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Polyhydroxyalkanoates (PHA)-based responsive polymers

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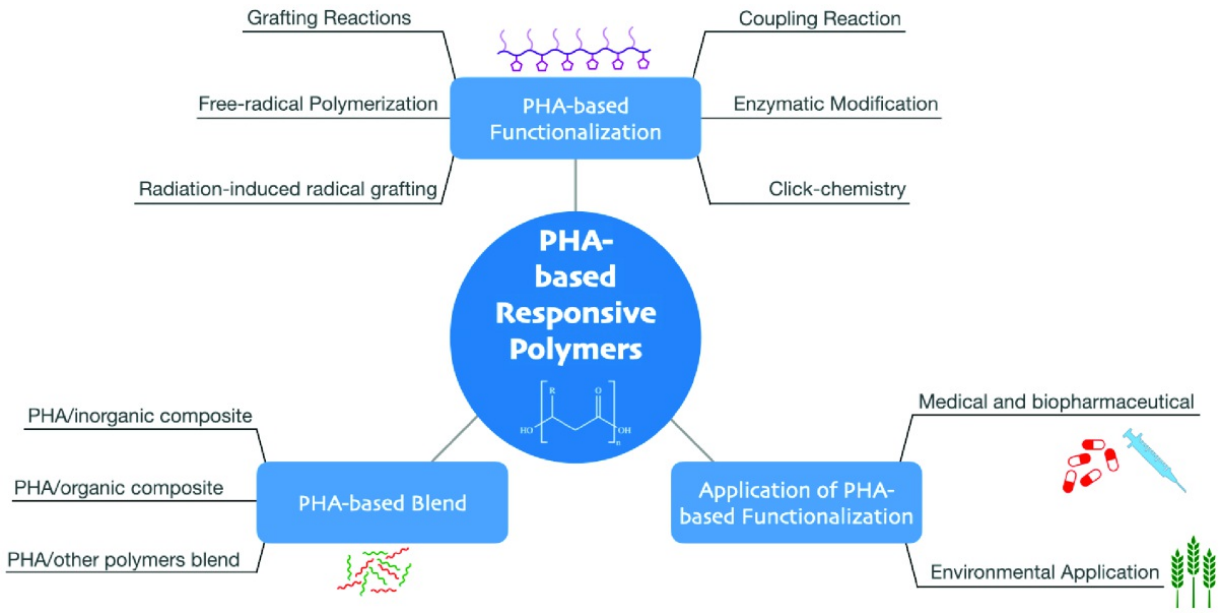
Abstract

Polyhydroxyalkanoates (PHA) envisage a potential biomaterial alternative to replacing synthetic polymers for their biodegradability and biocompatibility. Modification approaches exploit the attributes and adjust the intrinsic hydrophobic properties, such as blending to produce a new polymer mix with novel properties. Functionalization of PHA, especially chemical grafting, perform to introduce additional compounds covalently to PHA. As these methods address PHA potential and enable extensive utilization as a responsive material, reports are readily available in academia as confirmation. There are also substantial responsive PHA-based material applications in the biomedical area and agriculture materials, packaging materials, and nanocomposite materials.



Keywords: Biomaterial; biopolymer; chemical grafting; polyhydroxyalkanoates; polymer blending; polymer functionalization

1. Introduction

The increasing demand for environmental protection has led to the rapid development of greener and biodegradable polymers. Bacterial-synthesized PHA has attracted attention because they can be produced from various renewable resources and are truly biodegradable and highly biocompatible thermoplastic materials[1]. Microorganisms can accumulate various types of PHA in the form of homopolymer, copolymer, and polymer blends[2]. The properties of PHA copolymers depend strongly on the type, content, and distribution of comonomer units that comprise the polymer chains and the molecular weight distribution[3]. Besides the nature and proportion of different monomers, the bacterial strains, type, and relative quantity and quality of carbon sources supplied to the growth medium[4].

Biodegradable polymers classified into four categories according to their synthesis process which is (i) natural polymer (e.g., starch, cellulose, chitosan, and protein), (ii) polymers synthesized from natural monomers such as polylactide acid (PLA), iii) polymer conventionally and chemically synthesized from monomers obtained from petrochemical products (e.g., polycaprolactone, PCL) and iv) polymers synthesized by microbes such as PHA[5]. The inherent shortcomings of some biodegradable polymers, such as weak mechanical properties, narrow processing windows, and low electrical and thermal properties, can be overcome by composites reinforced with various nanofillers. These biodegradable polymer composites

have wide-ranging applications in different areas based on their large surface area and excellent aspect ratio. Moreover, the polymer composites that exploit the synergistic effect between the nanofiller and the biodegradable polymer matrix can lead to enhanced properties while still meeting the environmental requirement[6].

Recently, there has been explosive growth in bioresponsive polymers researches, which has significantly influenced materials science, molecular pharmaceutical, and nanobiotechnology. Future advances in polymer science mainly based on modifying

the chemical and physical properties of the polymer by creating creative combinations of copolymers and bioresponsive components that can deliver a wide variety of bioactive ingredients. Thus, modification of bioresponsive polymer, particularly PHA, is one of the promising candidates for future advances in polymer materials due to the structural properties of the polymers themselves. The variation in pH, temperature, stress, redox, enzyme, and ATP can trigger various responses such as swelling, shrinkage, assembly, disassembly, degradation, sol-gel transition, and crosslink, and the stimulus-responsive systems exhibit many promising applications[7]. The stimuli-responsive functions achieved through proper molecular design, including protonated groups, polar side chains, reducing groups, and enzyme-substrate units, and supramolecular self-assembly[8].

The construction of bioresponsive systems depends on several integrated steps, such as designing the responsive forms, determining the biological stimuli, and incorporating the responsive units. The prepared system should exhibit a desired responsive performance with high sensitivity and selectivity as a material for biomedical applications. The system should also have satisfying stability and biocompatibility properties. Good biocompatibility is required to avoid the possible systematic toxicity and immunogenicity, and it is crucial to control the timing for sufficient interaction between the carrier and the target tissues[8].

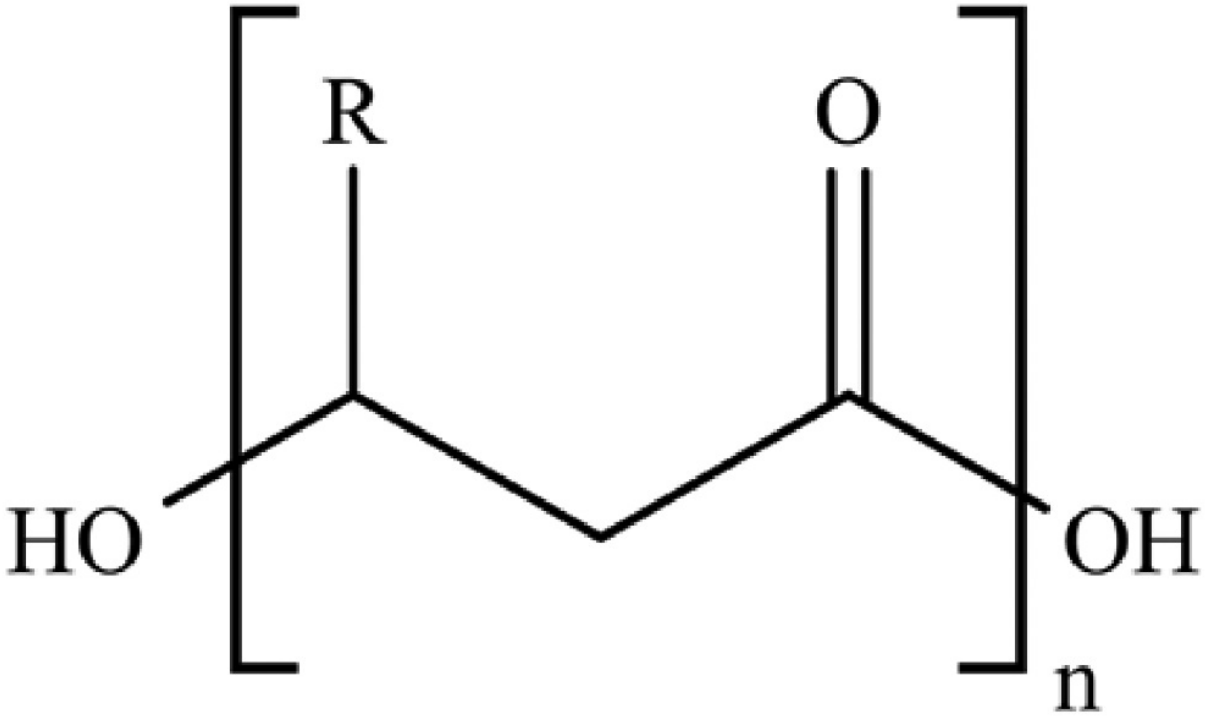
PHA has been emerged as potentially useful materials in the biomedical field for different applications due to their unique properties of being biodegradable and biocompatible. *In vivo* implants of PHA have been made possible due to their nontoxic degradation products, biocompatibility, desired surface modifications, a wide range of physical and chemical properties, cellular growth support, and attachment without carcinogenic effects[9]. Besides, lower acidity and bioactivity of PHA pose minimal risk compared to other biopolymers such as poly-lactic acid (PLA) and poly-glycolic acid (PGA). However, direct application of these polyesters, mcl-PHA included, has been hampered by their strong hydrophobic character and other physical shortcomings[10]. Hence, native PHA needs to be modified to improve its performance in specialized applications such as environmentally biodegradable polymers and functional materials for biomedical and industrial applications[11].

