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Review Article

Designing Novel Interfaces via Surface Functionalization of Short-Chain-Length Polyhydroxyalkanoates

S. Vigneswari,[1,2] **J. M. Chai**,[1] **K. Shantini**,[3] **K. Bhubalan**,[2,4,5] **and A. A. Amirul**[2,3,6]

[1] *School of Fundamental Science, Universiti Malaysia Terengganu, Terengganu, Malaysia*
[2] *Malaysian Institute of Pharmaceuticals and Nutraceuticals, NIBM, Malaysia*
[3] *School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia*
[4] *School of Marine and Environmental Sciences, Universiti Malaysia Terengganu, Malaysia*
[5] *Institute of Marine Biotechnology, Universiti Malaysia Terengganu, Malaysia*
[6] *Centre for Chemical Biology, Universiti Sains Malaysia, 11900 Bayan Lepas, Penang, Malaysia*

Correspondence should be addressed to S. Vigneswari; vicky@umt.edu.my

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Polyhydroxyalkanoates (PHA), a microbial plastic has emerged as promising biomaterial owing to the broad range of mechanical properties. However, some studies revealed that PHA is hydrophobic and has no recognition site for cell attachment and this is often a limitation in tissue engineering aspects. Owing to this, the polymer is tailored accordingly in order to enhance the biocompatibility *in vivo* as well as to suit the intended application. Thus far, these surface modifications have led to PHA being widely used in various biomedical and pharmaceutical applications such as cardiac patches, wound management, nerve, bone, and cartilage repair. This review addresses the surface modification on biomedical applications focusing on short-chain-length PHA such as poly(3-hydroxybutyrate) [P(3HB)], poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) [P(3HB-*co*-4HB)] and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3HV)].

1. Introduction

Surfaces of biomaterial play a crucial role in depicting suitable interface which is able to enhance the surface and cellular integration [1]. Tailor-made surface functionalization in improving the biomaterial is an important breakthrough in tissue engineering [2]. The goal of tissue engineering will be to engineer materials with suitable structural framework to enhance or replace biological tissues which will eventually degrade, leaving natural tissues in place without causing any severe immune response to the recipient. Biomaterials are designed to meet both nutritional and biological needs for specific cell proliferation involved in new tissue formation. Thus, the developed biopolymer as biomaterials with the basic properties of nontoxicity, noncarcinogen, nonallergen, nongenotoxicity, and biocompatibility are widely studied as potential candidate for tissue engineering [3, 4].

Over the past 70 years, polymers have revolutionized the global economy, manufacturing, and, mainly, the fields which require biocompatible material [5]. Among the variety of biodegradable polymers, polyhydroxyalkanoate (PHA) has been attracting a lot of attention. However, like most polymers, the surface of PHA is hydrophobic with no recognition sites for cell attachment. Therefore, it is crucial to alter the surface properties or promote cell recognition sites onto the biomaterial surfaces to promote specific cell adhesion and proliferation [6]. Despite many studies on PHA over the last few decades, continuous efforts to develop the applications of this biopolymer are still at its nascent stage [7]. In this regard, several approaches have been taken in improving scaffold-cell interactions by modifying the surface architecture of the scaffolds at a micrometer or nanometer scale [8]. Chemical modification of PHA surfaces is one of the approaches that enhance biocompatibility with increased cell attachment and proliferation. Physical blending, biological macromolecules grafting, and nanofabrication are other similar surface functionalization method that have been employed successfully for the alterations of the surface properties of PHA[9].

Modified PHA can be used in medical tissue engineering because modified PHA possessed enhanced properties such as increased hydrophilicity, biocompatibility, bioabsorption, and physical and mechanical properties. This modified PHA was fabricated to mimic native extracellular matrix via nanofabrication, which increased the surface area for the attachment of cells thus increasing their efficiency on the cell attachment and proliferation so that it make them suitable biomaterials to be applied in medical tissue engineering. In terms of other modification like solvent-cast porogen-leached polymer scaffold, the modifications lead to porous surface which increased surface area and contour that formed rough microporous pore wall surface which is more favorable conditions for the evolution of cell populations. In tissue engineering, desirable scaffolds should possess certain level of surface porosity, surface roughness, biodegradability, biocompatibility, and mechanical stability which can provide a suitable microenvironment for cell–cell interaction, cell migration, proliferation, and differentiation. In recent years, PHAs were developed as a good candidate for cardiac patch which even used in the cardiovascular diseases treatment [10].

This review serves to give an insight of the many kinds of surface modification carried out in tailoring the surface properties of PHA as a desirable material. There are many methods employed in improving the surface properties according to desired use which have been extensively researched. This has improved the applications of PHA scaffolds in the forefront. This review aims to highlight the various surface functionalization carried out using short-chain-length PHA (SCL_{PHA}) polymer, which demonstrates ways of using surface modification strategies to effectively improve the performance of PHA for a wide variety of applications.

2. Polyhydroxyalkanoate (PHA)

Generally, PHA is a microbial polymer made up of a variety of hydroxycarboxylic acids and stored as energy and carbon material in some bacteria [11, 12]. PHA is stored as insoluble granules and inclusion bodies in the bacterial cytoplasm of the bacterial cell [13–15]. The accumulation of these insoluble PHA granules can be identified using specific stains such as Sudan black or light fluorescent stains like Nile blue and Nile red [16, 17]. The production of PHA is carried out by feeding bacteria with carbon substrates such as simple sugars or complex plant oil [18–20]. PHA synthase (*PhaC*) is the key enzyme in the conversion of carbon sources to PHA granules as inclusion bodies of the bacterial cells [21]. Basically, the carbon substrates fed and the substrate specificity of the *PhaC* enzyme in the bacteria directly correlate to the structural confirmation of the PHA synthesized [22, 23].

PHA falls in three main categories, which are SCL_{PHA} with 3 to 5 carbon atoms, medium-chain-length PHA (MCL_{PHA}) with 6 to 14 carbon atoms, and hybrid polymer that consists of a combination of short-chain-length and medium-chain-length PHA (SCL-MCL_{PHA})[24]. The most abundantly found PHA is poly(3-hydroxybutyrate) [P(3HB)] [25]. Other than P(3HB), members of SCL_{PHA} are made up of 3-hydroxyvalerate (3HV) and 4-hydroxybutyrate (4HB) comonomers and are the most extensively researched PHA. The bacterial genera of *Cupriavidus*, *Alcaligenes* and *Escherichia coli* transformants are often linked in the biosynthesis of these SCL_{PHA} [26].

