



# Properties and Applications of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) Biocomposites

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## Abstract

Polyhydroxyalkanoates (PHAs) are biopolyesters accumulated by microorganisms as intracellular storage materials and they have attracted attention as “green plastic” alternatives to their petrochemical counterparts. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate), PHBV, is one of the most studied members of the PHAs family, with numerous applications. PHBV has three main features, biodegradability, biocompatibility and it is a biobased polymer (biosynthesis starting from renewable resources). These three features altogether qualify PHBV as a very promising polymer that has great potential to replace conventional non-degradable polymers, and to play a significant role in the circular economy concept. However, PHBV has some prominent disadvantages that limit its wide utilization for commercial use, these drawbacks are mainly weak mechanical properties, low thermal stability, difficult processability and considerable hydrophobicity. In order to overcome the properties issues, to produce materials with more desirable features and to engineer purpose-specific PHBV-based systems, much research has been focused on improving its properties by forming composites and to utilize these produced composites for a wide spectrum of very promising applications. The purpose of the current work is to compile and classify the research accomplished in the field of PHBV biocomposites and their applications in different disciplines. It was found that many different types of nanofillers, natural fibers, agricultural waste, clay, silicate, wood and cellulose derived natural materials have been successfully incorporated into PHBV matrix. The resultant biocomposites were characterized, tested and found promising to be utilized in a wide spectrum of applications, namely packaging, tissue engineering and drug delivery systems. The potential benefits of PHBV-based biocomposites make a strong case for research into this area. Therefore, further research works need to be conducted in order to find new PHBV biocomposite materials for advanced applications.

**Keywords** Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) · Biocomposites · Drug-delivery · Tissue engineering · Thermal and mechanical properties

## Introduction

Biopolymers are considered as very promising replacement for the conventional synthetic polymers that are derived from the petrochemicals industries. The key features of these biopolymers are their known biodegradability and biocompatibility [1] which qualify them to be utilized as

environmentally friendly materials and for therapeutic applications in human bodies [2, 3].

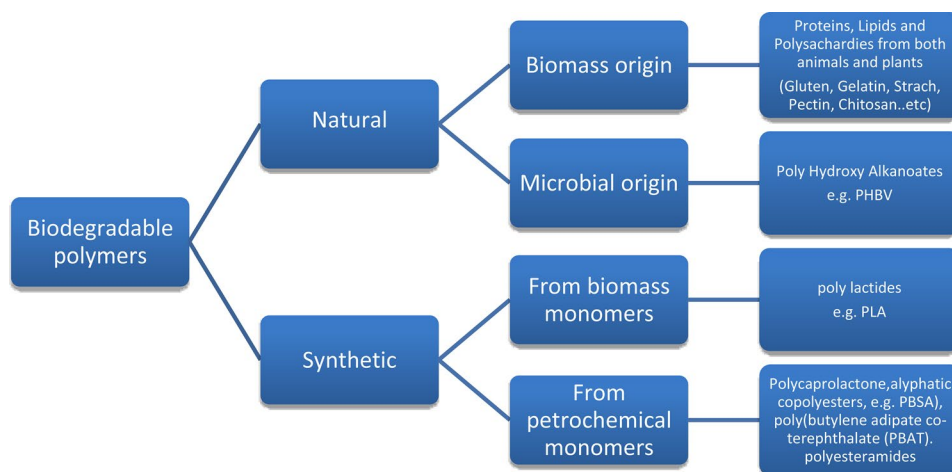
Expanding the utilization and dependence on biodegradable polymers will definitely help protect the environment, respond to the planet threats and counteract against environmental challenges such as global warming and the accumulation of non-biodegradable plastic debris in oceans and elsewhere on the planet. Also, considering the well-known problem of toxic and carcinogenic monomers and tetramers leaching from the walls and surfaces of plastics in direct contact with humans, biocompatible polymers offer a key advantage to be used in commodities like toys, food cans, dishes, cups, other kitchen utensils, water and juice bottles in addition to all types of food packaging materials.

Biodegradable polymers could be classified as natural and synthetic based on their origin [1] (Fig. 1). Cellulose,

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**Fig. 1** Biodegradable polymers classification

chitosan, starch, collagen and gelatin are good examples of natural biodegradable polymers. Synthetic biodegradable polymers can be polyurethanes, poly amino acids, polyesters like polylactones and poly ( $\alpha$ -hydroxyl acids) and many others. Microbial biodegradable polymers are mainly polyhydroxyalkanoates such as PHBV and poly (3-hydroxybutyrate) (PHB) in addition to poly ( $\gamma$ -glutamic acid).

Low carbon footprint, renewability and biodegradability are the driving forces to develop and expand the use of biobased materials, and to transform them into high-volume applications instead of limited niche markets. However, the narrow processing window, low heat deflection temperatures, poor barrier and many other properties are inherent drawbacks in many biobased plastics, those weaknesses limit the desired wide exploitation of such materials. Anyhow, such mentioned drawbacks and others can be overcome by forming biocomposites or blends [4].

Polyhydroxyalkanoate (PHA) are a very interesting family of biopolymers that are biosynthesized from different types of microorganisms, such microorganisms use different renewable carbon resources for the production process, and this makes it a good model for sustainable economy and environment. PHA's are intracellularly accumulated as carbon and energy reserves by several archaea and bacteria [5]. PHA's are biosynthesized as granules under stressed environmental conditions, mainly in the excess of carbon and deficient supplies of other essential growth nutrients like phosphate and nitrogen [5]. The recyclable biological process of PHAs is embedded into the closed carbon cycle of nature, which implies that their degradation does not cause an increase of the atmospheric CO<sub>2</sub> level; hence, PHA does not amplify or accelerate climate change [6] (Fig. 2).

