topography control of the materials and achieve the practical structure–property relationship in biomaterial applications.[68]

PPG is a thermoresponsive polymer that undergoes a hydrophilic to hydrophobic transition when the temperature changes across their corresponding lower critical solution temperatures (LCSTs). Our group reported the synthesis of multiblock amphiphilic and thermosensitive poly(ether ester urethane)s, which consist of PHB, PEG and PPG blocks (Figures 14a and b).[69] At PHB concentrations below 11.4 wt%, PHB/PEG/PPG poly(ether ester urethane)s were soluble in water and self-assembled into micelle formation with extremely low concentrations. When the concentration increased from 2 to 5 wt%, the PHB/PEG/PPG poly(ether ester urethane)s solution underwent sol-gel-sol transition as the temperature increased from 4 to 80 °C. The temperature-dependant sol–gel transition of the copolymer solution was attributed to the increased association of the micelles, which was caused by the multiple segments that link the micelles in a network-like gel structure.[69,70] The hydrolytic degradation of PHB/PEG/PPG poly(ether ester urethane) hydrogels was characterized by an initial incubation period, followed by the erosion of the polymer gel and the random chain scission of the ester bonds in PHB segments.[71] The relationship between the protein release and the hydrogel degradation process was also correlated. Due to control of the gel formulation and polymer composition, the release duration of the protein from 20 to 80 days was successfully demonstrated (Figures 14c, d).[71] In addition, the cell growth on the PHB/PEG/PPG poly(ether ester urethane)s hydrogel surface exhibited a typical spindle-like fibroblast morphology; a healthy morphology was maintained after 72 h of incubation. Conversely, the hydrophilic surface of F127 gels inhibited the growth of cells for longer time periods, which suggests that the incorporation of the PHB segment in PHB/PEG/PPG poly(ether ester urethane)s copolymers significantly increased the hydrophobicity of the gel surface, which enhances the cell adhesion capability and facilitates cell growth.[70]

PHA-poly(methacrylate) block copolymers and others. The incorporation of poly(meth)acrylates to PHA to form block copolymers is another important strategy for making the newly designed PHA materials accessible to controllable hydrophilicity, pH and temperature responsiveness, as well as therapeutic functions by simple variation of the substitute groups. Our group previously reported the synthesis of thermoresponsive amphiphilic PNIPAAm-PHB-PNIPAAm triblock copolymers by the combination of esterification and atom transfer radical polymerization (ATRP) (Figure 15a).[72] The obtained PNIPAAm-PHB-PNIPAAm triblock copolymers were water soluble with a LCST range of 28–29 °C in water.[72] At temperatures below the LCST, PNIPAAm-PHB-PNIPAAm self-assembled into core-shell micelle aggregates at 50 mg l^{-1}. At elevated temperatures, the hydrophilic to hydrophobic transition caused the PNIPAAm chains to collapse in the micelle corona, which enabled the formation of smaller particles (Figure 15b). The temperature responsiveness of the micelles was subsequently employed to design a PNIPAAm-PHB-PNIPAAm copolymer-coated surface for nonenzymatic thermal-induced cell detachment in tissue engineering (Figure 15c).[73,74] In