The degradability of PHA depends on the intracellular PHA depolymerase which can internally degrade the PHA accumulated in the bacteria or extracellular PHA depolymerase excreted by PHA-degrading microorganisms. [27, 28]. Generally, most types of PHA are widely studied due to their biocompatibility and hence are a remarkable candidate for biomaterial [29, 30]. Moreover, it has been widely reported that the *in vivo* biodegradation of PHA did not result in the formation of toxic compounds [30]. Besides, PHA breaks down to hydroxyacids which are naturally found in the metabolites of animals. It was previously reported that the copolymer P(3HB-*co*-4HB) commonly used in biomedical applications is biodegraded into 3-hydroxybutyric acid and 4-hydroxybutyric acid, which are normal constituents of human blood. Thus, the biomaterials possessing 4HB monomers can be degraded more easily in living systems without eliciting any toxic effects [31]. Moreover, the degradation rate of PHA copolymers is much faster than that of the P(3HB) homopolymer due to porous surface and low crystallinity [32–34]. Various PHA polymers including P(3HB), poly(4-hydroxybutyrare) [P(4HB)], P(3HB-*co*-4HB), poly(3-hydroxyoctanoate) [P(3HO)], poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) [P(3HB-*co*-3HHx)], and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3HV)] have gained much attention due to their great potentials in tissue engineering applications such as implants, surgical sutures, osteosynthetic materials, bone plates, stents, gauzes, and bone dowels and also as matrix material assisting slow release of drugs and hormones [24, 30, 35–37].

PHA possesses flexible mechanical properties from hard crystalline to elastomeric polymer materials. The properties of PHA are influenced by functionalized groups such as hydroxyl, carboxyl, halogen, phenoxy, and epoxyl in the monomer's side chain [24, 38, 39]. P(3HB) homopolymer is reported as a brittle, highly crystalline, and stiff material [40]. In addition, the molecular weight, M_w of P(3HB) synthesized from wild-type bacteria is typically between the range of $1 \times 10^4 - 3 \times 10^6$ Da with a polydispersity of approximately two. On the other hand, P(4HB) homopolymer is classified as an elastic material due to its high tensile strength and elongation at break with 104 MPa and 1000%, respectively [41]. This is similar to ultrahigh molecular weight polyethylene. Hence, PHA can be tailored to suit various applications and is a desirable alternative to petrochemical-derived plastics as well as beneficial in biomedical and pharmaceutical fields [42].

2.1. Poly(3-Hydroxybutyrate-co-3-Hydroxyvalerate), P(3HB-co-3HV). The synthesis of P(3HB-*co*-3HV) copolymer involves the incorporation of 3HV monomer into the 3HB backbone[12, 43, 44]. Biopol™ is the trade name that has been producing P(3HB-*co*-3HV) in a large scale for many years which has been the central PHA marketing strategies by Zeneca and Monsanto[45, 46]. The chemical structure of P(3HB-*co*-3HV) is shown in Figure 1.

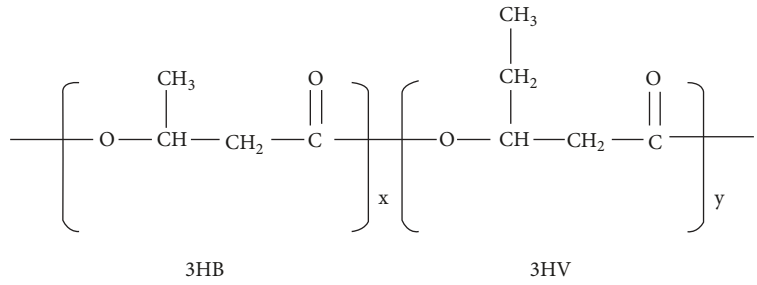


Figure 1: The chemical structure of P(3HB-*co*-3HV).

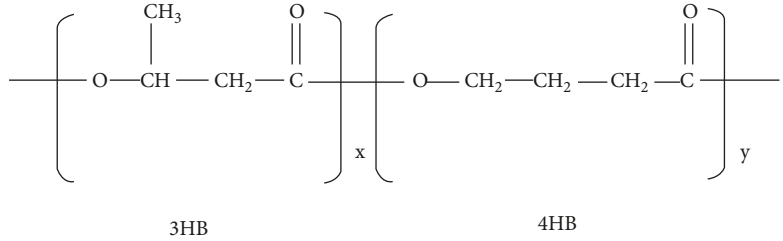


Figure 2: Chemical structure of P(3HB-*co*-4HB).

It was reported that the crystalline formation of P(3HV) is very similar to P(3HB). P(3HV) has a repeating unit volume of 0.13 nm^3 and a fiber repeat of 0.556 nm compared with 0.11 nm^3 and 0.596 nm, respectively, for P(3HB) [47]. It is reported that the P(3HB-*co*-3HV) copolymer is less crystalline, more flexible, and more readily processable. Generally, the changes in mole percentage of the 3HV monomer influence the properties of the copolymer. A trend of an increase in impact strength and decrease in Young's modulus can be observed as the fraction of 3HV monomer increases. This contributes to a tougher and more flexible copolymer [9, 48].

The rate of crystallization was found to decrease with increasing 3HV content. The rate was reported to increase by the adding of nucleating agents, which reduces brittleness and increases elongation at break[49]. The incorporation of 3HV into P(3HB) was reported to influence the nucleation rate, degree of crystallinity, growth rate, and aging effect [50, 51].

As compared to P(3HB), the P(3HB-*co*-3HV) copolymer has a lower melting point as compared without changing its degradation temperature. This allows the thermal processing of the copolymer for various applications [12]. The comparison of the thermal and mechanical properties of synthetic polymer to 3HV copolymer with varying monomer compositions is listed in Table 1. P(3HB-*co*-3HV) copolymer with more than 20 mol% of 3HV units is suitable for making films and fibers with varying elasticity by controlling the processing conditions [42]. It was reported that the P(3HB-*co*-3HV) copolymer exhibits isodimorphic characteristics. Below 30 mol% of 3HV monomer composition, the 3HV units crystallize in the P(3HB) lattice whereas above 30 mol% of 3HV monomer composition the 3HB units crystallize in the P(3HV) lattice [11, 52].

The potential of this biodegradable copolymer relies on the biodegradability of this polymer in the natural environment. Degradation was reported to occur due to the extracellular enzymes produced by various microorganisms [53]. There are a few factors that determine the biodegradability of polymer. Polymers that have low molecular weight and low melting point degrade faster. Besides, the chemical structure of the polymer also influences the rate of biodegradation. Polymers that have high crystallinity have lower rate of biodegradation [42].