Biosynthesized PHA was first reported in 1926 by Lemaigne [7], and that was the homopolymer PHB which is currently the most well-known member of the PHA family. PHB is a highly crystalline and hence a brittle polymer, and thermally unstable with a small window of thermal

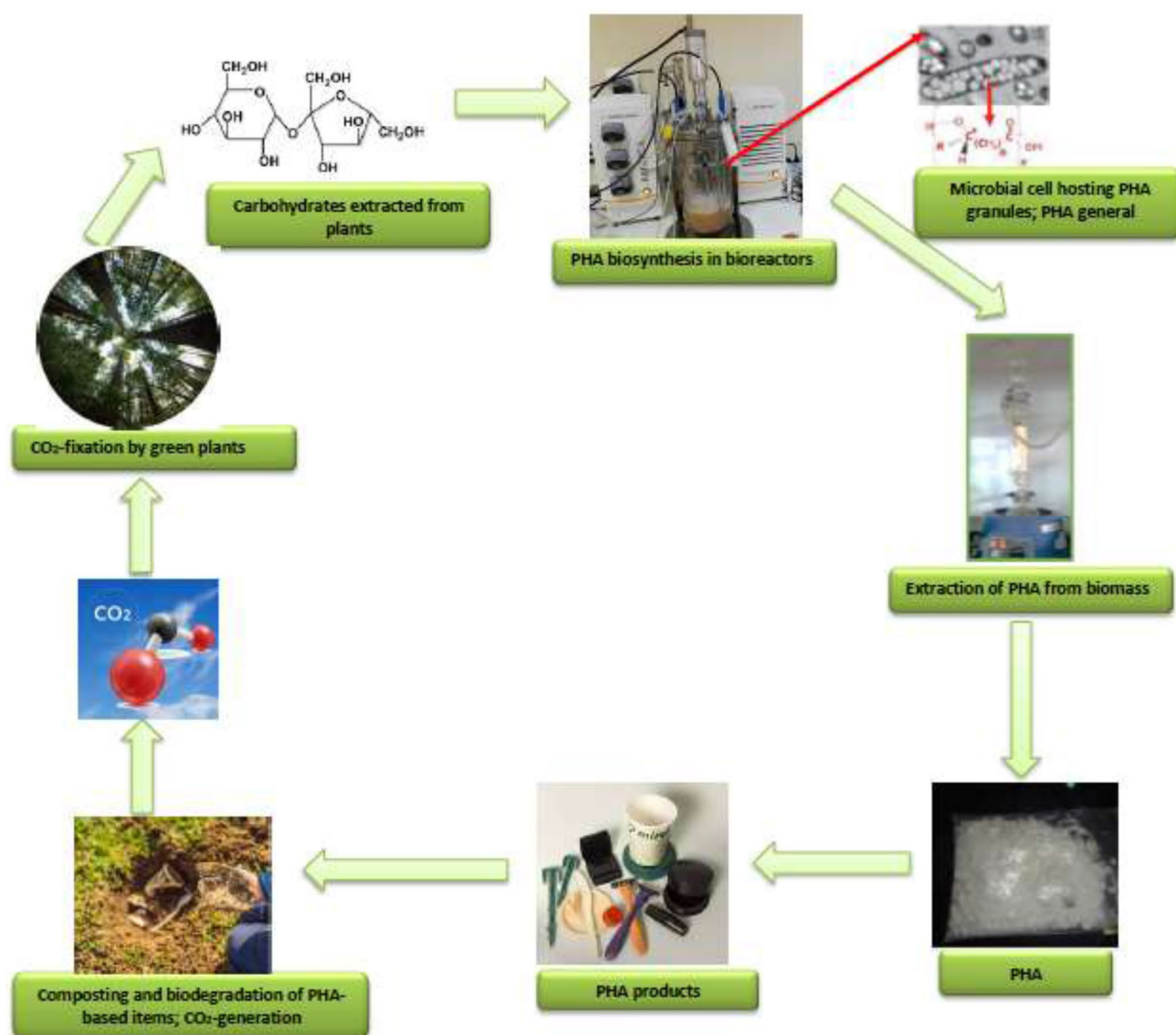
processing stability. PHA polymer is usually composed of 600 to 35,000 (R)-hydroxy fatty acid monomer blocks [8]. Each monomer unit contains usually a saturated side chain group but can also be a branched alkyl group, an unsaturated alkyl group and a substituted alkyl group, which are less frequent [9]. So far, there are more than 100 biosynthesized PHA types produced either by fermentation or genetic engineering [10], and there are more than 150 different PHA monomers identified [11].

By referring to the number of carbon atoms involved in their monomer structures, PHAs have been classified into three main categories [8]:

- (A) Short chain PHAs; 3–5 carbon atoms.
- (B) Medium chain PHAs; 6–14 carbon atoms.
- (C) Long chain PHAs; 15 and more carbon atoms.

PHAs can also be classified as homopolymers and copolymers, homopolymers with only one single monomer as the building block (Fig. 3), and PHAs copolymers which are composed of two or more different building block monomers, examples include PHBV, poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHx), poly(hydroxybutyrate-co-hydroxyoctanoate) (PHBO), poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyhexanoate) (P3HB3HV3HHx) [12, 13] and poly(3-hydroxybutyrate-co-4-hydroxybutyrate-co-3-hydroxyhexanoate) (P3HB4HB3HHx) [13, 14] poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-4-hydroxybutyrate) (P3HB3HV4HB) [15].