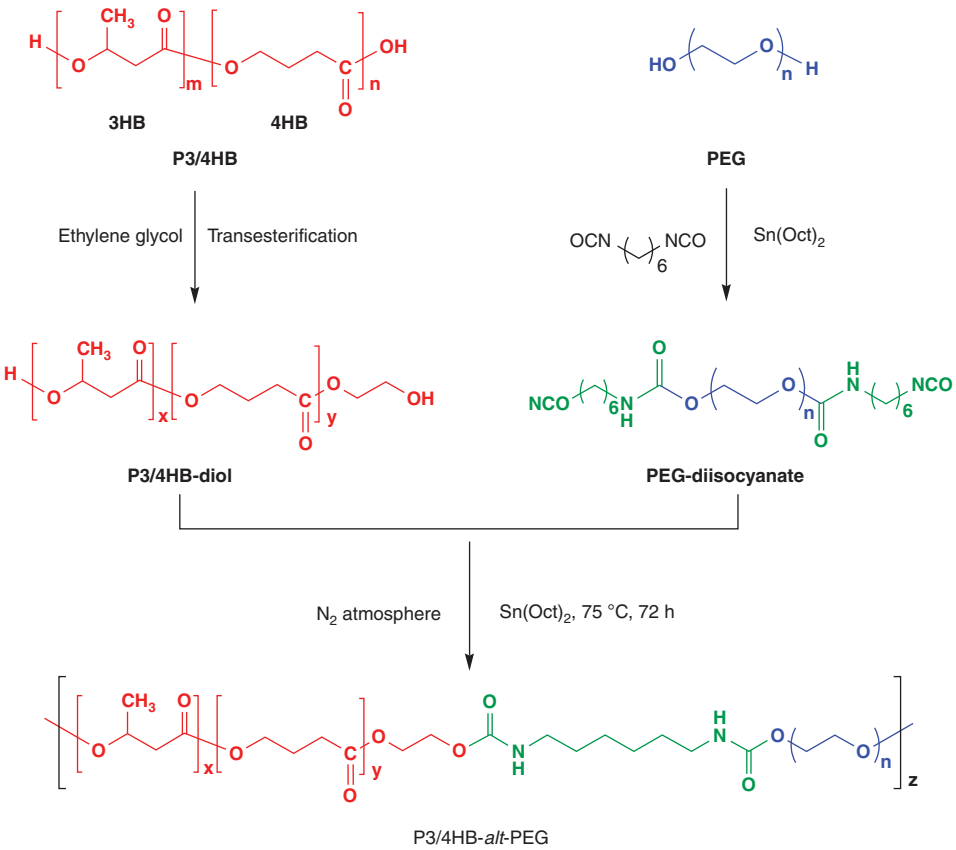

Figure 13 Synthesis of alternative P3/4HB-*alt*-PEG poly(ether ester urethane)s block copolymers. Adapted from reference 68 (2009 Elsevier).

this application, the micelles formed stable coatings on the polyester substrate surface via hydrophobic interactions, which caused the coated surface to exhibit thermoresponsive behaviors, that is, they were hydrophobic at higher temperatures and became hydrophilic when cooled to lower temperatures. Embryonic stem cells and human mesenchymal stem cells were employed in the cell culture to prove the concept of temperature-induced cell detachment. The hydrophobic surface at temperatures above the LCST supports cell adhesion and growth and the detachment of cells at 4 °C without trypsin digestion. Specifically, the embryonic stem cells demonstrated cell morphologies similar to the morphologies cultured using standard cell culture procedure, which indicates that the coatings do not affect the viability of the cells.[74] Human mesenchymal stem cells showed strong intercellular associations, and a sheet of cells was obtained. The preservation of cell–cell and cell–extracellular matrix interactions by this mild technique indicates the high potential of PNIPAAm-PHB-PNIPAAm coating as rapid culture and detachment surfaces, which may be very useful for cell harvesting in tissue regeneration therapies.

Temperature and pH-sensitive amphiphilic triblock copolymers with a central hydrophobic PHB block flanked by two hydrophilic poly(2-(di-methylamino)ethyl methacrylate) (DMAEMA) blocks was prepared.[75] The micelles were employed for drug encapsulation. The effectiveness of pH- and temperature-triggered drug release was investigated. The results revealed a slow rate of drug release at 20 °C, whereas a faster release rate was obtained when the micelle solution was incubated at 37 °C (Figure 16a).[75] The investigation of pH in triggering the drug release demonstrated that a faster rate was obtained at pH 5 compared with the rate at pH 7 (Figure 16b) because the micelles formed at pH 5 were smaller than the micelles formed at pH 7 and possessed a greater surface area for the enhancement of the release rate from the system.[75] These smart micelles were effectively internalized by HeLa cells and exhibited higher *in vitro* drug delivery efficiency than the commercially available Pluronics F127 (Figures 16c and d).[75] With the presence of a positively charged tertiary amino group of poly(2-dimethylaminoethyl methacrylate) (PDMAEMA), we also demonstrated that PDMAEMA-PHB-PDMAEMA copolymers can have significant potential as a gene delivery vector to transport plasmid to cells (Figure 16e).[76]

Other types of PHA-modified functional polymers, such as PHA-protein amphiphilic block copolymers were reported via a biosynthetic process. The protein component in this new type of block copolymer can be designed with a specific sequence and end-functionality by genetic engineering. This feature enabled the micelle system to self-assemble from a PHA-protein to a drug carrier with targeting capability (Figure 17). For example, Cys-Asp-Cys-Arg-Gly-Asp-Cys-Phe-Cys (RGD4C) was conjugated to the protein in PHA-protein block copolymer and exhibited strong molecular affinity with integrin overexpressed in cancer cells. The use of PHA as the reservoir of hydrophobic drugs caused RGD4C ligated PHA-protein micelles to exhibit an effective uptake to the targeted MDA-MB-231 breast cancer cells.[77]