The performance of these biodegradable polymers is usually enhanced and altered *via* blending. The incorporation of fillers in PHA composites expands their applications by improving the performance of the polymer significantly. The application of reinforced filler as composite agents is attractive because the filler

improves polymer crystallization and the gas-barrier, thermo-mechanical and physicochemical properties, surpassing those of the native biopolymer. Polymer blending has attracted the attention of researchers because polymer with extraordinary properties obtained by chemical synthesis more expensive than existing polymer and blending operations. Furthermore, a wise choice and combination of the polymeric materials' specific amounts may lead to the fabrication of blend materials with desirable properties. There are various numbers of polymers that can be combined to form blends with different physical properties. The polymeric blend characteristic easily influenced by the nature of the dispersed and dispersion phase, the volume ratio of the phases, the sizes, and the size distribution of the dispersed phase particles and interfacial adhesion particles. The issue that commonly addressed regarding the polymer blend is the miscibility between the component. The blends formed can be miscible, partially miscible, or fully immiscible. The miscible polymer blend is formed by choosing polymers with compatible chemical structures capable of specific interactions[5].

PHA comprised of diverse natural biodegradable polyesters synthesized by microorganisms. Among the different types of microbial PHA, medium-chain-length PHA (mcl-PHA) consisting of 3-hydroxyalkanoates (C6–C14 carbon atom length) is a promising biomaterial for temporary implant applications in surgery, scaffolds in tissue engineering, drug carriers in pharmacology, and as a component of paramedical disposables (Figure 1). The potentialities of PHA curtailed by its strong hydrophobic character and associated physical shortcomings[12]. In expanding the range of its versatilities, other properties such as mechanical strength, surface features, amphiphilicity, and thermostability need amendments to match the requirements of specific applications[13]. Amphiphilic copolymers with desirable properties often produced through simple modification reactions by inserting the hydrophilic segments into the mcl-PHA.

Figure 1. The general molecular structure of PHA, with *R* as alkyl side group and *n* is an integer of the repetition unit.



In general, bioresponsive materials can be deconstructed into functional motifs with biological sensitivities, which can be built into the desired formulations, scaffolds, or devices in a controlled manner using appropriate fabrication methodologies[8]. Tailoring biomaterials polyhydroxyalkanoates *via* blending and functionalization are practical approaches to mimic the natural bioresponsive processes in the body.

2. PHA-based blend

2.1. PHA/inorganic composite

2.1.1. PHA/hydroxyapatite

Synthetic and natural hydroxyapatites (HA) or $HCa_5O_{13}P_3$ have a similar chemical composition and crystallographic properties to a human bone[14]. Their biocompatibility and osteoconductive behavior are suitable for making bone implants. Studies have shown that incorporating HA into biomaterials could help enhance mechanical performance and osteoblast responses[15]. Currently, composites of polymers and ceramics developed to increase the mechanical scaffold stability and improve tissue interactions. Besides, efforts have also invested in developing scaffolds with

drug-delivery capacity. These scaffolds allow for the local release of growth factors or antibiotics and enhance bone in-growth to treat bone defects and even support wound healing[16].

Generally, polymers from the PHA family are not osteoconductive; thus, they generally overlooked for bone tissue engineering application. One of the significant limitations is the inability of PHA to form solid interfacial bonding with the surrounding bone tissue through forming a biologically active apatite layer on the implant surface[17]. Therefore, one of the approaches to overcome this lack of osteoconductivity and mechanical competence is combining mcl-PHA with inorganic bioactive particles or fibers. Incorporating inorganic phases may lead to mcl-PHA composites with different mechanical properties suitable for tissue engineering application. Extensive research carried out on developing bioactive and biodegradable composite materials in the form of dense and porous systems, where the bioactive inorganic phase incorporated as either filler or coating (or both) into the biodegradable polymer matrix[9, 17].

Concerning the development of PHA, researchers have looked into the possibility of designing composites in combination with inorganic phases to improve the mechanical properties further, rate of degradation, and also impart bioactivity. Poly(3-hydroxybutyrate) (PHB), poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate), (PHBV), and poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (PHBHx) are some of the polymers that extensively studied to fabricate composites in combination with hydroxyapatite, bioactive glass, and glass-ceramic fillers or coatings[17]. To improve the properties, PHA also blended with natural raw materials or other biodegradable polymers, including starch, cellulose derivatives, lignin, PLA, PCL, and different PHA-type blends[11].

Bioceramics are inorganic materials specially developed for medical and dental implants such as alumina and zirconia, bioactive glasses, glass-ceramics, hydroxyapatite, and resorbable calcium phosphates[17]. So far, only hydroxyapatite, wollastonite, and bioactive glasses have been extensively studied in combination with PHA to form composites[9]. The mechanical and biological performances of bioactive ceramic/polymer composites can be controlled using different particulate bioceramics and varying the number of bioceramic particles in the composite[18]. Hydroxyapatite is the primary mineral component of bone, and it is one of the most common biomaterials studied in bone tissue engineering[19]. The thermodynamic stability of hydroxyapatite at physiological pH and its ability to actively participate in bone bonding by forming chemical bonds with surrounding bone make it a suitable bioactive ceramic for preparing composites[20].

2.1.2. PHA/chitosan

Microbial PHA is a biodegradable polymer with good biocompatibility and good mechanical properties. However, PHA has several intrinsic deficiencies in use as scaffolds, including brittleness, thermal instability in a molten state, slow rate of degradation, and acidic degradation products. One of the considering ways to modify imperfections of this polymer is combining it with other polymers. Based on studies, combining with natural polymers could be a promising option[21]. Besides, the blends of commercial polymers with chitosan

have gained importance because synthetic polymers can overcome every disadvantage associated with natural materials [22]

Chitosan is a linear polysaccharide containing deacetylated and acetylated units. The acetylated unit contains *N*-acetyl-*D*-glucosamine, while deacetylated unit contains *β*-(1,4)-linked *D*-glucosamine. Chitosan most frequently obtained by treating crustacean material with an alkaline substance such as sodium hydroxide. In other words, chitosan is a polysaccharide extracted from crustaceans. Its chemical name is 2-amino-2-deoxy-*β*-*D*-glucopyranose with a molecular formula of $(C_6H_{11}O_4N)_n$. There is a wide range of properties of chitosan, such as biocompatibility, biodegradability, hydrophilicity, nontoxicity, and consist of antimicrobial activity. Based on the characteristics and properties, it is understandable that chitosan and PHA have mutual complementary potentials. Chitosan considered being ideal for reinforcing filler of polymer matrices due to functional groups of amine, amide, and hydroxyl groups[23, 24].

Moreover, chitosan exhibits mild antimicrobial activity from its cationic residue, an essential characteristic for its application as a biomaterial. However, some difficulties frequently encountered in PHA blending with chitosan due to two main problems, which are (i) melt processing technique cannot be applied since chitosan has a high melting point and PHA will start to decompose before melting chitosan; (ii) there are a very few common solvents available for chitosan and PHA[21]. Thus, methods actively modulated in obtaining well-blended copolymer PHA/chitosan for desired properties of the blended material. Besides, to achieve good dispersion levels and good bonding of the filler within the polymer matrix, engineering the polymer's surface needed[22].

Karbasi *et al.*[21] prepared PHB/chitosan blend using trifluoroacetic acid as a co-solvent followed by fabrication of scaffold using the salt-leaching technique. Fourier Transform Infrared Spectroscopy (FT-IR) test revealed that the crystallization of PHB in these blends suppressed when chitosan concentration increased from 10 − 40%. SEM images showed a thin and rough top layer with a nodular structure, supported with a porous sub-layer in blend scaffolds. The contact angle illustrates that increasing the salt content makes the top layer rougher, and the contact angle increases due to the presence of air pockets under the liquid drop. The water adsorption of the scaffolds increased with an increase in the chitosan concentration. *In vitro* degradability investigation indicated that the degradation rate of blend scaffolds was higher than pure PHB scaffolds, and the dissolution of chitosan could neutralize the acidity of PHB degradation products. The obtained results suggested that these newly developed PHB/chitosan blend scaffolds may serve as a three-dimensional substrate in cartilage tissue engineering[21]. The incorporation of chitosan in PHA composites was expanded their applications by improving the performance of the polymer significantly.

2.2. PHA/organic composite

2.2.1. PHA/nanocellulose

Cellulose is the most abundant biopolymer synthesized from plants, animals, fungi, and bacteria. Increased demand for high-performance materials with tailored mechanical and physical properties makes nanocellulose the most attractive renewable material for advanced applications. Nanoscale cellulose fibers can be isolated from various cellulose sources using various isolation methods or processed materials that have defined nanoscale structural dimensions[25, 26]. Nanocellulose usually divided into three types of materials which are (i) cellulose nanocrystals (CNCs), also referred to as nanocrystalline cellulose (NCC) and cellulose nanowhiskers (CNWs), (ii) cellulose nanofibrils (CNFs), also referred to as nano-fibrillated cellulose (NFC), and (iii) bacterial cellulose (BC).

Cellulose is a versatile starting material for subsequent chemical transformations because of its unique structure, significantly affecting its chemical reactions. Cellulose is a linear syndiotactic homopolymer composed of *D*-anhydroglucopyranose units linked by β-1,4-glycosidic bonds. The properties of cellulose depend on the degree of polymerization, which can vary depending on the cellulose source. Because of the high number of hydroxyl groups on the glucose rings and the skeleton, there is extensive hydrogen bonding between individual cellulose chains (intra- and inter-molecular bonds). These bonds result in the crystallization of multiple cellulose chains into insoluble microfibrils and two structural regions, i.e., crystalline and amorphous regions; this gives cellulose its high strength, stiffness, durability, and biocompatibility. The presence of three hydroxyl groups in each monomeric unit and their high reactivity gives cellulose properties such as hydrophilicity, chirality, and biodegradability. Amphiphilic copolymers with desirable properties often produced through simple modification reactions by inserting the hydrophilic segments into the PHA. Cellulose nanocrystals are ideal for nano reinforcement of polymer matrices because of the abundant hydroxyl groups on their surfaces and their high surface-to-volume ratios, making them suitable for many types of surface functionalization with various chemicals[26]. However, a key challenge is nanocellulose dispersion in hydrophobic polymer matrices. Hence, determining the particular methods for obtaining a uniform distribution of nanocellulose within nanocomposites is needed[27].