2.2. Poly(4-Hydroxybutyrate), P(4HB) and Poly(3-Hydroxybutyrate-co-4-Hydroxybutyrate), P(3HB-co-4HB). The P(4HB) homopolymer and P(3HB-*co*-4HB) copolymer have been intensively researched due to their biocompatible attributes. The chemical structure of P(3HB-*co*-4HB) is shown in Figure 2. Various precursor substrates such as γ-butyrolactone, 4-hydroxybutyric acid, and ω-alkanediols can be used to incorporate the 4HB monomer into the copolymer [54–56]. Besides, wild-type and recombinants of *Cupriavidus necator* and *Delftia acidovorans* are the good choices of strains currently studied for P(3HB-*co*-4HB) production using a variety of carbon precursors, but other potential strains have also been evaluated for higher copolymer yields. Meanwhile, genetically engineered *E. coli* is capable of producing this copolymer from unrelated carbon source such as glucose [57, 58].

Biosynthesis of P(3HB-*co*-4HB) can be conducted in shaken-flask or by batch and fed-batch culture systems in bioreactors. In shaken-flask cultivation, two-stage cultivation is usually preferred as it resulted in higher 4HB molar fraction [54, 59, 60]. 3HB monomers are typically generated by utilizing sugars such as glucose, fructose, sucrose, and acetic

Table 1: Thermal and mechanical properties of various composition of P(3HB-co-3HV) copolymer [18].

	Melting temperature (°C)	Glass transition temperature (°C)	Young's modulus (GPa)	Tensile strength (MPa)
Polypropylene	176	-10	1.7	38
Poly(ethylene terephthalate)	267	69	2.9	70
Nylon-6,6	265	50	2.8	83
Low-density polyethylene	130	-30	0.2	10
P(3HB)	179	10	3.5	40
P(3HB-*co*-3 mol% 3HV)	170	8	2.9	38
P(3HB-*co*-9 mol% 3HV)	162	6	1.9	37
P(3HB-*co*-14 mol% 3HV)	150	4	1.5	35
P(3HB-*co*-20 mol% 3HV)	145	-1	1.2	32
P(3HB-*co*-25 mol% 3HV)	137	-6	0.7	30

acid and butyric acid [59]. In contrast, the generation of 4HB monomers is triggered by 4HB precursors. There are various factors influencing the biosynthesis of this copolymer, which include inoculum concentration, pH, carbon to nitrogen (C/N) ratio, feeding of single or mixtures of carbon sources, and aeration [54, 57–59].

P(3HB-*co*-4HB) copolymer's physical properties vary based on the 4HB monomer composition. P(3HB-*co*-4HB) copolymers possess lower melting temperature (T_m) compared to P(3HB) homopolymer. T_m ranges between 50 and 178°C with raising 4HB molar fraction in the range of 0 to 100 mol% [41, 61]. Meanwhile, glass transition temperature (T_g) is reported to be in the range of 4 to -48°C. Besides, the number average molecular weight (M_n) of P(3HB-*co*-4HB) copolymer is varied between 10^4 and 10^6 Da [41, 55, 58, 61]. In previous studies, decrease in M_n was reported to increase 4HB molar fraction [54, 61, 62].

P(3HB-*co*-4HB) can be formed as random or block copolymers. Amirul et al. (2008a) demonstrated that P(3HB-*co*-4HB) with 32 to 50 mol% 4HB monomer composition were mostly random copolymers since they normally have polydispersity (D) values in the range of 1.3 to 1.8 [54]. Besides, the tensile strength of P(3HB-*co*-4HB) copolymers is between 17 and 104 MPa [41]. Their elongation to break values is between 5 and 1320% with the increment of 4HB composition. In this regard, P(3HB-*co*-82 mol% 4HB) achieved the highest 1320% of elongation to break, which was considered as a very flexible biopolymer [41]. Indeed, P(3HB-*co*-4HB) with higher 4HB monomer composition possessed desirable elastomeric properties. Thus, the elastomeric properties of P(3HB-*co*-4HB) facilitated the development of flexible and strong biomaterials to be used widely in various applications.

3. Surface Modification of SCL-PHA

Surfaces serve as a crucial platform especially in the fields of biology and medicine because most biological reactions normally happen at the surface and interfaces of materials. The cell surfaces comprise a great number of receptors that bind with other cells or proteins to compose the cell surrounding [77]. The interactions between the surfaces of materials and extracellular matrix (ECM) have been intensively investigated in previous studies, thereby developing ideal biomaterials which are able to enhance the tissue responses significantly. There have been reports of numerous reactions occurring between the biomaterial surface and the biological system when the biomaterials are exposed to the living organisms [78]. The reactions often differ based on the surface architecture and porosity as well as chemical composition of the cell-scaffold surface interface with the biological system.

Basically, water adsorption occurs at the surface of the biomaterial which subsequently attracts biomolecule adsorption which is followed by cell attachment onto the surface of biomaterials [79]. In fact, the quality of a biomaterial is influenced by its surface properties since reactions are initiated between biomaterial surfaces and ECM [80]. As biomaterials come in contact with the living organism or host, the cells are slowly adhered to the material of the surfaces, gradually initializing tissue regeneration. The foreign surface of the biomaterial is converted into a biological signal by the water and biomolecule from blood or serum adsorption towards the surface of the scaffold for accumulation of cell attachments [81]. Thus, an ideal material surface is capable of triggering a constructive cell response which is useful in wound repair and tissue engineering, while an unfavorable surface structure may indicate that the material needs to be removed or isolated [82]. However, both acute and chronic inflammatory responses can be activated by implanting the biomaterials into the living organisms.

Surface modification is considered as an excellent technique in designing scaffolds to meet the specific requirements for the development of tissue engineering scaffolds [83]. At present there are many surface modification methods that can be summarized in Table 2. However, the bulk properties of a material is not changed in the surface alteration, only the outermost surface composition and topography of the biomaterial [84]. The biological performance of the *in vivo* interaction between cells and scaffolds is determined by the quality of the scaffold [85].

3.1. Mechanical Modification of PHA. Mechanical modifications revolve around the surface topographical structures,

Table 2: Surface modification techniques employed in fabricating surface-modified biomaterials.

Types of modifications	Modifications of surfaces
Mechanical modifications	(1) Creating physical micro-rough, grooves or pores by enzyme degradation[63], alkali hydrolysis[64], particulate leaching or freeze-drying methods[65].
Physicochemical modifications	(1) Treatment with active gases, vapor or ion treatments to form hydrophilic groups on the surface[66].
	(2) Plasma treatment and grafting to form functional groups on surface[67, 68].
	(3) Photo-induced grafting to produce charged surface[69].
Biological modifications	(1) Immobilizing biomolecules (peptides, heparin, collagen, chitosan and gelatin) onto the surface[70–74]
	(2) Coating and entrapment of biomolecules onto the surface[75, 76].

such as grooves, ridges, and micro- and nanostructures [86]. The surface porosity or roughness of the designed scaffold, which mimics the ECM of natural tissue, is able to enhance their cellular responses effectively [87]. Presently, fabricating scaffold with porous surface is carried out through various methods of mechanical modifications.