CONCLUSIONS AND OUTLOOK

PHAs are a class of diverse polyesters that are naturally produced by various bacteria. These materials are at the forefront of biodegradable and biocompatible polymer research because PHAs and their degradation products are nontoxic after hydrolysis and demonstrate their potential for applications in the medical-related field. Many

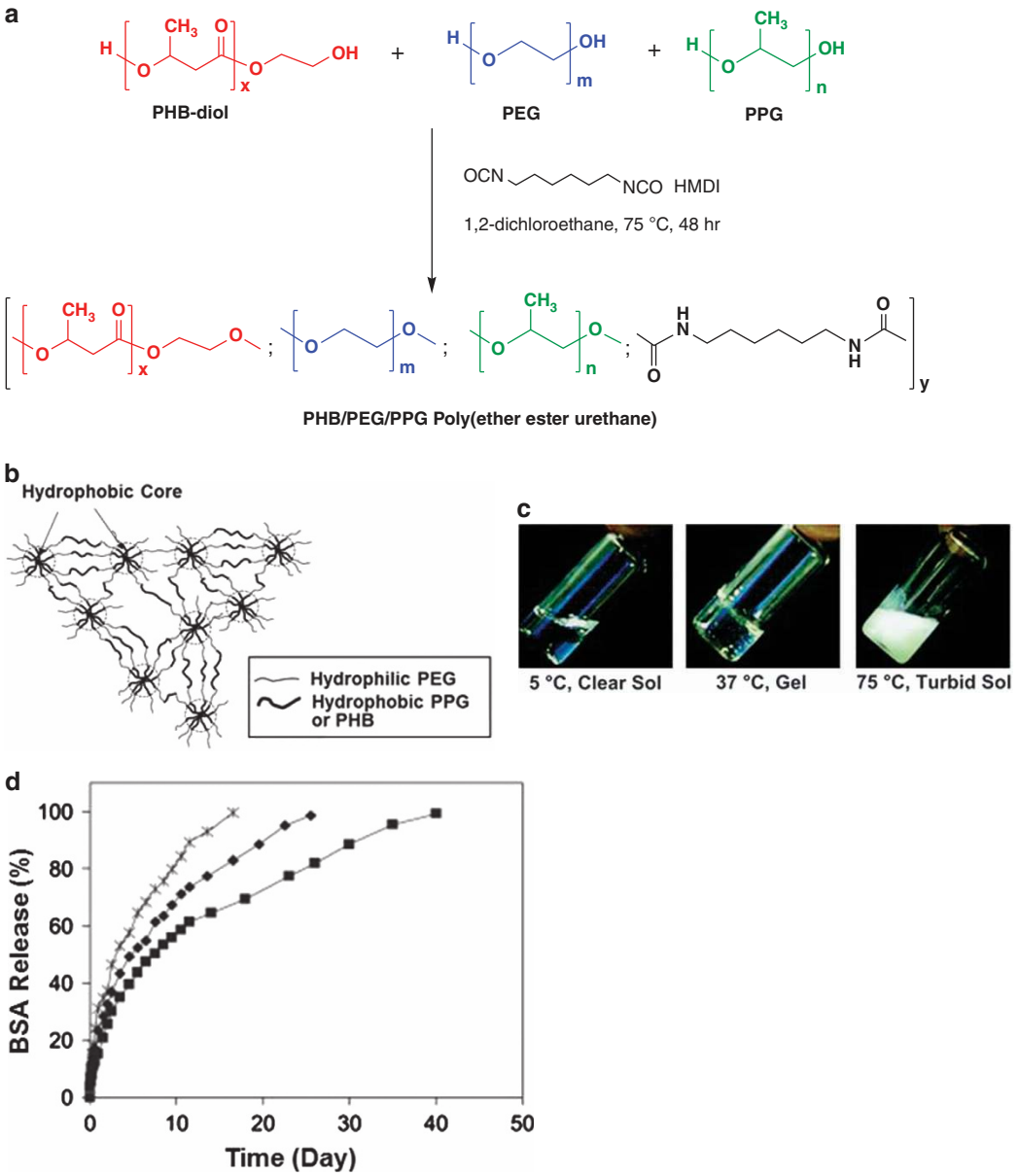

Figure 14 (**a**) Synthesis of PHB/PEG/PPG poly(ether ester urethane) block copolymers, and (**b**) Schematic of associated micelle model depicting network polymer packing gelation; (**c**) Sol-gel-sol transition of the copolymer aqueous solution with increasing temperature. Adapted from reference 69 (2007 American Chemical Society). (**d**) Protein release profiles for PHB/PEG/PPG poly(ether ester urethane) hydrogels of different copolymer compositions. (✻: 3 wt %, ◆: 4 wt%, ■: 5 wt%). Adapted from reference 71 (2007 Elsevier).