The homopolymer PHB is considered as the major member in the PHA family. Also, of the PHA family, the copolymer PHBV is a polyester with a more favorable properties compared to PHB like lower melting point and improved mechanical properties [16, 17]. Compared to PHB, the PHBV copolymer has shown a wider processing window by lower melting and glass transition points [18, 19], in addition



**Fig. 2** Cyclic biological process of PHA

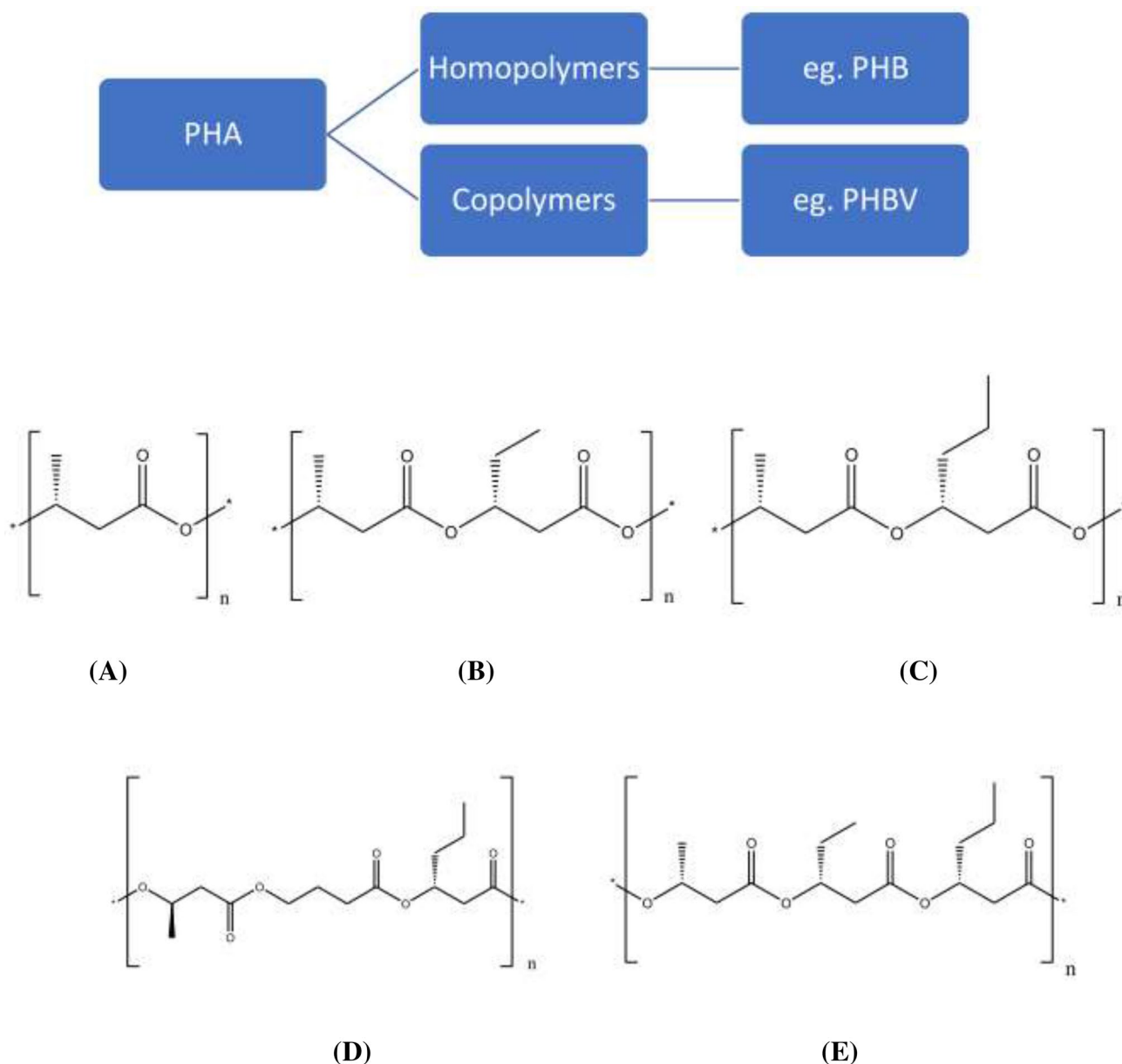
to lower crystallinity, less brittleness and more biodegradability as the valerate percentage increases [20, 21].

The PHBV is characterized by its biological origin, low cytotoxicity, piezoelectricity, thermoplasticity, biocompatibility with many types of cells, biodegradability, high absorption capacity, high degree of crystallinity, excellent oxygen barrier properties, high viscosity in a liquid state, chemical inactivity, ultraviolet radiation resistivity, solubility in chlorinated solvents, relatively low melting temperature, but it is still a rigid and relatively brittle polymer, it has low impact resistance, poor thermal stability, and considerable hydrophobicity [22–25].

Although PHB is considered as the major member in the PHA family, the PHBV copolymer—which was later discovered—has exhibited better biocompatibility, and this

has made it extensively exploitable for the biomedical and pharmaceutical applications such as drug delivery systems, bone scaffolds, implant coatings and tissue engineering in general [26]. The current market of PHBV is very small compared to that of petroleum based plastic. The major factors that restrict the utilization of PHBV on a wider scale are the expensive highly pure substrates, the discontinuous production in batch or fed-batch; the need to add expensive precursors such as valerate and propionate, the use of toxic solvents throughout downstream clean-up and possible microbial contamination [27].