Particulate-leaching is a commonly used method to fabricate scaffolds with rough surface architecture with grooves and microstructures. The particles often used are salts, sucrose, gelatin, or sodium bicarbonate ($NaHCO_3$) which are known as porogens to create the pores (Figures 3 and 4). Water-soluble particles (porogen) used in this method include salts [88], hydrogel [89], and ice particles[90] which provide ease of control on pore structure, pore size, and porosity. Fabricating scaffolds by the particle-leaching method involve casting a polymer solution with water-soluble particles (porogen). After the evaporation of the solvent, the water-soluble particles are leached out with water to form the pores. The porogen is leached out by rinsing the scaffolds with water, leaving behind pores left by porogen on the scaffolds [91]. Apparently, highly porous scaffolds with pore sizes ranging from 280 to 450 μm were achieved using various porogen with varied particle sizes. The bone generation on P(3HB-*co*-3HV) scaffolds based on the surface topography was investigated by Köse et al. (2003), who prepared P(3HB)-like scaffolds after solvent evaporation and solute leaching using sieved sucrose crystals (300-500 μm) as porogen [92]. The scaffolds were then treated with oxygen plasma to alter the surface chemistry and hydrophilicity in increasing cell adhesion. The cell adhesion strength of the stromal osteoblastic cells was enhanced through oxygen plasma treatment and the increased roughness or porous structure of the scaffold surface compared to the untreated surface. Similarly, P(3HB) bioresorbable porous patches were also fabricated using unsieved NaCl patches (300-500 μm) as porogen, which was added into the P(3HB) polymer solution. The degradation of the P(3HB) scaffolds was tested *in vitro* and *in vivo* [93].

Interestingly, recent research revealed a new fabrication approach of PHA macroporous scaffold by combining both the particulate leaching and enzyme degradation techniques. It was reported that the water uptake capability of the P(3HB-*co*-70 mol% 4HB) scaffold increased upon salt leaching followed by enzymatic degradation by the depolymerase isolated from *Acidovorax* sp. DP5 bacterium [94, 95]. Freeze-drying technique is a simple and economical approach in altering the surface topography of scaffolds by creating micro-rough or porous surface on scaffolds. This technique is based on the basic principle of sublimation whereby freezing polymer solutions at various concentrations. Later, this ice-polymer scaffold is freeze-dried leaving behind a porous structure on the surface of the synthesized P(3HB-*co*-4HB), where bacterial cellulose derived from the *Acetobacter xylinum* bacterium was prepared using the freeze-drying method. The P(3HB-*co*-4HB) copolymer was dissolved in 1,4-dioxane under vigorous agitation and kept frozen in -80°C which is later freeze-dried.

This scaffold exhibited accelerated cell proliferation with Chinese hamster lung (CHL) fibroblast cells. This scaffold, with enhanced degradation rate, has been targeted as a wound dressing or tissue engineering scaffold [96]. Immobilization of collagen onto P(3HB-*co*-3HV) films was carried out by aminolysis technique to create microrough surface structure conducive for cell adhesion and proliferation of sheep chondrocytes cells. The modified film was reported to demonstrate high water uptake capability as compared to P(3HB-*co*-3HV) films[97]. Apart from that, the biocompatibility of oligohydroxyalkanoates including oligo P(3HB) and oligo P(3HB-*co*-4HB) were fabricated by conducting polymer methanolysis and encapsulating them in liposomes. The biocompatibility was tested with mouse fibroblast cell line L929 [98]. This study showed that the biocompatibility of biopolymers for tissue engineering does not only depend on the polymer implant itself but also depend on the degradation of the polymers including oligomers and monomers [98].

Besides, Cheng et al. (2008) had evaluated the P(3HB-*co*-4HB) biopolymer as a potential candidate for artificial blood vessels. Solvent-cast P(3HB-*co*-4HB) scaffold containing 0-40 mol% 4HB was evaluated for growth and formation of elastin of rabbit blood vessel smooth muscle cells (RaSMCs)[99]. The *in vitro* culture of RaSMCs cells has proven good cytocompatibility of the scaffold with accelerated cell infiltration and proliferation [99].

Surface modification of biomaterials using enzymatic-catalyzed degradation has since garnered much interest. It has been reported that the macroporous structure of P(3HB) scaffold was fabricated using lipase enzyme as the degrading

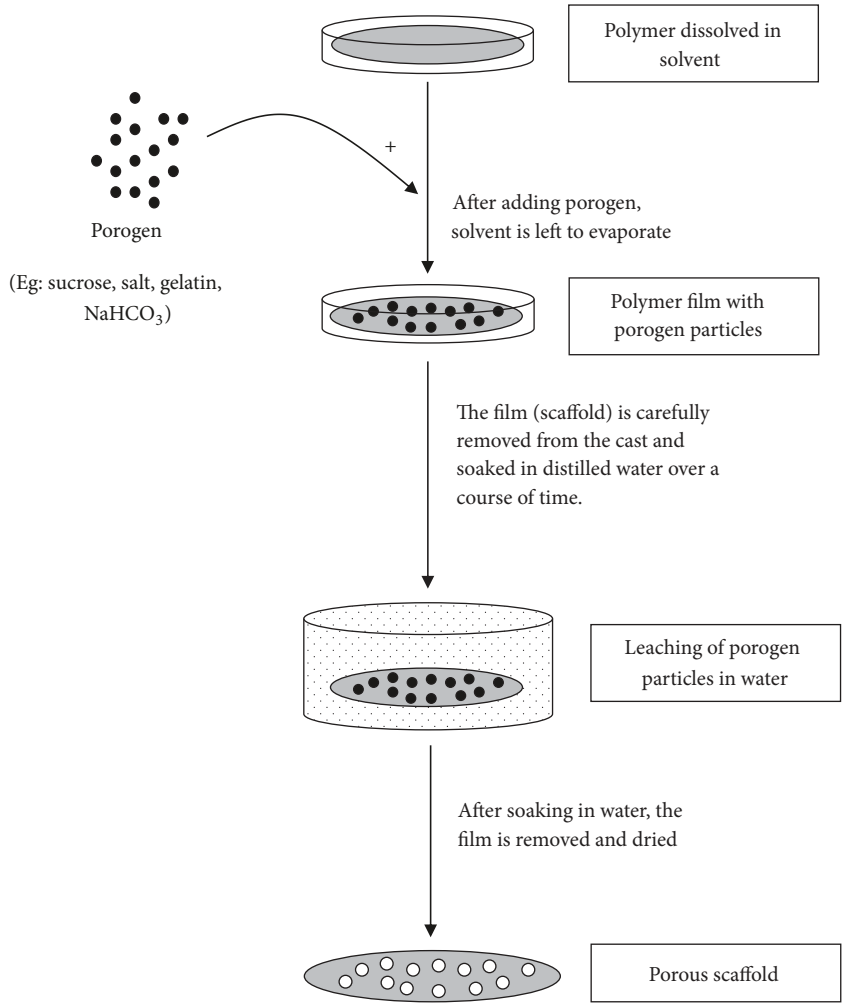
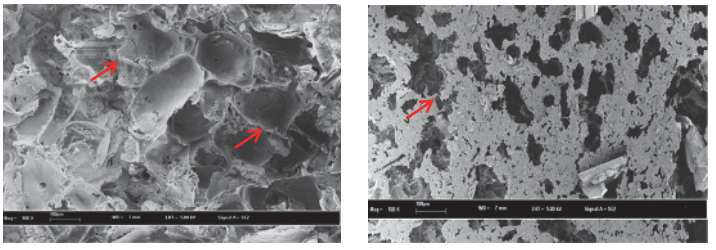


Figure 3: Schematic drawing showing the common fabrication for solvent-cast porogen-leached polymer scaffold.