different approaches have been employed to modify the polyester and its properties. Blending is highlighted due to its ease of processing. Blending enables the facile modification of physical properties, such as T_m, glass transition temperature, crystallinity and mechanical properties. These modifications often produce properties that fall within the extremes yielded by the polymers. This limitation indicates that outstanding properties cannot be formed by blending two different polymers. The blending of polymers is complicated by interfacial interactions between the two polymers. Poor interfacial interactions often cause the deterioration of properties and subsequently poorer mechanical behavior. For this reason, the polymers should be modified by grafting or copolymerization. In these cases, the total polymer property is affected. A hydrophobic polymer can be rendered amphiphilic by these approaches. Analyzing the modification of PHB with PEG enables an understanding of the difference between the two approaches: the blending of PHB with PEG produces a blend system that is inherently hydrophobic, whereas the PEG fraction will leach when exposed to an aqueous solution. The chemical conjugation of the PEG block segment to PHB causes the formation of an amphiphilic copolymer, which is soluble in water. This amphiphilic polymer can be employed in applications, which significantly differs from the potential of applications with simple blends. This polymer can be applied in biomedical applications, such as the solubilization of drugs, the delivery of therapeutics or as carriers of genetic materials. In Table 1, we summarize the properties of the blends and copolymers. In this table, we have also highlighted applications of these materials to showcase the possibilities of these materials.

Table 1 Material Characteristic Properties of Modified PHAs via Different Methods and Their Applications