The performance of these biodegradable polymers is usually enhanced and altered *via* blending. The incorporation of nanofillers in PHA nanocomposites expands their applications by improving the performance of the polymer significantly. The application of nanoparticles (nanofillers) as composite agents is attractive because it improves polymer crystallization and the gas-barrier, thermo-mechanical and physicochemical properties, surpassing those of the native biopolymers or its conventional micro composite. Moreover, nanoscale celluloses combine several unique properties, including large surface area, attractive strength and stiffness properties, hydrogen-bonding capacity, and eco-friendliness. Microbial polyesters utilization further diversified *via* physical and chemical blending[17]. The green nanocomposites of PHBV with various cellulose nanocrystals (CNCs) contents initially prepared using the solution casting method from a previous study[28]. CNCs act as an effective nucleation agent for the crystallization of PHBV, inducing an increase in the melt crystallization temperature of the nanocomposites. Non-isothermal crystallization kinetics showed that the overall crystallization rate of PHBV in the nanocomposites was

faster than that of neat PHBV but exhibited a decrease in the crystallinity decrease of the spherulite size of PHBV[29]. Besides, simultaneous enhancements on the mechanical property and thermal stability of PHBV after reinforcement of CNCs achieved through chemical composite. This property is due to a combination of CNCs reinforcement in the polymeric matrix, leading to the formation of strong intermolecular hydrogen bonding interactions by achieving the excellent dispersion of CNCs in the PHBV matrix*via* the solvent exchange procedure. Concomitantly, the formation of a six-membered ring ester during the degradation process of PHBV suppressed[29]. The reinforcing effect of the CNCs depends on their nature, content, and state of dispersion within the polymeric matrix and the intermolecular interactions between the above two components[13]. Solid hydrogen bonding interactions occur between carbonyl groups of PHBV and hydroxyl groups in CNCs[30]. A report also claimed that good dispersion of the CNCs in the polymer matrix was beneficial to form more hydrogen bonding interactions between copolymers[31]. Generally, the CNCs are obtained by the sulfuric acid hydrolysis of cellulose-based materials in an aqueous solution and then freeze-dried in a vacuum chamber[32]. It is challenging to avoid the aggregation of the CNCs during nanocomposite preparation when the CNC has dispersed into organic polymer solution again[31–33].

Besides, nanoscale celluloses combine several unique properties, including large surface area, attractive strength and stiffness properties, hydrogen-bonding capacity, and eco-friendliness[13]. Incorporating nanocellulose as nanofillers in PHA nanocomposites expands their applications by improving the performance of the polymer significantly[25]. Therefore, innovative modification with suitable functional groups on its surface topography is needed to minimize repellant interactions with the surrounding tissue.

2.3. PHA/carbon nanotube

Carbon nanotubes (CNT) belong to the fullerenes family and defined as a scaffold (sphere, ellipsoid, or a tube) made entirely of carbon which is only a few nanometers diameters but can be tens of microns in length. CNTs can be divided into a single wall (SWCNT), the basic cylindrical structure, or multiple walls (MWCNT) made of coaxial cylinders. Carbon nanotube's attractive physical properties, hardness, thermal stability, and electrical conductivity have engaged researchers interest in exploiting CNT as nanofillers to create new advanced polymer composites for various specific applications[34, 35]. Typically, three methods have used to synthesize polymer nanocomposites; (i) solution blending, (

ii) melt blending, and (iii) in situ polymerization. In solution blending, a solvent or solvent mixture used to disperse the nanoparticles and dissolve the polymer matrix. Meanwhile, instead of using solvent as a medium for melt blending, the nanoparticles can be directly mixed with the molten polymer. *In situ* polymerization can be done by tailoring interactions between the polymer, the surfactant, and the nanoparticles[36].

CNTs integration into the PHA polymer matrix acts as a way to introduce new material properties. PHB/CNTs nanocomposites exhibit electro-conductive properties; meanwhile, the thermo-mechanical

properties of PHB/CNTs nanocomposites reported extensively. Currently, there are active investigations of the use of PHB/CNTs in electronics and neurological applications. Valentini*et al.*[37] had investigated the optical and electrical properties of PHB/CNT nanocomposite for electronic applications such as electrical circuits. The PHB/CNT composite was prepared as thin films by solution blending (chloroform) with 0.25 wt% of MWCNT or SWCNT. The photo-responsive layer was prepared by drop-casting from a dichlorobenzene solution of the poly(*N*-9-heptadecanyl-2,7-carbozole-alt-5,5-(4′,7′-di-2-thienyl-2′,1′,3′-benzothiadiazole)) (PCDTBT): phenyl-C70-butyric acid methyl ester (PC70BM) (1:1 weight ratio) blend with the solution concentration of 20 mg/mL onto PHB/CNT composites. The conductivity of PHB/CNTs was measured using an electrometer. PHB/MWCNT exhibits lower electrical resistance (3×10^{7} Ohm) than PHB/SWCNT (2×10^{8} Ohm). They suggest that higher conductivity across PHB/MWCNT surface is due to the interconnected morphology instead of perpendicular to the substrate as demonstrated by PHB/SWCNT. On the other hand, the light transmittance of PHB/CNTs films (80% and 65% for SWCNT and MWCNT, respectively) was lower than pure PHB (90%). The lower transmittance of PHB/MWCNT was due to the larger polymer crystals and partial aggregation of MWCNT (observed from TEM cross-section).

Vallejo-Giraldo *et al.*[38] had assessed the suitability of mcl-PHA/MWCNT nanocomposite films for neural applications. The mcl-PHA/MWCNT with 0.1 − 1.0 wt% MWCNT prepared by solution blending using chloroform. They found that uniform dispersion of MWCNTs has obtained at 2 hours sonication at 40% of amplitude. The electrical resistance decreases following increasing MWCNT content, indicated by the final charge capacities of mcl-PHA nanocomposites were significantly higher than mcl-PHA. Biocompatibility evaluation by observing the growth of mesencephalic neuron cells conducted, the ventral mesencephalon (VM) cells, after ten days on the nanocomposites. They found that mcl-PHA was able to promote neuronal cell growth. However, mcl-PHA with 1.0 wt% MWCNT resulted in a lower percentage of neurons compared to 0.5 wt% MWCNT and pure mcl-PHA. Besides, the neurite length also decreased with the increase of MWCNT content. They suggested that this was due to increased rigidity or MWCNTs-mediated toxicity toward neurons.

PHA/CNTs nanocomposites prepared by solution blending or in situ polymerization exhibit enhanced material properties such as increased thermal stability, mechanical and water-barrier properties[37–45]. The incorporation of MWCNTs significantly increased the elastic modulus from 30 − 55 MPa compared to 8 MPa of pure mcl-PHA[38].

While CNTs can enhance a range of material properties, the inclusion of CNTs may affect the biodegradation of the polymer matrix. Also, there is the potential for CNT exposure and release into the environment due to microbial degradation. Furthermore, only a few isolates (*Burkholderia kururiensis*, *Delftia acidovorans, and Stenotrophomonas maltophilia*) exhibit biodegradability of CNTs at a prolonged rate optimized laboratory conditions[46–48]. Phan *et al.*[44] had investigated the effect of CNTs and CNTs compositions have on polymer biodegradation, structural changes of the polymer during biodegradation, and CNTs released during the biodegradation process. These nanocomposite films exposed to aerobic mixed

culture for up to 20 days. They had reported that incorporating MWCNTs (at different concentrations) did not affect the rate or extend of P4HB degradation. The residual MWCNT mat contained the same MWCNT mass initially present in the nanocomposite, indicate that MWCNT unable to degrade by the microbial communities and likely to remain persistent in the environment.

On the other hand, no inhibitory effect by MWCNTs across the entire CNT contents was found instead of a previous study using single culture, *Pseudomonas aeruginosa*. They suggested that the cytotoxic effect of CNTs possibly negated by microorganisms that are not affected by CNTs, or the diversity of mixed culture offered better adaptability to unfavorable conditions. Thus, the inhibitive effects of CNTs most likely insignificant in an environment with diverse microbial communities.

2.4. PHA/other polymers blend

2.4.1. PHA/PCL

Poly(ε-caprolactone) (PCL) is one of the earliest commercially available synthetic polymers; it consists of hexanoate repeating units. PCL is prepared either by (i) condensation of 6-hydroxyhexanoic acid[49, 50] and ring-opening polymerization (ROP) of ε-caprolactone[51–61], commonly derived from crude petroleum. Each method affects the resulting mechanical and thermal properties of derived polymers[62]. ROP is the preferred method as it results in higher molecular weight and lower polydispersity polymer.

PCL is a hydrophobic, semi-crystalline polymer with a degree of crystallinity up to 69%, low melting temperature (T_m) of 56 to 65 °C and glass transition temperature of −60 °C. Its degree of crystallinity tends to decrease with the increase of molecular weight[62]. The mechanical and thermal properties of PCL vary on its molecular weight and degree of crystallinity, as shown in Table 1.

Table 1. Properties of PCL.

Properties	Range	References
Number average molecular weight, M_n (g/mol)	3,000–80,000	[63]
Density, ρ (g cm^{-3})	1.071–1.200	[64]
Glass transition temperature, T_g (°C)	−65 to −60	[63–65]
Melting temperature, T_m (°C)	56–65	
Decomposition temperature, T_d (°C)	350	[66]
Tensile strength (MPa)	4–785	[64, 65]
Elongation at break (%)	20–1,000	
Young's modulus (GPa)	0.21–0.44	[64]

Properties	Range	References
Solubility	Highly soluble; chloroform, dichloromethane, carbon tetrachloride, benzene, toluene, cyclohexanone Low solubility; acetone, 2-butanone, ethyl acetate, dimethylformamide, acetonitrile Insoluble; alcohols, diethyl ether, water	[67, 68]

PHA is a promising material for biomedical applications due to its biocompatibility, nontoxicity, and biodegradability properties. *In vivo* degradation of PHB results in *D*-3-hydroxybutyric acid, which is a normal constituent of human blood. However, the application of PHB usually limited due to its brittleness; thus, blending of PHB with other

polymers was utilized for improved material properties. PCL described exhibiting good mechanical properties, biocompatibility, and miscibility with various polymers[69]. On the other hand, PCL has a more extended degradation period (three to four years) compared to PHB (days to weeks), suitable for long-term medical applications or consumables[65].