(a) (b)

Figure 4: SEM images of P(3HB-*co*-4HB) copolymer fabricated by (a) salt and (b) $NaHCO_3$ as porogens. Arrows refer to the surface area and contour that formed rough microporous pore wall surface which is more favorable conditions for the evolution of cell populations.

agent at 50°C and 120 rpm for 6 h. The lipase degradation generated functional groups to which gelatin and glucosamine were chemically coupled. The surface morphology examined using scanning electron microscopy (SEM) revealed a uniformly porous structure for potential application as tissue engineering scaffolds [100].

3.2. Physicochemical Alteration of PHA. Altering the surface chemistry and surface charge density and attaching specific chemical groups can accelerate cell adhesion, proliferation, differentiation, and synthesis of ECM [85]. In general, physicochemical modifications of surface biomaterial involve treatment with chemical crosslinking or aminolysis, active gases, vapor, radiation, or plasma treatment [86]. Plasma treatment is widely used in surface modification whereby it involves the use of electrons, ions, radicals, neutral molecules, and gases to modify the surface of materials [8]. Biocompatible P(3HB-*co*-3HV) films for tissue engineering that were fabricated by

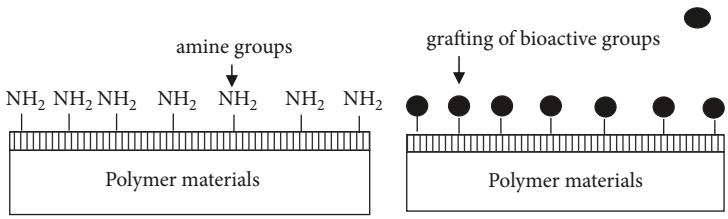


Figure 5: Illustration representing surface modifications using aminolysis technique.

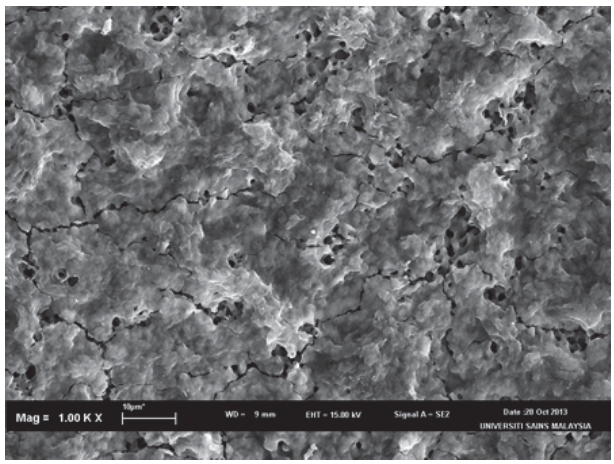


Figure 6: SEM image of P(3HB-*co*-4HB) immobilized with collagen peptides via aminolysis.

plasma treatment have been reported [88]. In the study, the oxygen plasma treatment had increased the oxygen content while the nitrogen plasma treatment enriched the surface with nitrogen atoms. It was also found that new bonds such as COOH (from oxygen plasma), C-N, C=N, and amide bonds (from nitrogen plasma) were generated after plasma irradiation. An increase in hydrophilicity and cell adhesion of dog bone marrow stromal cells were observed using the plasma treated P(3HB-*co*-3HV) scaffolds [88].

Photo-induced grafting is another form of well-known technique used in surface modification of polymer material. Photo-induced grafting involves the use of ultraviolet (UV) or gamma radiation to initiate graft polymerization of hydrophilic groups, such as hydroxyl (-OH), carboxyl (-COOH), or amide (-$CONH_2$), onto the polymer surface to enhance hydrophilicity, thus enhancing the biocompatibility of the biopolymer materials [89]. Gamma radiation has been utilized for the modification of P(3HB) surface for numerous biomedical applications. Many reactions occur when the polymer material was exposed to gamma radiation. The radiation causes chain crosslinking to form small molecular by-product, leading to changes in the surface properties of the polymer material [101].

Aminolysis is another versatile method of surface modifications by introducing the amine group (–NH_2) or other functional groups onto the polymer surface (Figures 5 and 6). Functional moieties are later conjugated or grafted onto the polymer surface via these active sites [102]. Many studies have been conducted to fabricate biomaterials grafted with a series of functional surfaces on various types and forms of polymers through the aminolysis process. These functional surfaces are capable of improving cell adhesion, proliferation, and cellular functions. Amine groups were conjugated onto the surface of P(3HB-*co*-3HV) films by the aminolysis process which was later grafted with collagen. The amount of amine groups grafted on the P(3HB-*co*-3HV) film was found to significantly enhance the hydrophilicity of scaffolds. The modification of collagen-loaded P(3HB-*co*-3HV) film served as a scaffold to promote the growth of bone cells demonstrating the versatility of the aminolysis-based polymer surface in biomedical applications[103]. Aminolysis has also been carried out by immobilizing fish scale collagen peptides onto P(3HB-*co*-4HB) scaffolds [91].

3.3. Biological Modification of PHA. Biomacromolecules are mainly protein, polysaccharides, lipids, ligands, nucleotides, and proteoglycans. These biomacromolecules are attached to the polymer surfaces to prompt cellular responses. Immobilizing these biomacromolecules on biomaterial surfaces requires several approaches such as physical adsorption, covalent attachment and physical-entrapment chemical grafting, surface coating, and entrapment. There are many studies on immobilization of collagen, chitosan, gelatin, and RGD peptide (L-arginine, glycine and L-aspartic acid) onto polymer surfaces to improve the scaffold-cell interaction and proliferation [104]. Nowadays, the studies on the immobilization of biopolymer with biomolecules such as chitosan, collagen, and gelatin have been conducted to improve the properties of the polymer in designing a more suitable biomaterial which even can be implanted into body for tissue repairs [105].