Material type	*Polymers*	T_m *(°C)*	T_g *(°C)*	X_c, *(%)*/T_d*(°C)*	*Mechanical properties*	*Applications*	*Reference*
Polymer blends	PHB/starch	165.4 to 167.9	67.2 to 87.4	T_d = 200.5 219.7	σ_{max} = 4.99 to 19.7 MPa, E: 578 to 1716 MPa, ε_{max} = 3.5 to 9.8%	Coating materials, cardboard for food package	10
	PHB/SA	171.0 to 175.9	8.6 to 9.9	ND	ND	ND	11
	PHBV/PGA-*g*-starch	ND	ND	ND	σ_{max} = 16.8 to 24.3 MPa, E: 458 to 539 MPa, ε_{max} = 13 to 18%	ND	12
	PHB/PVAc-*g*-starch (CSV)	167 to 171	3 to 25	$X_{c,PHB}$ = 32.9 to 66.4 T_d = 268 to 295	ND	ND	13
	PHB/EC	175.3 to 177.0	44.6 to 56.1, annealed samples	$X_{c,PHB}$ = 15.4 to 64.3	ND	ND	15
	PHB/CAB	178.5 to 189.5	6.3 to 12.5	$X_{c,PHB}$ = 40.6 to 53.2	σ_{max} = 13.3 to 29.3 MPa, E: 592.4 to 2288.3 MPa, ε_{max} = 2.2 to 7.3%	ND	16,79
	PHB/lignin	152 to 174	7.0 to 43.0	$X_{c,PHB}$ = 21 to 61	G´ = 3–5 × 10^4 Pa at ω of 1 to 100 rad s^{-1} G´´ = 50-1 × 10^5 Pa at ω of 1 to 100 rad s^{-1}	ND	18
	PHB/soda lignin	ND	8.6 to 39.5	T_d = 162 to 212	ND	ND	20
	PHB/PHBHHx	Approximately 152 to 165	Approximately 0.8 to 5.0	ND	E: 500 to 1210 MPa	Scaffolds for tissue engineering with improved biocompatibility	22,23
	PHBV/PLA	PLA:150, PHBV: 170	PLA: 53.2 to 57.6 PHBV: ~3	ND	G´ = 20-1.2 × 10^4 Pa at ω of 0.01 to 100 rad s^{-1}, η = 500 to 1030 Pas at ω of 1 rad s^{-1}	ND	29
	MCL-PHA/PLA	50 to 180	PLA: 55.7 to 57.7, PHA: −32.8 ~ −33.5	$X_{c,PHA}$ = 25 to 58	σ_{max} = 30 to 50 MPa J = 8.1 × 10^{-2}–26.0 × 10^{-2} J	Ductile and tough properties for plastics applications	30,31
	PHB/PLA	174.6 to 175.1	PHB: 9.6 to 11.0 PLA: 48.3 to 54.9	$X_{c,PHB}$ = 55.0 to 56.3	E: 274 to 366 MPa σ_{max} = 6.5 to 8.7 ε_{max} = 3.7 to 27.7%	ND	32
	P3/4HB/PLA stereo-complex (SC)	PLA SC: 218	P3/4HB: -12.5	$X_{c,PLA\ SC}$ = 38.1 to 45.1	σ_{max} = 4.2 to 6.6 MPa, E: 30.8 to 46.7 MPa, ε_{max} = 362.7 to 949.0	Enhanced processabiltiy and enzymatic hydrolysis rates	34
	PHBHHx/PCL	PHBHHx: 95.4 PCL: 61.1	ND	$X_{c,PHBHHx}$ = 10 to 60	E: 190.9 to 324.6 σ_y = 7.7 to 12.2 MPa, T: 0.3 to 3.6 J cm^{-3}	Improved cell adhesion and proliferation for musculoskeletal tissue engineering	35,36
	PHBV/PCL	PCL: 57.0 to 57.5 PHBV: 137 to 152.4	PHBV: 1.3	$X_{c,PHBV}$ = 5 to 40	E: 170 to 1200 MPa σ_y = 9.0 to 12.5 MPa ε_{max} = 8.0 to 25%	Hollow fibers and tubular scaffold in tissue engineering	37,38
Graft copolymer	PHA-*g*-chitosan PHA: PHB, PHBV, PHO	PHB: 150 to 173 PHBV: 117 to 131 PHO: 80 Chitosan: 105 to 116	ND	T_d = 290 to 308	ND	Drug delivery and tissue engineering	39,40
	PHOU-*g*-PEG	ND	ND	ND	σ_{max} = 219 to 263 kPa ε_{max} = 379 to 621%	Blood contact device, drug delivery	41,51,52
	PHB-*g*-PMMA	PHB: 171.8 to 176.8	PHB: 2.5 to 6.0 PMMA: 100 to 102	$X_{c,PHBV}$: 12 to 27	E_b = 3170 to 3300 MPa Gc = 0.7 to 2.3 KJ m^{-2}	Orthopaedic applications.	43,44,49
	PHBV-*g*-HEMA	138 to 156	2.3 to 63	T_d = 297 to 316	ND	Improved cellular interactions with film	46
	PHBV-*g*-PVP	140.0 to 158.4	7.7 to 8.6	$X_{c,PHBV}$: 35.5 to 45.9	ND	Increased antibacterial activity	47
Block Copolymer	PHOU-*b*-PCL	60 to 61.5	−57 ~ −60	ND	ND	Facilitating biomolecules conjugation	55
	PHB-*b*-PLA-*b*-PCL	123.4 to 130.2	−23.5 ~ −31.2, 11.2 to 32.8	$X_{c,PHB}$: 12.1 to 49.7	ND	Blood contact device	56
	PHB-*r*-PCL	PCL: 38.0 to 62.7 PHB: 133.0 to 141.0	−18.3 ~ −62.0	$X_{c,PHB}$: 36.6 to 53.8	σ_{max}: 11.0 to 30.5 MPa ε_{max}: = 117 to 990%	nd	57
	P3/4HB-*r*-PCL	32.3 to 78.4	−21.5 ~ −40.1	$X_{c,P3/4HB}$: 3.2 to 20.9, T_d = 251.6 to 279.5	ND	Wound-healing materials	58
	P3/4HB-r-P3/4HB[a]	49.8 to 95.4	−10.8 ~ −48.4	$X_{c,P3/4HB}$: 0.5 to 26.8, T_d = 247.7 to 286.3	ND	Hemostatic materials	59
	P3/4HB-r-P3-/4HB/PHHxHO	44.9 to 75.6	−9.0 ~ −44.6	$X_{c,P3/4HB}$: 0.4 to 16.9, T_d = 241.9 to 264.8	ND	Hemostatic materials	60
	PHO-*r*-PHB	61 to 178	−45 – 4	ND	E: 200 to 1560 MPa σ_{max} = 7.0 to 38 MPa ε_{max} = 6.3 to 490%		61