The ideal and most feasible PHBV version would result from a cheap carbon source [28, 29] used as a substrate to feed highly efficient microbial strains that could produce the polymer with high efficiency [30] and with much reduced



**Fig. 3** **a** Poly(3-hydroxybutyrate) (PHB), **b** poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), **c** poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHx), **d** poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P3HB4HB3HHx), and **e** poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyhexanoate) (P3HB3HV3HHx)

droxybutyrate-co-3-hydroxyhexanoate) (P3HB4HB3HHx), and **e** poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyhexanoate) (P3HB3HV3HHx)

cost in the downstream processing and extraction of the polymer [31]. In fact, these are the major three disciplines of current intensive research to commercialize PHBV on a wide scale.

Imperial Chemical Industries (ICM) was the first to commercialize PHBV in 1990, it has been commercialized in production as shampoo bottles (trademarked as Biopol®) that were available in supermarkets in Europe, but later the patents were sold to Monsanto and further to MetaboliX [32].

Regardless of the previously mentioned concerns on PHBV properties and process costs [33], PHBV has been

investigated to be exploited in a wide range of very promising applications. In order to overcome the properties issues, much research works have been focused on forming biocomposites where fillers have enhanced or even totally introduced new features [34]. Most of the applications were oriented towards packaging, reinforced materials and biomedical applications including drug delivery, tissue engineering and a few others (Fig. 4).

There have been many reported works to review the scientific progress achieved in the field of PHA family applications and biocomposites, such as their applications in



**Fig. 4** PHBV various applications



therapeutics field [35, 36], in tissue engineering and bone scaffolds [37, 38], their copolymers [39] and many others [16, 40, 41]. On the other hand, and despite of the very promising features and huge amount of published research on PHBV biocomposites and their applications, there have been very limited published works compiling, organizing and classifying these achievements and scientific progress in different arenas [34, 42].

### PHBV Composites for Packaging Applications

PHBV has been used extensively in packaging applications [43–45]. The well-known antimicrobial properties of zinc oxide (ZnO) and silver nanoparticles (AgNPs) have been introduced into PHBV for food packaging applications [38–48]. For example, zinc oxide has been used with reduced graphene oxide and introduced onto glycerol-plasticized PHBV in different percentages to form hybrid

nanocomposites that were melt-extruded for food packaging purposes [49]. Also, zinc oxide alone was used with different sizes (micro and nanoparticle sizes) and different morphologies to reinforce PHBV films. The results showed that in addition to the expected antimicrobial properties of the filler which qualifies it to be used in food packaging, the incorporation of the zinc oxide nanoparticles enhanced the thermal and optical properties of the nanocomposite films [47]. Composite films of PHBV and ZnO nanoparticles were prepared by solvent casting, the optimum percentage of added ZnO was found to be 4 wt%, This ratio showed the highest crystallinity, the best barrier properties in addition to the maximum tensile strength and Young's and storage moduli. The antibacterial tests were also good as expected. The extreme compatibility between the particles and the matrix and the high enhancement on the properties can be attributed to the formed hydrogen bonding [50]. For the purpose of enhancing the antimicrobial and mechanical properties and with the lowest immigration rate to improve the shelf life of poultry items, the leaf extract of *Thymus vulgaris* was

used for the fabrication of zinc oxide–silver nanocomposites which were incorporated into PHBV–chitosan. These have shown great antimicrobial activity that suggest them as replacement for the traditional petrochemical-based polymers which are currently in use for food packaging of poultry items [51].

Stabilized silver nanoparticles 0.04 wt% have been incorporated into PHBV to result in a nanocomposite with 56% reduced oxygen permeability compared to neat PHBV, and the produced films had a prolonged—up to 7 months—antimicrobial activity against the prominent food borne pathogens, *Salmonella enterica* and *Listeria monocytogenes*. The outcome results have shown very promising biodegradable composites that could be potential films and coatings in the field of active food packaging [46].

Another study has investigated ternary composites with nanohybrids of silver and cellulose nanocrystals incorporated in PHBV via solution casting, higher silver nanoparticles percentage was associated with better mechanical, overall migration, barrier and antibacterial properties, in addition to better thermal stability. The results of biocompatibility, barrier properties, lower migration levels and the high antibacterial effect of 99.9% suggest that these composites can be promising in food packaging [52].

A nanohybrid combination of cellulose nanocrystals and zinc oxide has been composited in PHBV matrix by simple solution casting, the produced nanocomposites showed excellent antibacterial ratio of 95.2–100% for both bacteria *Escherichia coli* and *Staphylococcus aureus* and exhibited degradation of 9–15% after 1 week. The incorporation of cellulose nanocrystals and zinc oxide showed enhanced hydrophilicity and barrier properties. The nanocomposite of 10 wt% fillers have improved Young's modulus (183.1%), tensile strength (140.2%) and enhanced thermal stability by increasing the maximum decomposition temperature ( $T_{\max}$ ) value by 26.1 °C. The composites have also been investigated in terms of in vitro degradation [53].

Recently, green nanocomposites based on PHBV with cellulose nanocrystals have shown better physical and mechanical properties [54]. Also, biodegradable films of PHBV with cellulose nanocrystals have been prepared by solvent casting method, the nanocomposites with 4 wt% of cellulose nanocrystals showed enhanced barrier properties against oxygen and water vapor suggesting the possible utilization of these nanocomposite films in packaging applications [55].