In general, the miscibility of PHB/PCL blends influenced by the polymer molecular weight. High molecular weight PCL immiscible with PHB[70–73] while PHA blends with low molecular weight PCL (M_w = 2,000 or 600, respectively), found to be partially miscible[74]. Although PHB and PCL are immiscible, these polymers still formed mechanically compatible films. PHB/PCL blends exhibit an enormous change in the material elasticity (Table 2), which overcome PHB brittleness. Jenkins *et al.*[72] had introduced the use of supercritical carbon dioxide (CO_2) during processing showed improved miscibility of PHBV/PCL blends. Garcia-Garcia *et al.*[75] and Przybysz *et al.*[76] reported that the elasticity significantly increases for PHB/PCL blends compatibilized by peroxides (dicumyl peroxide and di-(2-tert-butyl-peroxyisopropyl)-benzene) at 275% and 305%, respectively.

Table 2. Mechanical and thermal properties of PHA/PCL blends.

Polymer	Blend composition (wt%)	M_w (g mol^{-1})	M_n (g mol^{-1})	Polydispersity	T_g (°C)	T_m (°C)	Tensile strength (MPa)	Young Modulus (GPa)	Elongation at break (%)	References
PHB/PCL	100/0	652,000	362,222	1.8	1	179	38	1.56	5	[73]
Solvent-casting	77/23	–	–	–	1	179/59	21	0.73	9	
	49/51	–	–	–	2	178/59	4	0.11	18	
	25/75	–	–	–	1	178/60	8	0.22	11	
	0/100	68,000	35,790	1.9	−70	60	15	0.22	24	

Polymer	Blend composition (wt%)	M_w (g mol^{-1})	M_n (g mol^{-1})	Polydispersity	T_g (°C)	T_m (°C)	Tensile strength (MPa)	Young Modulus (GPa)	Elongation at break (%)	References
PHB/PCL	100/0	–	222,000	–	–	180	29.4	2.10	1.0	[71]
Melt-blending	20/80	–	–	–	–	180/63	18.0	0.53	5.5	
	40/60	–	–	–	–	180/63	21.4	0.86	4.4	
	60/40	–	–	–	–	180/63	23.7	1.47	1.8	
	80/20	–	–	–	–	180/63	25	1.92	1.3	
	0/100	–	43,000	–	–	63	18.4	0.36	7.4	

PHA/PCL hydrogel also investigated for wound healing application[77]. The PHA/PCL macromer was crosslinked with polyethylene glycol methacrylate (PEGMA) to give a hydrogel. Their findings showed that PHA/PCL-PEGMA hydrogel accelerates the wound healing process (similar healing rate with Intrasite® gel, positive control), thus a potential medium in wound treatment. Prior, the pH-responsive behavior of PHA/PEGMA hydrogel was studied in phosphate buffer solution with pH ranging from 2.4 to 13.0[78]. They reported that the gel swelling behavior is pH-dependent; an increase in pH results in the increase of hydrogel pore size, increasing the protein release with time. The pH sensitivity was attributed to active ionizable moieties in the copolymer network, making PHA/PEGMA hydrogel responsive to the surrounding hydrogen ion concentration. Under alkaline pH, the carboxylic groups were ionized, leading to dissociation of interpolymer complexes due to electrostatic repulsion result in swelling of hydrogel.

2.4.2. PHA/PLA

Polylactic acid or polylactide (PLA) is one of the most attractive biopolymers because of its biodegradability and biocompatibility with material properties similar to standard plastic. PLA is widely used and well-studied among other biopolymers. PLA produced through combined biological-chemical processes; ROP of lactide, a dehydrated cyclic dimer derived from lactic acid fermentation. PLA is highly soluble in dioxane, acetonitrile, chloroform, methylene chloride, 1,1,2-trichloroethane, and dichloroacetic acid; partially dissolve in ethylbenzene, toluene, acetone, and tetrahydrofuran; thoroughly if heated to boiling temperatures. PLA is insoluble in water, alcohols (i.e., methanol and ethanol), hexane, and propylene glycols[79]. It is already commercialized mainly for single-use disposal packaging applications such as bottles, cold drink cups, lid containers, blister packages, and lamination films[80]. Although PLA homopolymer has high tensile strength and good biocompatibility, it also has undesirable material properties such as poor barrier and mechanical (brittle) performance, low thermal stability, and lack of reactive side-chain groups[81].

For the PHB/PLA blends, there are reports on their miscibility, crystallization, melting, and physical characterization[82–92]. Zhang *et al.*[91] reported that PHA/PLA blends prepared by melt-blending showed better miscibility than blends prepared by solvent-casting; due to the transesterification between PHB and

PLA chains at high temperature (190 °C), which leads to the formation of PHB/PLA copolymers *in situ*. However, the thermal treatment also caused polymer degradation and changed the crystallization kinetics. Zhang *et al.*[90] indicate that PLA crystals heavily influence the crystallization dynamics of PHB in both miscible and immiscible blends. Besides, the miscibility of PLA and PHB is strongly dependent on each polymer's molecular weight[86]. PHB is only miscible with low molecular weight PLA[93]. The blending of PLA with different PHA derivatives (PHB, PHBV, and P4HB) results in different material properties (Table 3). With increasing PLA content, PHB/PLA blends exhibit increase elasticity and decrease in tensile strength; PHBV/PLA blends exhibit an increase in tensile strength and elasticity while PH4B/PLA blends exhibit an increase in tensile strength and Young's modulus while the elasticity decreased.

Table 3. Thermal and mechanical properties of various PHA/PLA blends.

Polymer	Blend composition (wt%)	M_w (g mol^{-1})	M_n (g mol^{-1})	Polydispersity	T_g (°C)	T_c (°C)	T_m (°C)	Tensile strength (MPa)	Young Modulus (GPa)	Elongation at break (%)	References
PHB/PLA	100/0	300,000	–	–	9.6	–	175.1	85.3	3.6	3.7	[91]
	80/20	–	–	–	11	–	175.4	–	–	–	
	60/40	–	–	–	10.3	–	175	63.7	2.69	27.7	
	40/60	–	–	–	10.6	–	174.6	–	–	–	
	20/80	–	–	–	10.4	–	174.7	–	–	–	
	0/100	81,700	43,000	1.9	–	–	–	–	–	–	
PHB/PLA	100/0	425,000	169,323	2.51	5.2	48	179	31	1.95	7.3	[82]
	25/75	–	–	–	1.7, 62	115	173	16	1.27	7.1	
	0/100	52 000	27,368	1.9	63	115	173	42	1.4	7.2	
Lapol	100	80,000	3,008	26.6	−0.7	–	–	–	–	–	
PHB/PLA/Lapol	25/75/5	–	–	–	2.7, 59	125	172	13	1.15	15.5	
	25/75/7	–	–	–	0.6, 58	120	173	15	1.12	15.1	
PHB/PLA	0/100	–	14,200	–	60	95	150	40	1.3	100	[83]
PHB/PLA	15/85	–	–	–	55.4	103.6	144, 150, 170	40	1.22	140	
OLA	–	–	957	–	−37	–	–	–	–	–	
PHB/PLA/OLA	15/70/15	–	–	–	49.4	111.5	143, 148, 168	31	1.12	35	
	15/65/20	–	–	–	47.2	108	141, 148, 169	23	0.95	220	
	15/55/30	–	–	–	36.2	95.6	135, 146, 165	16	0.59	370	

Polymer	Blend composition (wt%)	M_w (g mol^{-1})	M_n (g mol^{-1})	Polydispersity	T_g (°C)	T_c (°C)	T_m (°C)	Tensile strength (MPa)	Young Modulus (GPa)	Elongation at break (%)	References
PHBV/PLA	100/0	–	–	–	2.8	109.3	174	33.9	2.6	1.5	[88, 89]
8%HV	75/25	–	–	–	3.0, 57.1	107.1	173.1, 151.0	37.3	2.45	1.6	
	50/50	–	–	–	3.3, 57.9	105.6	172.8, 153.2	44.6	2.36	4.7	
	25/75	–	–	–	4.5, 58.4	101.3	170.1, 152.6	45.1	2.2	7	
	0/100	–	–	–	59.8		154	55.7	2.18	5.5	
PHBV/PLA	100/0	–	–	–	–	–	–	38.8	2.31	3.9	[94]
	90/10	–	–	–	–	–	–	39.8	2.32	3.8	
	80/20	–	–	–	–	–	–	42.7	2.38	3.4	
	70/30	–	–	–	–	–	–	46.1	2.42	3.7	
	60/40	–	–	–	–	–	–	47.5	2.41	6.6	
	50/50	–	–	–	–	–	–	53.6	2.61	4.1	
	40/60	–	–	–	–	–	–	56.4	2.61	5.5	
	30/70	–	–	–	–	–	–	57.0	2.58	9.8	
	20/80	–	–	–	–	–	–	56.5	2.49	50.7	
	10/90	–	–	–	–	–	–	58.0	2.36	204.3	
	0/100	–	–	–	–	–	–	67.5	2.63	4.8	
P4HB/PLA	100/0	362,700	195,000	1.86	−10.7	–	–	14.0	0.078	2233	[85]
24%4HB	30/70	–	–	–	−14.0, 60.4	–	–	29.0	1.2	214	
	20/80	–	–	–	−13.9, 60.6	–	–	37.7	1.6	317	
	10/90	–	–	–	−13.1, 61.0	–	–	43.1	1.74	273	
	5/95	–	–	–	−6.9, 60.9	–	–	48.6	1.89	96	
	0/100	360,180	207,000	1.74	61.5	–	–	64.7	1.98	5.5	
P4HB/PLA	100/0	362,700	195,000	1.86	–	–	–	5.3	0.085	2,122	[84]
	70/30	–	–	–	–	–	–	14.5	0.239	145	
	30/70	–	–	–	–	–	–	29.6	1.23	186	
	0/100	360,180	207,000	1.74	–	–	–	63.4	2.0	5	
P4HB/PLA/OOCP	100/0/0.1	–	–	–	–	–	–	5.6	0.092	1.86	
	70/30/0.1	–	–	–	–	–	–	7.8	0.174	593	
	30/70/0.1	–	–	–	–	–	–	28.2	0.8	317	
	0/100/0.1	–	–	–	–	–	–	69.4	2	58	
P4HB/PLA/OOCP/TAIC	70/30/0.1/1	–	–	–	–	–	–	11	0.3	410	