Table 1 (Continued)

Material type	Polymers	T_m (°C)	T_g (°C)	X_c, (%)/T_d(°C)	Mechanical properties	Applications	Reference
	PHB-*b*-PEG	PEG: 47 to 56 PHB: 122 to 152	ND	ND	ND	Excellent thermoplastic properties and mechanical properties for medical applications Self-assemblies for drug delivery	62
	P(3HV-*co*-4HB)-*b*-MPEG	PEG: 53.5	ND	ND	ND	Drug delivery in cancer therapy	63
	PEG-*b*-PHB-*b*-PEG	PEG: 23 to 57 PHB: 140 to 153	ND	$X_{c,PHB}$: 53.3 to 73.6 T_d = 382.9 to 391.0	ND	Supramolecular hydrogel for drug delivery	64,80
	PHB-*r*-PEG	PEG: 31.4 to 61.6 PHB: 82.2 to 148.5	−49 ~ −33	$X_{c,PHB}$: 9.3 to 44.5 T_d = 260.2 to 279.4	E: 21 to 120 MPa, σ_y: 5.9 to 10.2 MPa, ε_{max}: 11-1912%	Biomaterials with programmed degradation rates	66,67
	P3/4HB-*alt*-PEG	32.01 to 53.21	−24.16 ~ −44.26	T_d = 241.7 to 277.9	ND	Hemocompatible materials	68
	PHB-*r*-PEG-*r*-PPG	ND	ND	T_d = 227 to 303	η: 0.05 to 0.2 mPa s at 5 °C; 43 to 55 Pa s above 30 °C.	Thermal-responsive hydrogels for protein release and 3D cell culture	69–71
	PNIPAAm-*b*-PHB-*b*-PNIPAAM	124.3–142.2	ND	$X_{c,PHB}$: 7.3 to 42.6 T_d = 235.9 to 291.9	ND	Temperature-induced smart surface for cell detachment	72–74
	PDMAEMA-*b*-PHB-*b*-PDMAEMA	ND	ND	T_d = 230 to 293	ND	Stimuli-responsive drug delivery and gene delivery	75,76

Abbreviations: CAB, cellulose acetate butyrate; EC, ethyl cellulose; MCL-PHA, medium-chain-length polyhydroxyalkanoates; PGA, polyglycolic acid; PHB, poly(3-hydroxybutyrate); PHBV, poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate); PLA, Poly(lactic acid); PVAc, poly(vinyl acetate); SA, starch acetate.
σ_{max}: Tensile strength, E: Young's modulus, ε_{max}: Elongation at break, G′: Storage modulus, G″: loss modulus, ω: Frequency. *J*: Total absorbed energy in Charpy impact test, σ_y: yield stress. T: toughness, E_b: Bending modulus in Charpy impact test. G_c: Critical energy release factor, nd: not determined.
#P3/4HB with different 4HB contents were employed as prepolymers.

Figure 15 (**a**) Synthesis of PNIPAAm-PHB-PNIPAAm triblock copolymer using PHB-diBr as a macroinitiator in ATRP and (**b**) Thermoresponsive demonstration of PNIPAAm-PHB-PNIPAAm triblock copolymers. Adapted from reference 72 (2009 American Chemical Society). (**c**) Schematic of the temperature-induced cell detachment using a surface that was coated with PNIPAAm-PHB-PNIPAAm triblock copoylmers. Adapted from reference 74 (2009 WILEY-VCH Verlag GmbH & Co. KGaA).