PHBV reinforced with PHBV-grafted multi-walled carbon nanotubes (PHBV-*g*-MWCNTs) were prepared through a simple solution casting method, the films were transparent in the visible light, and have shown improved thermal stability and mechanical, barrier, and migration properties for the food packaging application [56].

Copper oxide nanoparticles and PHBV were used to form bilayer–structure nanocomposite, the first bottom layer was PHBV with 3% mol of 3-hydroxyvalerate (3 HV) that was compression molded, it was then coated with an electrospun nanofibrous composite mat which is composed of PHBV (18% mol 3 HV) and copper oxide nanoparticles. In this study, considerable virucidal and bactericidal performance was reported against the food-borne pathogens murine norovirus, *S. enterica* and *L. monocytogenes* with low copper oxide loading. The composites were also biodegradable [57].

Paper is considered as the most environmental friendly and economical material for food packaging. Because of the inadequacies in the nature of paper, oil-based polymers have been extensively used as paper coating materials. However, the produced coated paper loses the biodegradability and recyclability properties [58]. Nanocomposites of PHBV were prepared with a commercial montmorillonite Cloisite® 30B (CS30B) and a novel modified clay to be coated on paper, for food packing application. Also the water vapor transmission rates (WVTRs) of the PHBV/nanocomposite-coated papers were measured. Overall, PHBV and its composites coating treatments lowered the WVTR values by up to 118 times. Due to an improved moisture barrier, the resulting coated paper is considered as a promising green-based food packaging material [58]. A similar study has been reported later on (PHBV) where solution intercalation process was applied using two types of organomodified clay minerals. Ring opening polymerization of polyhydroxybutyrate in the presence of Cloisite®30B was applied to synthesize intercalated clay mineral (PHB-C30B), the effect of the intercalated mineral and Cloisite®30B on PHBV films in terms of wettability and moisture absorption was investigated, and expressed as contact angle and water vapor transmission rate (WVTR) of the composites [59].

The modified clay vinyl triethoxy silane grafted Sepiolite has been used to prepare a nanocomposite with PHBV by solvent casting method. Thermal and mechanical properties were improved, and hence the authors suggested that the clay polymer nanocomposite films could be used in the packaging field [60]. Also, nanocomposite films based on PHBV and chemically modified Sepiolite have been developed by solution casting method. Water-barrier properties in addition to thermal and biodegradation tests suggested the possible utilization of these biocomposites in the field of food packaging [61].

Purified alfa micro-cellulose fibers were used as filler to form PHBV biocomposite with enhanced barrier properties for packaging and membrane applications [62]. Another biocomposite was prepared using bamboo fibers by injection molding following extrusion compounding, The tensile modulus of the prepared biocomposites at 40 wt% fiber ratio was improved by 175% compared to neat PHBV [63].

Agricultural by-products of wheat straw, corn straw and soy stalk were composited with PHBV (total of 30 wt% ratio) to improve the tensile and storage modulus of PHBV by maximum of 256% and 308% when it is reinforced with of the agro-residues. Also, for equal amounts of the three biomass fibers (10 wt% ratio each, total 30 wt% ratio), and compared to the corresponding poly propylene composites, the tensile and flexural behavior of PHBV composites showed much higher values. In addition, the impact strength and strain at break have been enhanced by around 35% through alkali treatment of wheat straw fibers [64]. Another study has investigated a composite of wheat straw alone with PHBV for food packaging materials to respiring fresh products [65].

Coconut fibers that are impregnated with oregano essential oil were used to reinforce PHBV and form green composite sheets by compression molding. The biocomposite exhibited bacteriostatic effect against *S. aureus* with even low fibers content 3 wt%. The results suggest that these can be used as vehicles entrapping extracts or compounds and could be applied in the field of shelf life extension by packaging [66].

Lignocellulosic fibers from three different types of agricultural residues have been used as fillers within PHBV to investigate their potential use in packaging, brewing spent grains and olive mills in addition to wheat straw were tested. The results showed that all composites have degraded mechanical properties. The authors concluded that PHBV/wheat straw fibers composites could be promising to reach the requirements of respiring food products, whereas PHBV/olive mills composites would be more suitable for water sensitive products [67].

Natural rubber has also been used to prepare a biocomposite with PHBV by melt blending and found to enhance toughness and flexibility but with a decreased tensile strength, so trimethylolpropane triacrylate (TMPTA) coagulant and peroxide were both used to synergistically optimize the mechanical properties. The optimum blend had a notched impact strength of 27.5 J/m, tensile strength of 28.1 MPa, flexural modulus (1% secant modulus) of 8679 MPa and was comparable to polypropylene in terms of sealability and water vapor permeability, in addition it was claimed to be safe for food-contact applications [68].

## PHBV Composites for Drug Delivery Applications

PHBV and all PHB copolymers have been widely investigated in the field of drug delivery [69].

In addition to its well established biodegradability and biocompatibility, many other properties have qualified PHBV to be a superior candidate for drug delivery

applications, it has more amorphous structure compared to PHB, and that is considered more convenient for drug release because drugs can diffuse easily through the chains of a polymeric amorphous area. Also, as long as PHBV can be highly and easily dissolved in chloroform and dichloromethane while it has poor solubility in other solvents, it can readily form nanoparticles through the emulsification-solvent evaporation method [70]. In addition, PHBV biodegradation is relatively slow and this makes it a potential long term drug release candidate [71]. Very recently, a research work review was published on the utilization of PHBV in the specific field of antitumor applications [42].