Chemical grafting is a method of immobilizing biomacromolecules like RGD peptide and proteins through the plasma graft or photochemical method [93]. P(3HB-*co*-3HV) films were activated by ammonia plasma treatment on the surface, followed by the chemical grafting of RGD-containing peptides, where the RGD-containing peptides were covalently grafted onto the P(3HB-*co*-3HV) films. The modified P(3HB-*co*-3HV) films grafted with RGD exhibited a distinctly improved cellular compatibility [106]. Covalent immobilization onto the P(3HB-*co*-3HV)-collagen film surface to improve its cell compatibility was also developed by Wang and coworkers [97]. Amide groups were photografted on P(3HB-*co*-3HV) films and collagen was then chemically bonded to amine groups to form the collagen-modified P(3HB-*co*-3HV). Surface wettability or hydrophilicity of the

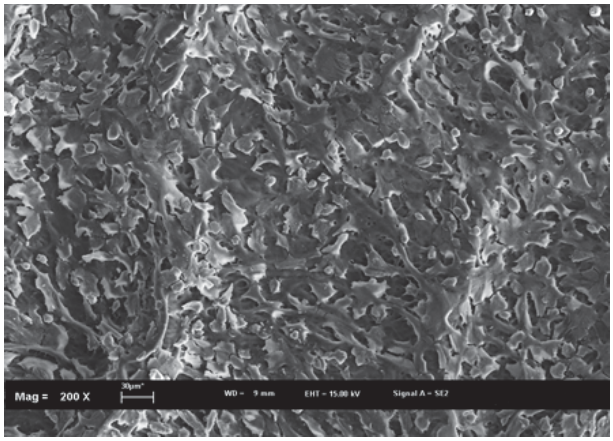


Figure 7: SEM image of V79 fibroblast cells proliferation on P(3HB-*co*-4HB)-collagen peptide.

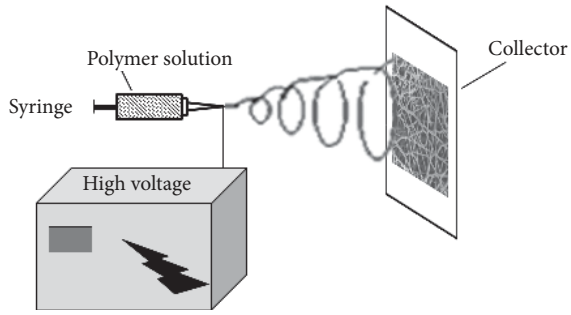


Figure 8: Illustration representing fabrication of nanofibers using electrospinning.

modified P(3HB-*co*-3HV) films was enhanced remarkably. Sheep chondrocytes cultured on the modified P(3HB-*co*-3HV) films showed that the collagen-modified P(3HB-*co*-3HV) film had good cell adhesion and proliferation. Chondrocytes on the collagen-modified P(3HB-*co*-3HV) film adhered and formed cell layers, indicating that the collagen-modified P(3HB-*co*-3HV) was a promising biomaterial for cartilage tissue engineering [97]. The blending and covalent bonding of biomacromolecules, such as collagen, gelatin, and chitosan, on P(3HB-*co*-4HB) were also previously described (Figure 7) [91, 107].

The technique of coating a biopolymer surface with biomacromolecules is considered as one of the simplest surface modification approach. A better adhesion between biomaterial surfaces and cells can be provided by certain proteins including collagen, fibronectin, and vitronectin [3]. The sol-gel process is a wet-chemical technique which was used to apply bioactive coatings onto the material surface. This alters the biological behavior of proteins and cells hence can be applied as implants. In this process, the sol (or solution) gradually forms a gel-like network containing both the liquid phase and the solid phase on the surface of the material. There are several works on the use of the sol-gel process for production of biomaterials [95]. Among this, Li et al. (2005) had fabricated scaffolds of P(3HB-*co*-3HV) coated with sol-gel-derived bioactive glass. They investigated the coating of bioactive glass via sol-gel technique onto P(3HB-*co*-3HV), which promoted the hydrophilicity properties of the composites [108]. It was found that the modified P(3HB-*co*-3HV) coating delayed the degradation of P(3HB-*co*-3HV) in the composite scaffolds during the period investigated. This not only showed a useful method to prepare scaffolds with improved properties but also a way of tailoring the *in vitro* degradation behavior of the scaffolds.

The entrapment method which was invented by Desai and Hubell (1992) is a versatile method to modify biomaterial surfaces with improved hydrophilicity and compatibility [94]. The cytocompatibility of P(3HB-*co*-3HV) scaffolds was enhanced by surface modification of scaffolds through physically entrapping gelatin onto the scaffold surfaces and heparin was subsequently immobilized on the entrapped gelatin. The surface modification method enhanced the wettability of scaffolds and provided the active binding site onto them for cell attachment. Consequently, the formed scaffold possesses desirable properties towards individualized tissue engineering especially bone tissue regeneration [109].

4. Nanofabrication of PHA

Nanofabrication or commonly known as nanotechnology revolves around devices and materials produced in nanometers [110]. In recent years, nanofabrication garnered much popularity due to the nano- and microtopographies which closely mimics the natural surroundings for extracellular matrix. Electrospinning is a popular technique in the fabrication of fibrous biomaterials (Figure 8) [111–114]. Basically, electrospinning process comprises the syringe to extrude the polymer solution, high-voltage source to form the surface charges, and the collector to collect the polymer nanofiber. The application of high-voltage source causes the formation of surface charges that causes the polymer solutions to emerge as jet polymer solution at the needle tip to form Taylor cone. The shape of the cone determines the shape of the cone. The polymer jet is stretched based on the accelerated electric forces which contribute in the synthesis of elongated fibers. The collector is where the nanofiber polymer scaffolds form when the solvents evaporate [112, 115, 116].

These fabricated electrospun matrices (Figure 9) were known to support the attachment and proliferation of a wide variety of cell types. The use of innovative collectors and spinning techniques resulted in scaffolds with aligned fibers, different compositions, improved mechanical properties, varying degradation rates, or functional moieties to be produced [117]. Although the fundamental comprehension on the fiber formation has been emphasized in several previous studies, one of the limitations is the fiber diameter uniformity which still required much effort to be addressed [112].