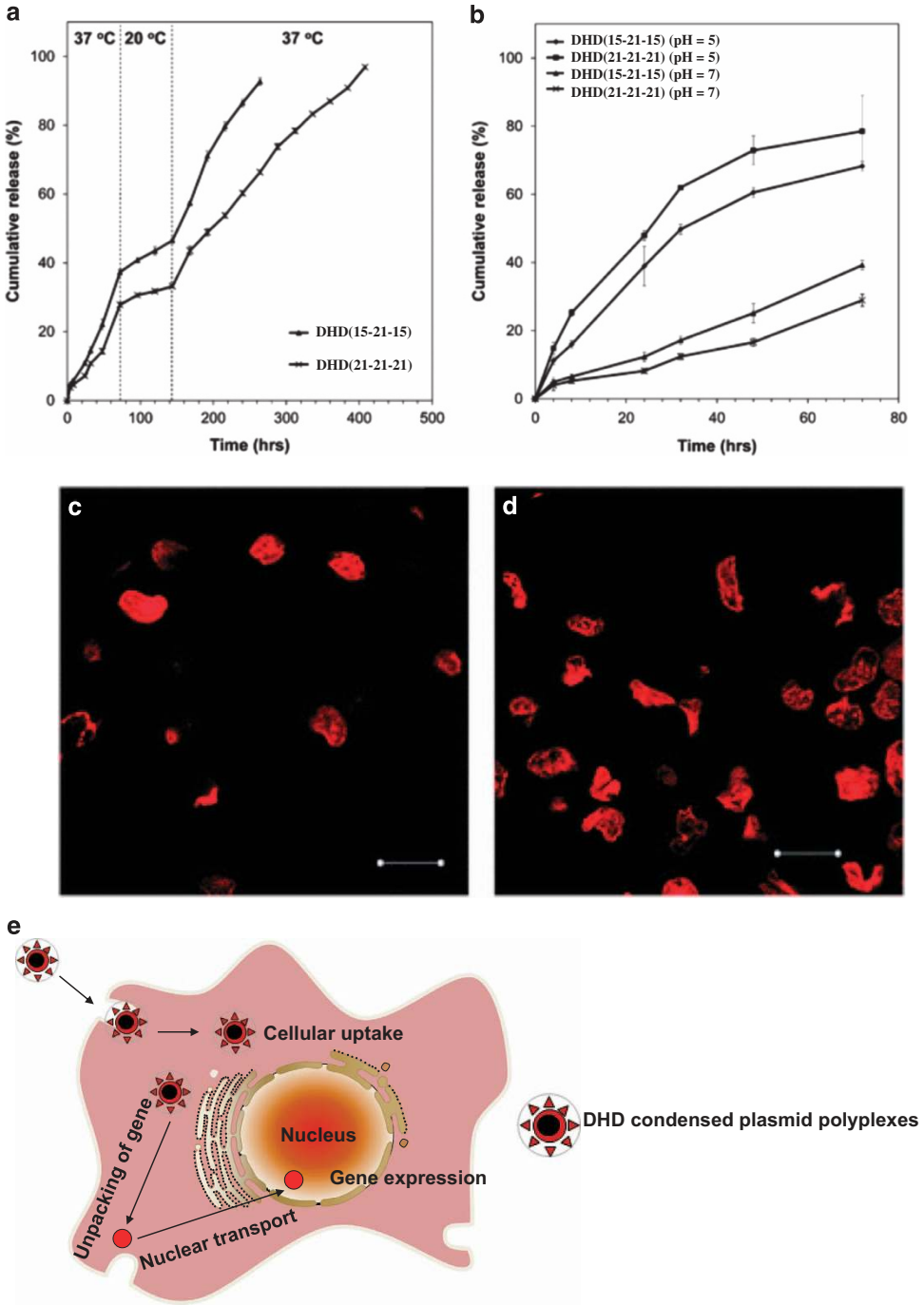

Figure 16 PDMAEMA-PHB-PDMAEMA triblock copolymers are denoted as DHD, and the numbers in brackets indicate the indicative molecular weight of the respective block in hundred g/mol. The effect of temperature (**a**) and pH (**b**) on the drug release profile of PDMAEMA-PHB-PDMAEMA micelles. Confocal images of HeLa cell uptake by incubating with (**c**) Pluronic F127 micelles and (**d**) PDMAEMA-PHB-PDMAEMA, DHD(21-21-21) micelles. The scale bar denotes 20 mm. Adapted from reference 75 (© 2013 Royal Society of Chemistry). (**e**) Schematic of the DHD condensed plasmid polyplexes in gene delivery. Adapted from reference 76 (2013 WILEY-VCH Verlag GmbH & Co. KGaA).

In addition, some of the PHAs have exhibited features that are similar to the features of conventional synthetic plastics, but with a significantly lower environmental impact on the production process. With an increase in the energy crisis, the renewable and sustainable behaviors of PHAs render them the new generation of eco-friendly plastics based on non-petroleum chemicals. However, many challenges have restrained PHA applications, including complicated genetic engineering processes for producing new PHAs, inadequate mechanical strength and a lack of functionalities. The high production cost and poor processability of PHA have limited their competitive forces to substitute traditional synthetic plastics. Therefore, modification of PHAs to achieve better performance has attracted increasing attention from industry and scientific research organizations and has gained excellent achievements in materials and medical applications.