Polymer	Blend composition (wt%)	M_w (g mol^{-1})	M_n (g mol^{-1})	Polydispersity	T_g (°C)	T_c (°C)	T_m (°C)	Tensile strength (MPa)	Young Modulus (GPa)	Elongation at break (%)	References
	70/30/0.2/0.2	–	–	–	–	–	–	11.6	0.34	289	
	30/70/0.1/0.1	–	–	–	–	–	–	29.3	1.0	310	
	30/70/0.2/0.2	–	–	–	–	–	–	31.2	1.12	251	

Plasticizers are often incorporated into polymer blends to improve their processing properties for a specific application. Plasticizers exchange the intermolecular bonds among polymer chains to the bond between macromolecules and small molecular weight compounds, thus encouraging conformational changes, resulting in increased polymer structure deformability. Consequently, these changes affect the glass transition and processing temperature by lowering the said temperature, enabling the melt processing of heat-sensitive polymers at a lower temperature. Abdelwahab *et al.*[82] reported that the addition of polyester plasticizer Lapol 108 to PHB/PLA with a 25:75 ratio has a significant effect on the PHB/PLA molecular structure, which resulted in the decrease of glass transition temperature for both PHB and PLA while the glass transition peak for Lapol disappeared. However, the thermal decomposition properties of the blends were not affected by the addition of Lapol 108. The mechanical behavior of PHB/PLA blends with Lapol showed a slight decrease of Young's modulus and tensile strength, increasing elasticity compared to pure PHB and PLA. This behavior is similar to the mechanical properties of PHB/PLA blends plasticized with different lactic acid oligomer (OLA) compositions[83]. PHB/PLA with 30% OLA enhanced the oxygen and water barrier properties (37% reduction) compared with only PLA/PHB blends, an essential aspect for food packaging application.

Incorporating carvacrol, an active compound, into the blend produces an antimicrobial plasticized PHB/PLA with improving material elasticity suitable for industrial application such as antimicrobial/antioxidant active packaging films[83]. The plasticized PHB/PLA-OLA blend with carvacrol was effective against *Staphylococcus aureus* and *Escherichia coli,* while PHB/PLA blend with carvacrol did not show any inhibitory effect. The presence of OLA enhanced the antimicrobial activity by increasing the mobility of the macromolecular chains, thus promote the diffusion of carvacrol. Also, decreased hydrophobicity of PHB/PLA-OLA due to the hydroxyl groups of carvacrol lead to a higher release of active agent in the medium[95]. Arrieta *et al.*[96] investigated the use of acetyl (tributyl citrate) (ATBC) as a plasticizer for PHB/PLA blend with antioxidant catechin for fatty food-packaging application. They reported that the incorporation of catechin enhanced the thermal stability of the PHB/PLA-ATBC blend, whereas its release improved by the addition of ATBC as a plasticizer.

Bian *et al.*[84] reported that modification of P4HB/PLA blends with crosslinking agents, dicumyl peroxide (DCP), and triallyl isocyanurate (TAIC) increase the miscibility. The addition of only DCP promotes branching reaction to occur while the addition of DCP and TAIC, the crosslinking reaction became dominant. From the DMA and DSC analysis, the glass transition temperature of P4HB and PLA component of P4HB/PLA blends with crosslinking agent showed significant shifts toward each other, indicating these ccrosslinkedP4HB/PLA blends were partially miscible. Besides, P4HB/PLA blend with

DCP (0.1%) exhibits improved elasticity of the material.

Zhang and Thomas[92] reported that the biodegradability of PHB/PLA blends improved with increased PHB composition compared to pure PLA at room temperature. A similar observation by Han*et al.*[85] through enzymatic degradation using protease from *Amycolatopsis orientalis*, further increase of P4HB content significantly enhances the enzymatic degradation rate P4HB/PLA blends. Complete degradation of P4HB/PLA blend with 30%4HB was observed on the second day, whereas more than five days needed for pure PLA. Thus, enzymatic degradation of PLA improved after the incorporation of P4HB.

3. PHA-based functionalization

PHA envisaged replacing conventional plastics. However, scientists realized the limitations of raw PHA produced by bacteria and started to explore and design methods to modify them to obtain desirable characteristics. The processes in altering PHA molecular structure to produce specific characteristics for targeted usage are known as functionalization of PHA. This process typically related to some techniques, including chemical modification, physical modification, and enzyme modification[97].

3.1. Grafting reactions

There are many works of literature on the functionalization of PHA chemically, for example, through halogenation, carboxylation, hydroxylation, epoxidation, and grafting. The focus here directed to grafting, a primary method specified in PHA's functionalization to produce a range of responsive polymers. It is well-known as one of the extensive modification methods and widely established by offering precise and easily modified experimental designs to alter the PHA macromolecule. Furthermore, this approach aims to yield a uniform and bulk production of the functionalized PHA[98]. The main idea is to promote changes in introducing an

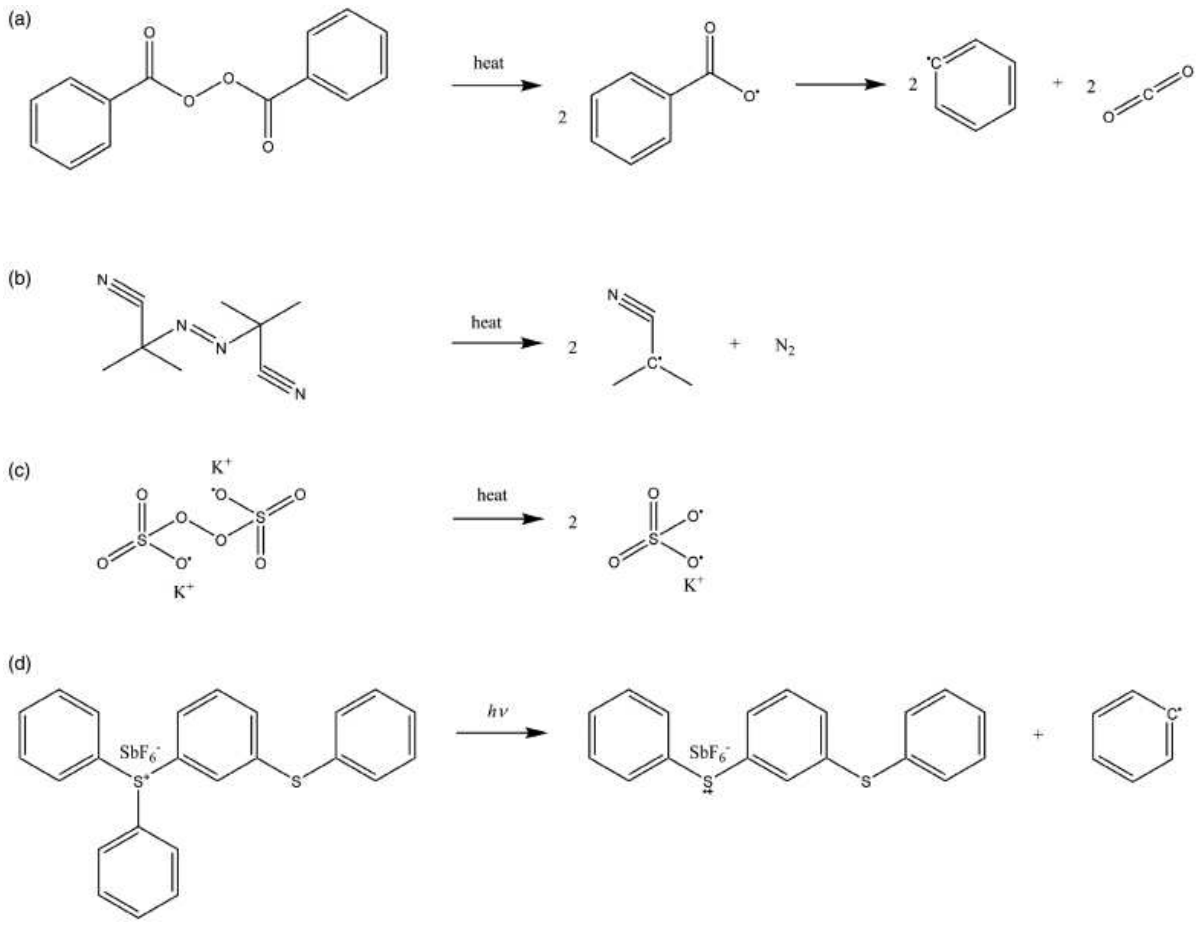
additional chemical group, which leads to enhancement of functionality, hydrophilicity, wettability, and surface charge[99].

Generally, grafting is an approach adopted by the researchers to lessen, neutralize, or alter the hydrophobicity attribute widely associated with PHA. PHA grafting is defined by introducing additional species as a side chain to the linear backbone chain chemically and ultimately producing the compound of interest at the end of the reaction[100]. Multiple methods were developed and established accordingly to cater for the specific needs of functionalization based on the chemical nature of PHA and also that of the extra material during the modification process.