In a recent study, electrospun P(3HB-*co*-4HB) copolymer with collagen peptide showed variation in the size of nanofibers depending on 4HB monomer composition [114]. In general, the electrospun nanofibrous matrix was found to enhance surface wettability and cell growth, showing desired properties as biodegradable wound dressing. The properties of electrospun scaffolds can be improved prominently by

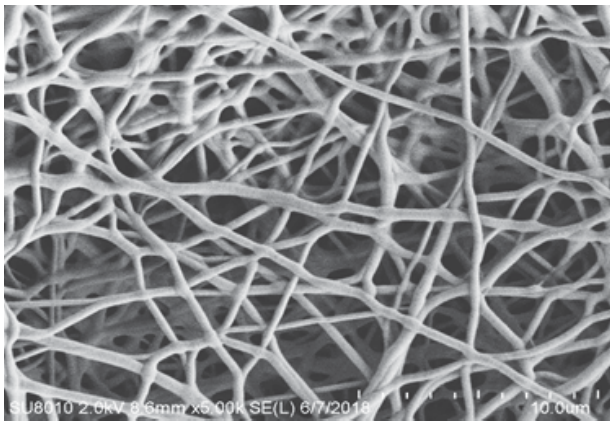


Figure 9: SEM image of nanofiber scaffold fabricated by electrospinning technique using P(3HB-*co*-4HB) copolymer. The nanoscale network structure mimic ECM.

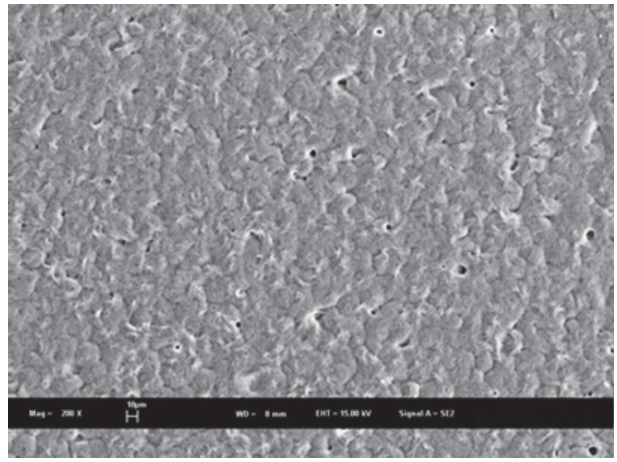


Figure 10: SEM image of P(3HB-*co*-4HB)-collagen peptide scaffolds fabricated via facile blending.

controlling the morphology of the nanofibers. In addition, the development of ideal scaffolds with clinically relevant dimensions and the homogeneous distribution of cells within them also required to be considered in medical application especially tissue engineering [118].

5. Blends and Composites of PHAs

In order to vary the properties of P(3HB-co-4HB) copolymers for specific applications, they have been incorporated with other suitable bio-based materials to devise new blends or composites. Based on a study by Yu et al. (2012), P(3HB-*co*-4HB) containing 4 mol% 4HB was melt-mixed with short glass fibers (SGF) through a corotating twin-screw extruder [119]. They proved that the properties, such as tensile modulus, tensile strength, and impact strength of P(3HB-*co*-4HB)/SGF composites, were increased by 6.6 times greater compared to the P(3HB-*co*-4HB) copolymer. Apart from that, another study regarding new blends of polybutylene succinate (PBS)/P(3HB-*co*-4HB) have also been investigated in detail [120]. The analysis using differential scanning calorimeter (DSC), universal material testing machine, and rheometer has been conducted to determine the mechanical properties, crystallinity, and rheological behavior of PBS/P(3HB-*co*-4HB). As a result, the addition of P(3HB-co-4HB) increased the crystallization temperature of PBS. The storage modulus (G') and loss modulus of PBS/P(3HB-*co*-4HB) blends were directly proportional to the content of P(3HB-*co*-4HB). It was strongly hypothesized that the addition of P(3HB-*co*-4HB) could enhance the properties of PBS [120].

Furthermore, the blends of biodegradable polymer polylactide (PLA) with P(3HB-*co*-4HB) have been synthesized via melt compounding [121]. The impacts of P(3HB-*co*-4HB) on the properties of PLA/P(3HB-*co*-4HB) blends have been well studied and understood. Both P(3HB-*co*-4HB) and PLA are immiscible and this decreased the glass transition temperature (T_g) of P(3HB-*co*-4HB) compared to that of the original P(3HB-*co*-4HB). This is due to the presence of phase interface between PLA and P(3HB-*co*-4HB), which results in promoted chain mobility near the interface. Moreover, the elongation at break of the PLA/P(3HB-*co*-4HB) blends was enhanced considerably after adding P(3HB-*co*-4HB), indicating an improvement in the inherent brittleness of PLA. Meanwhile, it was affirmatively that PLA biodegradability was improved via the synthesis of the blends [121]. In another study, collagen was incorporated onto the P(3HB-*co*-4HB) copolymer by using facile blending combining two different solvents, chloroform and acetic acid solution (Figure 10) [70]. This technique of blending was found to increase hydrophilicity and L929 cell proliferation on the surface significantly.

Another study on the preparation of P(3HB-*co*-4HB) and cobalt-aluminum-layered double hydroxide (LDH) by melt intercalation has been investigated [122]. The bionanocomposites have been analyzed systematically for their thermomechanical properties, thermal stability, and thermal combustion. As a result, the reading of the heat release capacity (HRC) of the bionanocomposites from the microscale combustion calorimetry was shown to reduce pointedly by adding LDH. In addition, dynamic mechanical analysis indicated that the storage modulus of the bionanocomposites with little LDH has improved greatly [122]. Besides, a simple blending has been used to fabricate the composite membrane of porous P(3HB-*co*-4HB)/calcium metaphosphate (CMP) to improve the properties of P(3HB-*co*-4HB) significantly[123]. Apart from that, syntheses for random block polyurethanes (PU3/4HB-ran-PEG) and alternating block polyurethanes (PU3/4HB-alt-PEG) were carried out using 1,6-hexamethylene diisocyanate (HDI) as the coupling agent, based on P(3HB-*co*-4HB) and poly(ethylene glycol) (PEG) with similar chemical compositions. Comparatively, PU3/4HB-alt-PEG possessed better hemocompatibility on platelet adhesion due to microstructure presence and hydrophilic surface. Meanwhile, rat glial cells and fibroblasts exhibited a more favorable compatibility towards PU3/4HB-alt-PEG films for cell attachments based on cell culture assays. Therefore, PU3/4HB-alt-PEG offered better

biomaterial structures and desirable properties for medical applications [124, 125].

6. Applications of Surface Modified PHA in Medical and Pharmaceutical Fields

The biocompatibility of PHA usually depends on its shape, surface porosity, chemistry of materials, and incorporated tissue [30]. P(3HB) exhibits good biocompatibility and is a normal metabolite found in human blood[29]. This polymer also exhibits negligible cytotoxic response. However, due to the low degradation of P(3HB), copolymer P(3HB-*co*-3HV) has been chosen for medical studies [53].