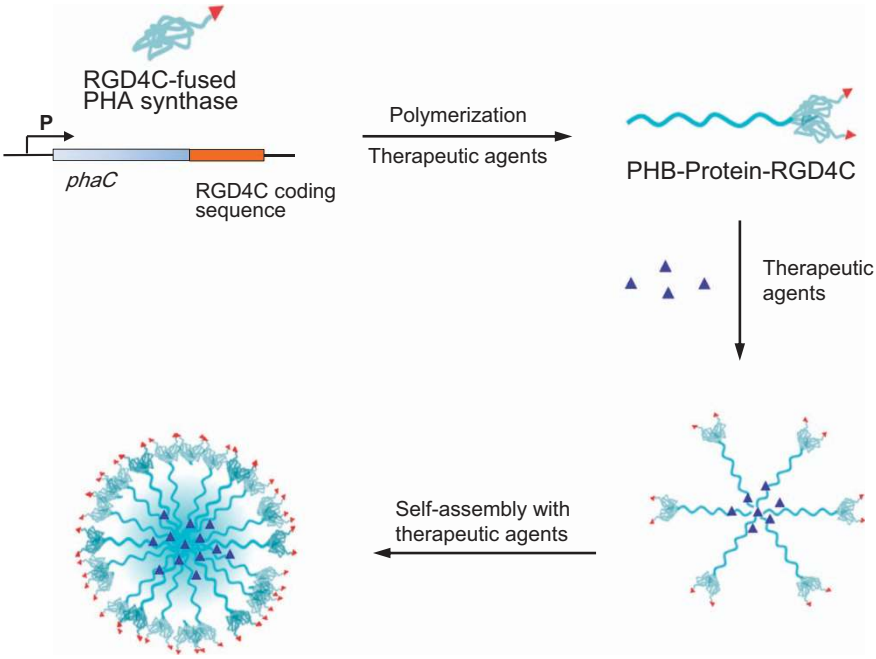

Figure 17 Schematic of PHA-protein block copolymers synthesis with RGD4C as a tumor-targeting ligand in drug delivery. Adapted from reference 77 (2013 Royal Society of Chemistry).

This article summarized the well-established modification approaches of PHAs using two important strategies: physical blending and chemical functionalization. The PHA blends with different types of biodegradable polymers, such as starch, cellulose derivatives, lignin, PLA and PCL, and the chemical modification PHAs to form copolymers in various block/graft architecture were discussed. With significantly altered material properties, modified PHAs have been employed to develop engineering material resins by regulating various physicochemical properties and to enhance the robustness of their use in different biomedical applications, including advanced drug delivery for cancer therapy, gene delivery and tissue engineering in emerging fields. The use of PHAs in the biomedical fields is particularly promising. For biomaterial applications, stringent requirements exist for an implantable system: first, a benign material is required; second, very low toxicity is needed; and third, the material should exhibit long-term stability in a wet, oxygen- and enzyme-rich environment. The living body possesses glutathione and other related enzymes (such as glutathione reductase and glucose-6-phosphate dehydrogenase), which protect various organs from chemical and oxidative stress. The presence of these enzymes can affect the stability of the materials that are implanted in the body. Future PHA material development should emphasize the consideration of these factors. Biodegradable and polymeric soft materials present the future of tissue engineering substrates due to their high water content, density, degradability and rheological properties, which mimic the characteristics of human tissue. Stimuli-responsive PHA-based soft materials have emerged as the material class with the most potential to succeed as an ideal implantable scaffold substitute because it possesses not only the properties of polymeric hydrogels but also added functionalities, such as the change from reversible sol to gel, due to external stimuli that facilitate easy implementation during surgery. In addition, the majority of these hydrogels have shown their efficacy as a drug delivery system. The mechanism and rate of drug release of these systems should be examined if their use for drug release in the body is desired. Additional tests to prove the biocompatibility of PHA with cells should also be performed before *in vivo* studies are conducted. To examine the suitability of the polymeric hydrogels, their material properties and the clinical signs of the study subject must be accurately and comprehensively examined. A collection and thorough analysis of all vital signs and clinical information will enable the well-informed clinician to determine a material's ultimate suitability as a successful long-term biomaterial. With continuous efforts to obtain a large-scale supply of PHA, the development of PHA as new biodegradable material will open doors for a new future.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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