Superparamagnetic iron oxide (magnetite) has been encapsulated into PHBV nanoparticles for the purpose of biomedical applications. The results showed that these can be potential nanocarriers for site-specific delivery of drugs and as contrast agents for magnetic resonance imaging, also promoting hyperthermia [72]. In a similar research, iron oxide has been used to prepare PHBV nanocomposite particles that are biodegradable and superparamagnetic for the purpose of magnetically-guided drug delivery systems [73].

Cerium oxide nanoparticles were incorporated into electrospun PHBV membrane for healing applications of diabetic wound. This study has shown that membranes of PHBV with incorporated cerium oxide nanoparticles have great potential to be utilized as wound dressings that enhance cell proliferation and vascularization in addition to promoting the diabetic wounds healing [74].

In another study, bioactive wollastonite and PHBV were prepared as composite microspheres where gentamicin drug was encapsulated into them by the absorption method. This has resulted in microsphere composites with controlled drug release behavior, which could be used as bone filling implantations for bone repair [75].

Hydroxyapatite particles (HA) have been incorporated into microspheres of PHBV and loaded with the osteoporosis preventing drug alendronate. This composite with controlled release behavior can well support the proliferation of mesenchymal stem cells and is a potential candidate for bone repair applications [76].

Using the selective laser sintering technique, the biomolecule bovine serum albumin was loaded into nanocomposite microspheres based on calcium phosphate and PHBV (Ca-P)/PHBV scaffolds. It was suggested that these nanocomposite scaffolds with controlled architecture obtained through selective laser sintering could be incorporated with biomolecules which empowers them with more functions for the purpose of bone tissue engineering application or to qualify them to be used for drugs localized delivery [77].

A composite based on the flavonoid Icaritin and PHBV was fabricated and applied onto an anodic oxidized titanium plate as a biodegradable coating for drug delivery. The

conclusion is that icariin/PHBV coating can enhance the bioactivity of titanium based orthopedic implants [78, 79].

Highly porous scaffolds of 45S5 Bioglass®-based glass–ceramic were coated with drug loaded solution of PHBV and vancomycin. The coated material enhanced the mechanical properties, the drug releasing profile and other results suggested that the fabricated loaded drug biocomposites represent potential scaffolds candidates for application in bone tissue engineering [80]. In a very similar study by the same author, the PHBV was coated onto the porous 45S5 as microspheres where vancomycin drug was successfully encapsulated, the resultant bioactive composites showed good sustainable prolonged drug-releasing profile [81].

Utilizing matrix assisted pulsed laser evaporation, biodegradable coatings of the silk fibroin and PHBV biocompatible composite were grown on titanium substrates for biomedical uses. The results showed that increasing PHBV content on the expense of silk fibroin leads to slower degradation, slightly lower hydrophilicity and a more stable behavior of the coatings, the authors also infer that the prepared coatings can be potential systems for localized drug delivery where a prolonged delivery profile can be achieved by larger PHBV content [82].

3D fibrous composite scaffolds have been produced via co-electrospinning system of PHBV/poly ( $\epsilon$ -caprolactone), diatom shell incorporated pullulan, also, the antibiotic cefuroxime axetil was loaded and its controlled release was investigated. The co-electrospun scaffold showed better osteocompatibility, cell distribution and cell spreading. The authors suggest that these can be promising for bone tissue engineering [83].

PHBV composites membranes reinforced by functionalized cellulose nanocrystal-poly[2-(dimethylamino)ethyl methacrylate] have been processed by electrospinning to form a double stimuli-responsive drug delivery system. The incorporated particles enhanced crystallization, hydrophilic and thermal properties of PHBV and resulted in an intelligent and long sustained drug release system [84].

Nanoprecipitation method was used to prepare drug-loaded PEGylated PHBV and PHB nanoparticles as drug delivery systems with epirubicin as the model drug, a longer sustained release profile of 8 days was found at pH 4 compared to pH 7. The tests showed that the antibacterial properties against *E. coli*, *Pseudomonas aeruginosa* and *S. aureus* bacteria were significant compared to the free drug of equivalent amount [85].

In an attempt to overcome the undesirable and adverse systemic toxic effects of the lung anticancer drug sunitinib, it has been loaded on PHBV nanoparticles, and the tests showed that it kept its pharmacological activity. The authors conclude that this formulated inhalable powder could represent a potential biocomposite of local therapy medication for lung cancer [86].

In order to accomplish a dual drug delivery carrier as a novel composite hydrogel, PHBV microparticles encapsulating mupirocin and ketoprofen were prepared and embedded in a physically crosslinked ( $\kappa$ -carrageenan/locust bean gum) hydrogel. The composite has also been tested in terms of biocompatibility, and the overall results qualify this hydrogel to be a potential biomaterial composite for dual drug delivery, mostly for applications of wound healing [87].

In another attempt to develop a double drug delivery system for cancer therapy, nanoparticles of PHBV/lactic-co-glycolic acid were loaded with both 5-fluorouracil and oxaliplatin to achieve co-delivery of both drugs. It was shown that the co-loaded nanoparticles exhibited much higher antitumor efficiency in comparison to the free drugs together [88].

A double-emulsion high-speed technique was used to obtain PHBV nanoparticles loaded with the anti-inflammatory, antimicrobial and antiviral quercetin, the encapsulation efficiency was found to be 51%, the nanoparticles showed biocompatibility with fast release of the drug (most of the drug released in 5 h) when immersed in water [89].