3.2. Free-radical polymerization

Free-radical polymerization is a robust yet straightforward radical-mediated reaction involving the functionalization of polymer. This reaction utilized to create a brand-new macromolecule consisting of supplemental species covalently bonded to the primary polymer, assisted by a sole radical initiator. Free radical grafting *via* radical initiators is also widely used to modify bacterial polyesters. The most widely used radical initiator, in this case, is benzoic peroxyanhydride or benzoyl peroxide (BPO) [10, 99, 101–105]. Other well-known initiators include 2,2′-azobis(2-methylpropionitrile) or azobisisobutyronitrile (AIBN), potassium persulfate, and dicumyl peroxide. The procedure is straightforward without the involvement of a metal catalyst or ligand. Thermal decomposition of the radical component will provide active anionic radicals with an unpaired electron during the initiation steps (Figure 2).

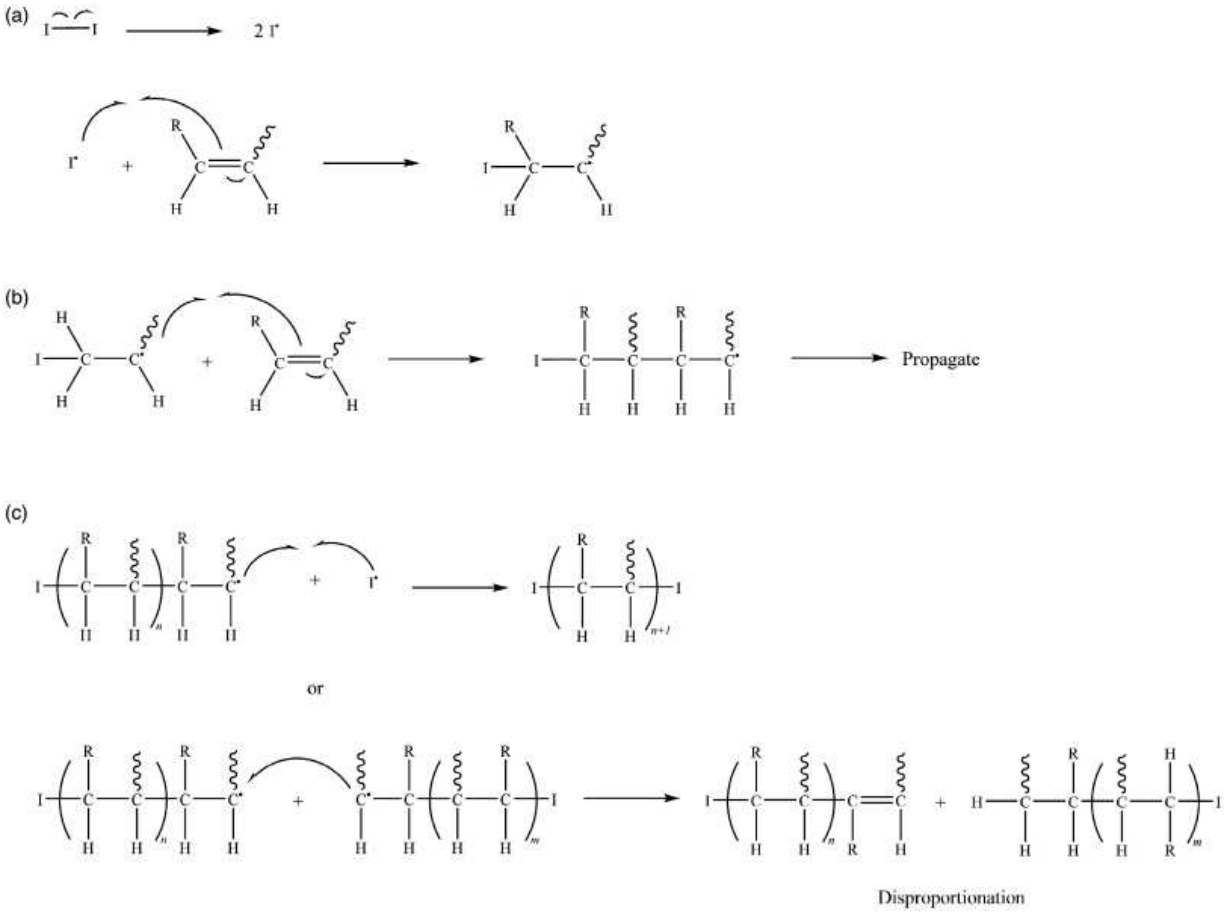
Figure 2. Thermal decompositions of (a) benzoyl peroxide, (b) AIBN, and (c) potassium persulfate, meanwhile irradiation-induced decomposition of (d) triaryl sulfonium salt.



The mechanism for graft copolymerization consists of three steps, namely, initiation, propagation, and termination (Figure 3). The mechanism can be described as an addition reaction of a radicalized unit to the

active site to form a higher molecular mass polymer. As the reaction going, monomer concentration will decrease, as they linked to the polymer backbone[107, 108].

Figure 3. The mechanism for graft copolymerization involving radical initiator and polymer species with alkenyl end group, which consists of three steps, namely (a) initiation, (b) propagation, and (c) termination [106], where *m* and *n* are integers and *R* is hydrogen or the rest of the polymer structure. Copyright © Springer Nature 2021, reused with permission.



The initiation step begins with the radicalization of monomer and polymer substrate by the action of radical initiator, for instance, benzoyl peroxide, and eventually generating radicals. Radicalized monomers later also play the role of undergoing chain transfer to the backbone of the polymer. Therefore, radical formation is the process to enable monomer transfer to the main polymer backbone[103, 109]. A particular polymer active site grafted with monomer will

propagate radicalization, radicalizing other backbone sites. This reaction will allow another chain transfer

reaction, forming successful graft copolymerization. However, radicalized monomers from the initiation step can also perform chain transfer to undergo an unintended homo-polymerization process[103]. Termination step will come ultimately, resulting in graft copolymer and homopolymer as final products due to recombination process. The occurrence influenced by several factors such as temperature and monomer concentration[110], primarily by increasing radical initiator concentration, which would later reduce the graft yield[99, 103, 105]. However, studies report that the radicals had no observable effect on polymer composition, for instance, when a micro-initiator such as AIBN usually used[111].

One of the essential bioresponsive material attributes necessarily achieved *via* radical grafting is the degree of wettability, especially in responsive surfaces—the significance of switchable wettability measured in the degree of water contact angle[112]. Ansari and Annuar[101] reported a successful grafting of glycerol 1,3-diglycerolate diacrylate (GDD) onto medium-chain length PHA by utilization of benzoyl peroxide as a sole micro-initiator. The introduction of hydroxyl groups-bearing GDD component improved the wettability up to 33%, hence contribute amphiphilicity on the grafted product. Earlier work by Kim *et al.*[10] describe a similar reaction strategy and components and managed to improve PHA wettability up to 28%. They also had some additional biocompatibility tests carried out by observing the growth and adhesion of Chinese hamster ovary cells, which results in improvement and the spread and growth of moderately hydrophilic grafted PHA. In the blood compatibility test, platelets have less tendency to stick to the polymer when higher GDD groups are available, leading to lesser deformation[10]. Another work worth mentioning was utilizing vinyl imidazole (VI) instead, yield a biocompatibility pattern with the increment of VI fractions, with additional antimicrobial properties with the ability to reduce viable cell counts of common Gram-negative bacteria more than 90%[113].

On the other hand, there were also works reported focusing on the fundamental properties of the functionalized product. Lao *et al.*[99] deployed a free-radical reaction assisted by benzoyl peroxide to graft a PHBV film with 2-hydroxyethyl methyl acrylate (HEMA) monomers. The product reported having improvement on wettability with water contact angle up to 37°. Another fundamental research published in a different report by Nguyen and

Marchessault[114] incorporated a free radical method in functionalizing PHA by utilizing AIBN as an initiator to prepare the grafted product of pretreated PHB with methyl methacrylate (MMA) monomer to yield comb polymer. In improving PHA biodegradability, Elsawy *et al.*[115] managed to graft a prominent short-chain length PHA, PHBV with*N*-isopropyl acrylamide, creating significant behavior change in different pH in terms of swelling degree within aqueous solution up to approximately 170%.

Besides graft copolymerizing reactions, interfacial grafting between PHA and other material to overcome restriction in blending also successfully demonstrated. Xu *et al.*[116] reported work on interfacial modification, initiated by dicumyl peroxide coated physically onto both P4HB and starch separately before heat-induced reaction, eventually producing thermostable gel with modified mechanical properties.

3.3. Radiation-induced radical grafting

Radiation is one technique that utilizes a light source to induce a chemical reaction to create a reactive site within a compound. The formation of monoradical allows the introduction of the interest component to covalently bonded onto target PHA. Typically, ultraviolet or UV radiation widely used besides gamma rays and plasma irradiation. The procedure is very straightforward by solely emitting the select radiation to the sample without the assistance of any catalyst or micro-initiator. However, Lao *et al.*[117] demonstrated that irradiation treatment, especially by UV, is prominent only for film surface modification, while the free-radical reaction preferred for bulk functionalization.

Important introduction of hydrophilicity characteristic to the hydrophobic PHA done by González*et al.*[118] in grafting vinyl acetate (VA) monomer onto PHB using ^{60}Co gamma rays. However, further modification by ion implantation, reported in a different publication, had further increased the degree of hydrophilicity of the grafted product by the bombardment of H^+, Na^+, and Ag^+, with Na^+ as the best candidate[119]. Torres *et al.*[120] have reported fundamental research on the usage of ^{60}Co gamma rays and graft poly(2-aminoethyl methacrylate hydrochloride) (PAEMA) onto PHB. The crystallinity of the grafted is inversely proportional with the degree of grafting, hence promoting a less brittle product that is commercially favorable.

Another surface modification of PHA film work also conducted by Zhang*et al.*[121] to increase the recalcitrant property of the grafted product toward enzymatic degradation. P4HB pretreatment by exposing the film directly to radiofrequency plasma or RF-plasma, a non-thermal plasma treatment, avoids the deteriorating effect onto the polyester. The plasma-initiated film soaked in an aqueous solution containing acrylic acid (AA) with different concentrations. The degradation rate reduced due to hydrophilic polyacrylic acid (PAA) that hindered the PHB depolymerase interaction with hydrophobic PHA.