PHA is used as nanofibrous scaffold material in tissue engineering where it was designed to provide strong support [111]. It functions to protect implanted cells from the body's immune system and offers necessary mechanical properties and bioabsorption properties. An example of scaffold application is a heart valve. Due to its biodegradable properties, the scaffold inserted in human body does not have to be removed [30]. Monofilament P(3HB-*co*-3HV) sutures were also used for healing of muscle-facial wounds. No acute vascular reaction was detected at the site of implantation. Throughout the healing period of the muscle-facial cuts, the sutures featured the necessary strength and this implantation fitted into the usual wound process [35]. The hemostasis system at the cellular response level was not affected by P(3HB) and P(3HB-*co*-3HV) as reported previously [35]. There was no acute vascular reaction at the site of implantation where P(3HB) or P(3HB-*co*-3HV) sutures were implanted in test animals for a year. The blend of hydroxyapatite with P(3HB) or P(3HB-*co*-3HV) demonstrated mechanical strength similar to that of human bones, which is particularly beneficial in bone tissue engineering [126].

Since PHA is biodegradable and biocompatible, it has become a potential candidate to be used as drug carriers. The rate of drug release depends on matrix porosity and copolymer composition [127]. P(3HB-*co*-3HV) was used in the construction of controlled antibiotic systems [128]. It was reported that, as the 3HV content increases, the encapsulation efficiency decreases. This is due to the increase in the amorphous portion as the 3HV content increases, causing the drug to partition into the amorphous region [128].

The P(3HB-*co*-4HB) is a promising biomaterial for diverse applications especially in tissue engineering. This copolymer gained attention from several researchers due to its superior biodegradability, biocompatibility, tailorable chemical and physical properties, and nontoxicity [13, 129]. Therefore, much efforts were taken to synthesize P(4HB) and P(3HB-*co*-4HB) with enhanced biomaterial characteristics. In a study by Xu et al. (2010), both nanofiber scaffolds and films of P(3HB), P(3HB-*co*-4HB) and P(3HB-*co*-3HHx), synthesized via the phase separation, were investigated for their potentials as medical materials [2]. Results showed that P(3HB-*co*-3HHx) possessed the greatest potential in enhancing the differentiation of neural stem cells (NSC) into neurons, which assists in the repair of the central nervous system (CNS). For the comparison of both nanofibers scaffolds and films on cell viability, nanofiber scaffolds undeniably exhibited higher efficiency for NSC attachments, synaptogenesis, and synaptic outgrowth. This is due to the continuous extensive fibrous network of nanofiber scaffolds, which formed highly interconnected porous structure thus enabling the penetration of neuritis to the inner scaffold matrices for better connection between the cells. Hence, the nanofiber scaffolds of P(3HB-*co*-3HHx) have been proven to be a potential biomaterial for treating severe CNS injury [2].

Furthermore, the technological progress in PHA studies had facilitated the development of electrospun nanofibers of P(3HB-*co*-4HB) and many other PHA constituents as *in vivo* medical scaffolds [129]. Based on a study by Ying et al. (2008), P(3HB-*co*-7 mol% 4HB) and P(3HB-*co*-97 mol% 4HB) were used to fabricate nanofiber scaffolds with improved bioabsorption and biocompatibility via electrospinning [130]. The width of P(3HB-*co*-97 mol% 4HB) nanofiber scaffold was 220 nm while that of P(3HB-*co*-7 mol% 4HB) was 190 nm; thus fiber width was shown to increase with the molecular weight of polymer. However, the surface of P(3HB-*co*-97 mol% 4HB) scaffold reduced in porosity whereas that of P(3HB-*co*-7 mol% 4HB) remained the same after sterilization. The degradation and bioabsorption rate of P(3HB-*co*-97 mol% 4HB) were higher than those of P(3HB-*co*-7 mol% 4HB). In addition, the periodic histological observation demonstrated that both scaffolds had triggered mild tissue responses. Their mechanical properties were comparable to those of human skin thus have potential in providing adequate biomechanical support. After 12 weeks of subcutaneous implantation, the thin connective tissues around P(3HB-*co*-97 mol% 4HB) were observed with no fibrous encapsulation. Moreover, there was considerable decrease in the number of inflammatory cells leading to minimal inflammation around the degraded scaffolds [129]. Besides, the electrospun nanofiber scaffolds of P(3HB-*co*-4HB) were developed with a combination of chloroform and dimethylformamide (DMF)[131]. Various methods such as enzymatic degradation and salt-leaching techniques were used to alter the surface structure of scaffolds by increasing porosity for application in biomedical fields [71].

Apart from that, the incorporation of PHA with nanoclay reinforcement was developed into nanobiocomposites with great potential in biomedicine. For example, P(3HB-*co*-70 mol% 4HB) with a reinforcement of 5% claytone had demonstrated enhanced thermal properties, mechanical properties, and optical transparency [131]. These biocomposites also showed positive improvement on antimicrobial performance with increasing clay concentrations. In general, the biodegradability and biocompatibility possessed by P(3HB-*co*-70 mol% 4HB) had touted its superior performance which broaden its usage in regenerative medical application while being developed as an environmentally friendly biomaterial [132, 133].

On the other hand, the development of P(4HB) and polyglycolide-contained composites as *in vivo* patches for pulmonary artery and heart valve had demonstrated positive results [6, 134–136]. In this regard, polyglycolide/P(4HB) composites used as pulmonary artery and heart valve patches were evaluated *in vivo* in sheep before clinical trials to better understand and control the effects of the composites [134, 136]. In a study by Rao et al. (2010), the fabrication of

P(3HB-*co*-4HB) with natural polymer collagen was carried out via a simple binding method with the addition of vitamin E [137]. The biocompatibility evaluation on chick chorioallantoic membrane (CAM) demonstrated that P(3HB-*co*-4HB)-collagen and vitamin E could reduce inflammation associated with biopolymer and facilitate angiogenesis. This blend could be utilized as potential wound healing dressings due to the components which exhibit accelerated wound healing properties [137].

7. Summary and Outlook

This review paper highlighted various approaches used to modify the surface of PHA for the purpose of improving their properties so that they can be used widely in a variety of applications including biomedical, agricultural, and aquaculture fields. These modification techniques are required to enhance biomaterials for better performance on cell attachments, cellular responses and implantations. Undoubtedly, the alteration of PHA surfaces via blending or binding with biomolecules promotes surface hydrophilicity, thereby enhancing their properties in designing ideal scaffolds mimicking the structure of native ECM. Moreover, advanced nanotechnology has been applied on PHA to produce outstanding electrospun nanofiber scaffolds possessing high nanoscale fibrous network to provide good environment for cell-cell interactions. Further understanding on cell-biomaterial interactions will provide vital ideas for future research in designing improved biomedical patches or scaffolds for better treatments. In addition, the development of enhanced biomaterials should be emphasized and implemented in a versatile and efficient way to open up a promising future for a wide range of applications. Hence, advanced surface modifications of PHA and the emergence of new fabrication methods are necessary to be further investigated and evaluated towards significant contributions for the progression of various PHA applications in the future.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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