Nano diamond and nano hydroxyapatite particles were used in PHBV to prepare biocomposites loaded with vancomycin drug by injection molding. The nanofillers enhanced the flexural elastic modulus by 34% that became similar to natural bone, and the composites showed a prolonged sustained release of the vancomycin. In addition, in vitro assays exhibited a good adhesion and growth of cells expressing good biocompatibility and bioactivity, these results show the potential of these composites to be used as bone defect filling material [90].

Hydrocortisone was encapsulated in PHBV nanoparticles that were prepared by emulsification–solvent evaporation, and the resultant drug delivery system was suggested for topical ophthalmic administration. The ocular toxicity profile showed that new system is neither irritating nor producing any alteration in the permeability and in the transparency of the cornea. Additionally, no cytotoxicity on bovine keratocytes has been reported. The results suggest that this drug delivery system could be a good option for topical ophthalmic administration of drugs [91].

The anticancer drug docetaxel has been loaded into drug delivery systems of polymeric nanoparticles based on PHBV and vitamin E polyethylene glycol succinate (PHBV–TPGS), and these have been prepared by a modified emulsification solvent evaporation method. The in vitro cytotoxicity tests and other results suggested that these systems are effective in enveloping and chaperoning to the site of action, they have also exhibited sustained and controlled release of the drug and hence reducing drug dose and toxicity [92].



## PHBV Composites for Tissue Engineering and Other Biomedical Applications

PHBV has shown great potential in many biomedical applications including biodegradable implants, biosensors, tissue patches, cartilage repair scaffolds, porous scaffolds for bone repair and engineering purposes [93–95], absorbable surgical sutures, medical packaging [96] and cardiovascular stents [97] and others.

### Tissue Engineering

A research has been conducted on PHBV with different valerate molar percentages (0, 8, 12 and 24%) where hydroxyapatite nanoparticles were used as nanofillers in the polymer matrix. Clearly, one of the resultant composites, namely the P(HB-co-8% HV)/HA (30% w/w) has shown mechanical strength in compression of 62 MPa, which is about the same of several human bones and so it is promising as a biomaterial for use in fixing some bone fractures. Moreover, this composition has shown the highest modulus among other different composites [98]. In order to enhance the tissue compatibility of the PHBV/HA composites for application in tissue engineering, collagen was immobilized on the surface of the porous scaffolds [99]. In another study PHBV was blended with poly lactic acid (PLA) and the blend was 3D-printed as a potential candidate for biocompatible material applications. Also, the 3D printed specimens have exhibited good biocompatibility with human embryonic kidney 293 (HEK293) cells, indicating real promise as biological scaffolds for tissue engineering [100].

A comparison have been accomplished between single and multi-walled carbon nanotubes in a composite with PHBV; (SWCNTs)/PHBV and (MWCNTs)/PHBV composites for biocompatibility and osteoinductivity. The composites were implanted in bone defects of rat femoral. The results showed that there were no observed differences between MWCNTs and SWCNTs in PHBV reinforcement, though MWCNTs/PHBV composites exhibited better biocompatibility and osteoinductivity both in vivo and in vitro [101].

Nanocomposites of hydroxyapatite nanoparticles and PHBV were fabricated with controlled pore architectures and high porosity. The authors suggest that these nanocomposite scaffolds may serve as a promising 3D substrates for bone tissue engineering [102]. Another more recent research paper reported the fabrication of nanofibrous scaffolds based on PHBV and hydroxyapatite nanoparticles. The produced composites were tested for bioactivity and biocompatibility and suggested accordingly

to be used for bone tissue engineering [103]. In another study, biocomposites coatings of PHBV, calcium phosphate,  $\beta$ -tricalcium phosphate and hydroxyapatite were synthesized by matrix assisted pulsed laser evaporation. The purpose was to investigate the prepared biocomposites for bone tissue engineering applications. The wettability and interaction with mesenchymal cells confirmed the suitability of some of the prepared composites for biomedical applications [104].

Another composite was fabricated for the purpose of bone tissue engineering, and this time it was based on integrating  $\beta$ - $\text{Ca}_2\text{SiO}_4$  nanoparticles with PHBV to produce scaffolds with high porosity and interconnected porous structure. The results of cells adhesion and proliferation indicated that the synthesized materials could be potential composites for application in bone tissue engineering [105].

In another interesting study, fibrous scaffolds of silk fibroin, PHBV and reinforced with hydroxyapatite nanoparticles were prepared by electrospinning method to mimic bone tissue structure. The fibrous nanocomposite membranes supported bone-like apatite crystal growth after 28 days, and also supported cell attachment, hence proving their bioactivity and biocompatibility, respectively [106]. In another later research, PHBV nanofiber mats have been modified by silk fibroin and plasma treatment. The treated PHBV-silk fibroin mats showed high hydrophilicity, cell proliferation and cell viability which support their utilization in the field of bone tissue engineering [107].

Bredigite ( $\text{Ca}_7\text{MgSi}_4\text{O}_{16}$ ), hydroxyapatite and mixture of both nanoparticles have been prepared by sol-gel method, and then used to reinforce PHBV fibers in an electrospinning process. The research concluded that incorporating 10% of mixture nanoparticles into the PHBV nanofibers has led to enhance the mechanical properties with high ability for apatite formation, and thus producing a promising candidate material for bone regeneration application [108]. In another study, bredigite nanoparticles were used alone to reinforce PHBV and produce nanofibers through electrospinning technique. The composite scaffolds had improved Young's modulus and tensile strength compared to PHBV alone, they have also shown good bioactivity and biocompatibility, the results altogether suggest that these scaffolds can be potential materials candidates for bone tissue engineering [109].