Besides direct radiation, photo-initiator such as benzophenone was utilized for surface grafting, as reported by Ke *et al.*[122] PHBV film was coated with benzophenone before soaked in an aqueous solution containing polyacrylamide (PAM) before UV treatment. The grafting was deemed successful with the biocompatibility test of sheep bone marrow stromal cell studies by observing an increment of cell attachment and growth. A similar method by Kim *et al.*[123] involving poly(3-hydroxyoctanoate) grafting with poly(ethylene glycol) methyl acrylate, just that the biocompatibility test carried out was about reducing protein and platelets adhesion to increase blood compatibility of the product.

On the other hand, the formation of radical reaction induced by UV irradiation utilizes cationic salt radical, such as triaryl sulfonium salts induced by UV irradiation[124] and ceric salt-redox system[125].

3.4. Coupling reaction

The development of new methods involving coupling reaction is widely adopted to create an amide or ester bridge between two species. It is essential to assist the synthesis of synthetic protein; however, it is further

applicable to create graft copolymer between non-miscible polymer. Carboxylic groups available within PHA, either innate at the end of the polymer chain or synthetically introduced based on the unsaturated side chain, are exploited to form this additional covalent bond bridge by carboxylic activation by forming PHA intermediate before further covalently bonded to the target compound. Materials fabricated by this reaction are usually have improved solubility as a potential drug delivery bioresponsive material.

One reason for this approach is to overcome the low interfacial interaction property between two different compound species. This factor opens up the possibility of the utilization of the coupling method. Huerta-Angeles *et al.*[126] worked on the formation of an ester bond between the carboxylic end group of pre-degraded poly(3-hydroxybutyrate) (PHB) and the hydroxyl group of hyaluronic acid (HA). In the reaction, it is vital to activate the PHA carboxyl group to form PHA-*co*-imadazolide before esterification, and *N,N'*-carbonyldiimidazole (CDI) was used with triethylamine as an activator and reported to yield higher efficiency. The esterification was continued by converting HA into its acidic or tetrabutylammonium salt form with triethylamine and 4-(dimethylamino)pyridine (DMAP) solubilization. The final product was asserted to be novel, well-defined, soluble in water, and confirmed for its *in vivo* biodegradability.

Another more complicated attempt in forming PHA conjugates with an additional hydrophilic component was carried out earlier by Renard *et al.*[127] Poly(3-hydroxyoctanoate-*co*-3-hydroxy-10-undecanoate) (PHOD) was involved in this work. Instead of activating the carboxylic end group of the PHA, as mentioned earlier, the alkenyl group of unsaturated 10-undecanoate monomer within the polymer chain manipulated for esterification. The double bond on the side chain was oxidized beforehand by potassium permanganate in ether crown solution to form a synthetic carboxylic group. Next, the activation step assisted by *N,N'*-dicyclohexylcarbodiimide (DCC). Methoxy oligomers of polylactic acid (PLA) and polyethylene glycol (PEG) used separately, prepared by protecting their

respective carboxylic end group with (trimethylsilyl)diazomethane by esterification to prevent crosslinking. The hydrophilic component was successfully incorporated directly onto the hydrophobic main chain of PHOD with DMAP as a nucleophilic catalyst in esterification. This process finally yielded a water-soluble end product observed for a high chain length of PEG. Extension of the work was published in a different report regarding the stability of these copolymers as nanoparticles in drug delivery systems in terms of esterifying and amidifying the remnant carboxylic groups with other bioactive compounds[128]. A report on the biocompatibility of the resultant product based on the growth of human bladder cells also made available later[129].

3.5. Enzymatic modification

The process involving enzymes typically serves as a pretreatment for PHA before further modification. Enzymes usually used able to cleaving the ester bond within the backbone of the polymer chain to yield a much lesser molecular weight oligomer.

However, it was different from a report by Iqbal *et al.*[130] utilizing lipase as a catalyst to graft the carboxylic groups of PHB onto ethylcellulose backbone hydroxyl groups by esterification. Lipase depolymerization effect on PHB was not a concern in this work; however, it was notable for reducing PHB molecular mass grafted onto cellulose due to interaction with lipase.

The latest work by Bhatia *et al.*[131] demonstrated the esterification of ascorbic acid onto PHBV by a lipase from *Candida antartica* with the presence of hydrogen peroxide as a micro-initiator. The resultant polymer has been introduced with additional antioxidant property from ascorbic acid and improved biodegradability due to the hydrophilicity, enabling the attachment of degrading bacteria. Similar to earlier work by Gumel *et al.*[132] used sucrose and medium-chain length PHA consisting of four different monomers, namely, 3-hydroxyhexanoate 3-hydroxyoctanoate, 3-hydroxydecanoate, and 3-hydroxydodecanoate, or poly(3-hydroxyhexanoate-*co*-3-hydroxyoctanoate-*co*-3-hydroxydecanoate-*co*-3-hydroxydodecanoate) producing poly(1′-*O*-3-hydroxyacyl-sucrose). The biodegradability of the functional product was improved 1.5-fold compare to neat PHA due to the favorable sucrose component for microbial attachment. The same research group further adds the variety of the functionalized product and introduces glucose instead of similar PHA to produce 6-*O*-glucosyl-poly(3-hydroxyalkanoate). However, the lipase-mediated reaction, in this case, a phospholipase, was conducted in mixed organic solvent without the assistance of a micro-initiator, also managed to yield a higher degree of biodegradability polymer product due to the availability of glucose component[133].

Lipase also used in the pretreatment of the PHA before functionalization with the target compound. A report by Guzmán *et al.*[134] used *Candida antartica* lipase A to modify PHB's surface to expose more carboxylic groups, eventually forming a porous scaffold. Further functionalization commencement facilitated by 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDA) and *N*-hydroxysulfosuccinimide sodium salt, a catalyst for amide bond formation with glucosamine and gelatin in phosphate buffer. The lipase-mediated treatment creates more hydrophilicity of PHB sample; meanwhile, the functionalized PHB-gelatin product managed to promote growth and cell proliferation of human fibroblast cells in biocompatibility test.

3.6. Click-chemistry

Click chemistry is another chemical synthesis method to conjugate two different molecules, and even crosslinking Unsaturated PHA has been widely demonstrated to bear thiol pendant group to make functionalization commencement *via* thiol click reaction possible[12]. Besides incorporating additional compounds, the utilization of crosslinking agent on thiol-bearing unsaturated PHA such as pentaerythritol tetrakis (3-mercaptopropionate) (PETMP) and radical initiator 2,2-dimethoxyphenylacetophenone (DMPA) also has been reported[135, 136].

Yao *et al.*[137] have reported the synthesis of comb-like temperature-responsive PHA polymer involving poly(3-hydorxydodecanoate-*co*-3-hydroxy-9-decanoate) (P(3HDD-*co*-3H9D)) *via* thiol-ene click reaction.

2-dimethylaminoethylmethacrylate (DMAEMA) monomer prepared with two steps before grafting; first, reversible addition-fragmentation chain transfer (RAFT) polymerization by controlled polymerization to create uniform oligomer of poly(DMAEMA) or PDMAEMA, and second aminolysis end group modification by breaking the chain transfer agent attached *via* RAFT using *n*-butylamine and eventually introduced thiol group. PMAEMA with thiol group then easily grafted to P(3HDD-*co*-3H9D)) given the availability of unsaturated side chains with the assistance of photo-initiator, DMAP. The product portrayed excellent temperature-responsive behavior in terms of adsorption of protein over the lower critical solution temperature of 47.5 °C. Another similar work by Ma *et al.*[138] produced another thermo-responsive P(3HDD-*co*-3H10U) grafted with poly(N-isopropyl acrylamide) (PNIPAm) prepared by RAFT polymerization with trithiocarbonate-based catalyst as chain transfer agent.

In another report, 3-mercaptopropionic acid-bearing carboxylic and 2-aminoethanthiol bearing amine groups introduced as thiol compound onto unsaturated medium-chain length PHA produced from undecylenate before thiol click reaction, as reported by Tajima *et al.*[139] Both components were treated separately by different click chemistry pathways. Carboxylic-bearing PHA was treated with 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC) to introduce active fibronectin fragment (GRGDS); meanwhile, TEA catalyzed amine-bearing PHA to introduce fluorescein. GRGDS introduction has a distinct advantage to creating a more biocompatible product. However, the fluorescein component acts as an indicator that emits light under UV irradiation, useful to determine homogeneity when blended with a different polymer.

A unique click reaction, copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction, has been established by

Lemechko *et al.*[140] PHBV and dextran, as an additional component, were undergone different pretreatment before grafting to introduce hydrophilicity. First, PHBV functionalized with propargyl alcohol, alcohol that bears a carbon-carbon triple bond, with the presence of dibutyltin dilaurate as a catalyst. On the other hand, dextran was functionalized with 4-toluenesulfonyl chloride (TosCl) or underwent tosylation with lithium chloride as a catalyst for introducing the thioalkyl functional group, eventually further modified to introduce azide group by nucleophilic displacement to produce deoxy azide tosyl dextran. Finally, both alkyne-bearing PHBV and deoxy-azide tosyl dextran were mixed with copper (I) iodide (CuI) catalyst and *N,N,N',N'',N''*-pentamethyldiethylenetriamine (PMDETA), enabling CuAAC to start a click reaction for the production of the dextran-graft-PHBV copolymer.

4. Application of PHA-based responsive material

Responsive polymer-based materials can modify their chemical or physical properties upon exposure to external stimuli (pH, temperature, mechanical force, the presence of various small molecules, or biomolecules) driven by the desire to mimic nature. The response results from capture biomolecules immobilized in the polymer interacting with the target, which results in network crosslinking or ionization.

These materials intensively studied for various applications, such as drug delivery, tissue generation/repair, biosensing, smart coatings, and artificial muscles[141].

PHAs demonstrated a good, responsive polymer-based material with degradability, biocompatibility, and piezoelectric[14, 142]. To further apply PHA in medical, biopharmaceutical, and environmental, PHA must undergo particular modifications to be more companionable with application requirements. PHA blends are one of the approaches to converted PHA to become highly stimulating for diverse applications while retaining the original advantageous properties of PHAs such as biodegradability and biocompatibility[143]. In this situation, PHA will be incorporated or impregnated with other suitable bio-based materials or bioactive molecules by physical mixing, chemical conjugation, or complexation to invent new blends or composite with innovative polymer system properties[144, 145].

4.1. Medical and biopharmaceutical

4.1.1. PHA-based responsive polymers as tissue engineering

In tissue engineering, PHA blends were designed and fabricated to mimic natural extracellular matrix (ECM) characteristics to facilitate cell seeding, adhesion, proliferation, differentiation, and neo tissue genesis[14]. For use in wound healing, a polymeric material must exhibit dimensions similar to ECM. Thus, nano-fabrication by incorporating P(3HB-*co*-4HB) and collagen peptides has shown significant cell adhesion and growth compared to PHA copolymer only. This collagen-based copolymer significantly affected responsiveness on wound contractions, with the highest percentage of wound closure of 79%[146].

Besides, bio blend film of P4HB and 20 wt% chitosan also found to provide actual microenvironment of ECM to wound dressing materials. It offered outstanding performance by increasing thermal stability, biocide activity, antimicrobial performance, cell attachment, and proliferation[14, 147]. Furthermore, Li and coworkers fabricated nanofibrous matrices of PHA blends PHB/PHBHx and PHB/P4HB as cell growth supporting materials for implant biomaterial development in skin engineering and nerve generation[14].

There have been various studies on scaffold preparation to address the problem of loss of bone tissue. In earlier studies of medical scaffolding material, blends of PHB and hydroxyapatite (HA) were used as scaffolds to treat bone defects[148]. Then, scaffolds from PHBHx and PHB mixed to type-I collagen have been tested for tissue engineering[149]. In another investigation, using a 3 D-bioplotter, the team of Zhao *et al.*[150] produced 3 D scaffolds of composites of PHBHx and mesoporous bioactive glass. In*in vivo* experiments targeting investigating these materials for improved bone regeneration, the robust and highly porous scaffolds featured excellent bioactivity, stimulated human bone marrow stromal cells adhesion, and stimulated bone regeneration[150, 151].

4.1.2. PHA-based responsive polymers as cardiovascular applications

Cardiovascular tissue engineering aims to provide new and better approaches for treating various diseases of

the cardiovascular system. Therefore, PHA blends pose multiple advantages over synthetic materials due to their biocompatible and responsive nature[152]. Currently, many successful scaffolds have been developing using PHA blends material in cardiovascular engineering. For example, a copolymer of polyglycolic acid (PGA) and PHB used to generate pulmonary valve leaflets and pulmonary artery scaffolds in sheep[148]. Similarly, PGA non-woven mesh dip-coated with P4HB shown as a suitable scaffold for creating tissue-engineered trileaflet heart valve substitutes in sheep[153]. Furthermore, PHA blends with hydrophilic polymer (polyethylene glycol, PEG) can enhance the wettability of PHAs-based materials and the resistance to platelet adhesion. PHB/PEG demonstrated satisfying cell-compatibility of the Chinese Hamster Lung fibroblast cells[142, 154].

Besides, Shum-Tim *et al.*[155] used a polymeric scaffold containing two components in a tubular conduit for tissue engineering the abdominal aorta in a lamb model. The inner layer comprises a non-woven PGA mesh and three layers of nonporous P(3HO-*co*-3HHx), with 10% of the 3-hydroxyhexanoic acid outer layer. The scaffolds were seeded with autologous cells and retained implanted for up to 5 months. The grafting outcomes were promising since all the P(3HHx-*co*-3HO)/PGA grafts initiated to permit unrestricted blood flow (except in one case), and no inflammatory reactions observed[155].

4.1.3. PHA-based responsive polymers as a drug release material

Drug delivery systems are applied to deliver drugs at the target sites inside the human body[156]. In this situation, PHA

is impregnated with a drug compound to adjust the site, and the degradation over time will release the compound, acting as an automatic dosing agent[148, 156]. For example, PHA matrices in bioactive compounds such as antibiotics or therapeutic drug delivery applicable by encapsulation. Drug delivery systems demonstrated and offered unique methods to control release[151]. Previously, rifampicin immobilized in PHB microspheres investigated as a potential chemoembolization agent that can release about 90% of rifampicin within 24 hours[157].

Conversely, the kinetics of release easily altered by changing the amount of drug loading. For instance, in an *in vitro* study of antibiotic release, PHBV rods impregnated with either gentamicin or Sulperazone, sustained release of the drug into aqueous solution was seen for two weeks. Furthermore, using a higher HV content copolymer (20% HV, compared to 7% or 14% HV), sustained release of Sulperazone observed for over 60 days. Higher levels of cumulative release seen using a copolymer with higher HV content[158].

Similarly, in a study of antibiotic delivery, gentamicin was incorporated into PHBV disks, and the release of the drug measured over time. The polymer containing higher HV content released more antibiotics into the solution than lesser (12% 3HV versus 8% HV). These PHBV disks containing gentamicin incubated in standard human blood samples were shown not to cause proliferation of white blood cells, red blood cells,

or platelets, indicating no adverse effects of the polymer/antibiotic combination[159].

Based on Shishatskaya *et al.*[160] an experimental dosage form of rubomycin developed by incorporating the drug in an absorbable polymeric (PHB) matrix in microparticles. The system demonstrated that the release of the anti-tumor drug rubomycin inhibited the proliferative activity of Ehrlich's carcinoma in mice and improved the survival of mice with tumors[160].

PHA blends PEG–PHB–PEG, PHB/PEG/PPG, and PHB/PEG/PPG triblock copolymers also found responsive in water-soluble PHAs system for controlled release of chemotherapy. Moreover, a water-soluble PHAs system for DNA/siRNA delivery also developed as gene delivery could potentially be used to treat diseases *via* the introduction of corrective genetic information to the malfunctioning cells[143].

4.2. Environmental application

4.2.1. PHA-based responsive polymers as agriculture materials

One of the specialized applications of PHA-based responsive polymers in the environment for the agriculture sector is in the controlled release of insecticides. Insecticides could be integrated into PHBV pellets and sown along with the farmer's crops. The insecticide released at a rate related to the level of pest activity since the bacteria breaking down the polymer would be affected by the same environmental conditions as that of the soil pests[161–163].

Besides, formulations of the fertilizer urea loaded in the PHB in films, pellets, and coated granules were created and investigated by Volova *et al.*[164] Nitrogen release into soil occurred as the polymer degraded, and the release rates can be controlled depending on the geometry of the carrier, the amount of nitrogen loaded in it, and the fabrication technique. PHB/urea formulations shown to have a favorable consequence on the soil microbial community also. Such slow-release formulations can decrease the amounts of chemicals in the environment and inhibit their adverse effects on the biosphere[164].

PHA blends with natural fiber also were studied for agricultural mulching and transplantation. Avella *et al.*[165] reinforced PHB with heat exploded wheat straw and hemp fibers, and the composites thus obtained can be used in agricultural mulching and transplantation[162, 165].

4.2.2. PHA-based responsive polymers as packaging materials

PHAs materials have a distinct property of gas barrier, CO2 and O2 only can permeate slowly, which have attractively potential application in the packaging[156]. Jost and Kopitzky[166] explored the blending of these two biopolymers PHBV and PLA (and copolymers). The outcomes suggested that a PHBV content of 20–35 % in PLA is the most suitable blend system for compatibility and high barrier properties.

Furthermore, combined with synthetic plastics or starch, PHAs make excellent packaging films[167]. Diez-Pascual and Diez-Vicente[168] dispersed ZnO into scl-PHA to obtain a composite film with antibacterial

properties applicable in the food packaging industry.

4.2.3. PHA-based responsive polymers as nanocomposite materials

Recently, nanocomposites are a new, promising generation of materials. The material properties of the polymers improve significantly after reinforcing with suitable filler materials. For example, PHO latex films were prepared and found to possess outstanding thermoplastic properties. When these latex films used as the host matrix for nanocomposite materials, on the other hand, a colloidal solution of hydrolyzed starch or cellulose whiskers used as fillers, 'high-performance' materials produced[162, 169]. Similarly, P4HB containing 4.0 mol % 4-hydroxybutyrate (4HB) was melt-mixed with short glass fibers (SGF)*via* a co-rotating twin-screw extruder. An optimum condition for the P4HB/SGF composites established with increase mechanical properties[170]. Hopefully, this nanocomposite will enable PHAs to compete more effectively with petroleum-based in the future.

5. Conclusion: challenges in PHA-based responsive polymers applications

Nowadays, bio-based polymers such as PHA, with outstanding properties (biodegradability, biocompatibility, or natural abundance) and advantages (low-cost of production, environmental-friendly or wide application) are attractive and

popular trends as responsive materials. Although PHA-based responsive polymer systems show great potential in the application, as mentioned earlier, we cannot ignore that many limitations are to be resolved or improved. Since PHA is typically produced in large quantities by Gram-negative bacteria, removing lipopolysaccharide and other cell wall material is necessary for medical applications, especially for contact with blood. Therefore, more involved polymer purification procedures followed significantly reduce the amount of bacterial cell wall material associated with the purified PHA.

For large-scale production of PHA-based responsive polymer for medical use, purification challenges will have to be addressed, as a polymer with close to 100% purity needed. Continuous dissolution and re-precipitation of polymer are acceptable at a laboratory scale, but the industry must formulate cost-effective and environmentally friendly processes[148, 151].

In contrast, high purity is not a primary criterion for ecological applications, but the low cost of PHA resource as a raw material is deemed essential. Therefore, the overall PHA production cost must be a significant challenge for environmental applications. Therefore, using an inexpensive carbon source as a substrate for biosynthesis and the simple, inexpensive, and efficient recovery process is also an added advantage for PHA production at an industrial scale[171, 172]. Besides, the blending of PHA with cheaper materials like starch and cellulose will further decrease costs without degradation and maintain sustainability[154].

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