For the purpose of application in bone repairing, pearl powder was mixed with PHBV and the resultant composites have been processed by electrospinning. The naturally non-toxic pearl powder acted as crystal nucleus for the deposition of HA particles. Cells activity and biocompatibility have been enhanced by introducing pearl powder, and the resultant material was considered as a potential osteoconductive composite [110].

In a simple solution blending method, tungsten disulphide inorganic nanotubes were composited with PHBV.

The composites had better thermal stability than that of PHBV, and a highly efficient nucleating effect for tungsten disulphide inorganic nanotubes was observed. The authors claim that the results qualify these composites for applications in packaging and biomedical fields like bone tissue engineering [111].

Electrospun nanofibrous scaffolds based on different compositions of PHBV, chitosan and hydroxyapatite were produced with different fibers diameters, the researchers concluded that chitosan and hydroxyapatite played a synergistic effect that promoted the regeneration of bone tissue, and hence making these scaffolds as potential biomaterials for the proposed application [112].

3D-printed scaffolds of PHBV/calcium sulfate hemihydrate were fabricated by fused deposition modelling and coated with chitosan hydrogel. In vivo investigation of the scaffolds suggested that these materials can effectively promote new bone formation and hence proposing them as potential products for bone defects repair [113].

For the purpose of engineering new biomaterials that can be used in orthopedic devices in the field of joint replacement, collagen immobilized PHBV film loaded with bovine serum albumin capped silver nanoparticles has been formulated, the target was to promote or retain cell viability and still inhibit bacterial growth. The antimicrobial tests showed that the film has a wide effect against *S. aureus*, *E. coli* and *P. aeruginosa* with low concentration compared to sulfamethoxazole trimethoprim and gentamicin, and still the released silver containing particles have no impact on cells viability [114, 115].

Another biodegradable and biocompatible composite based on PHBV, strontium carbonate nanoparticles and platelet-rich plasma was fabricated for the purpose of bone tissue engineering. The preliminary results indicated that the fabricated electrospun biocomposite scaffold could be used for bone regeneration [116].

PEGylated and acetylated cellulose nanocrystals have been incorporated into PHBV with 3 wt% ratio to furnish super hydrophilic scaffolds with improved water uptake and compressive modulus qualifying them for tissue engineering applications [117].

Nano diamond and nano hydroxyapatite particles were used in PHBV to prepare biocomposites loaded with vancomycin drug by injection molding. The nanofillers enhanced the flexural elastic modulus by 34% that became similar to natural bone, and the composites showed a prolonged sustained release of the vancomycin. In addition, in vitro assays exhibited a good adhesion and growth of cells expressing good biocompatibility and bioactivity, these results show the potential of these composites to be used as bone defect filling material [90].

Matrices of PHBV were produced to create a cartilage by tissue engineering. The authors showed that PHBV matrices

had good healing response, which was effective in cartilage regeneration and hence have great potential to be used in the repair of joint cartilage defects [118].

PHBV nanofibrous coatings were obtained by electrospinning onto sintered 45S5 Bioglass®-based glass–ceramic pellets, the coatings can be used to tailor the surface topography of bioactive glass–ceramics for applications such as scaffolds in tissue engineering [119]. In addition, highly porous scaffolds of 45S5 Bioglass® -based glass–ceramic were coated with drug loaded solution of PHBV and vancomycin. The PHBV coating enhanced the mechanical properties, also the drug releasing profile and other results suggested that the fabricated loaded drug biocomposites represent potential scaffolds candidates for application in bone tissue engineering [80].

For the purpose of application in cartilage tissue engineering, PHBV was composited with calcium silicate and tested through in vitro experiments of scaffold-cell interactions and observation of in vivo cartilage formation. The results suggested that PHBV/calcium silicate scaffolds can be more applicable for cartilage tissue engineering than neat PHBV scaffolds [120]. Also, PHBV with silicate containing hydroxyapatite have been electrospun to produce promising scaffolds for bone tissue engineering [121].

In the field of nucleus pulposus tissue engineering, PHBV, chitosan and chondroitin sulfate nanoparticles were prepared in a composite hydrogel for which the water uptake ability and viscoelastic properties were similar to native tissue. After testing the material in terms of mechanical and bioactivity properties, the authors concluded that these composites represent great potential for the targeted application [122].

Utilizing selective laser sintering, three-dimensional nanocomposite scaffolds based on calcium phosphate/PHBV were successfully fabricated. The in vitro studies have revealed that human osteoblast-like cell line (SaOS-2) had normal morphology and phenotype in addition to high cell viability after 3 and 7 days culture. Also, a significant improvement on alkaline phosphatase activity and cell proliferation was observed. Similarly, the biomolecule bovine serum albumin was loaded into nanocomposite microspheres using selective laser sintering technology. It was suggested that these nanocomposite scaffolds could be incorporated with biomolecules which empower them with more functions for the purpose of bone tissue engineering application or to qualify them to be used for drugs localized delivery [77, 123].

3D fibrous composite scaffolds have been produced via co-electrospinning system of PHBV/poly ( $\epsilon$ -caprolactone) and diatom shell incorporated pullulan. Also the antibiotic cefuroxime axetil was loaded on the system and its controlled release was investigated. The co-electrospun scaffold showed better osteocompatibility, cell distribution and cell

spreading. The authors suggest that these can be promising for bone tissue engineering [83].

Nanodiamond particles were embedded into molybdenum disulfide nanosheets, then the constructed co-dispersion nanostructures were incorporated into PHBV bone scaffolds, which were fabricated by selective laser sintering. Compared to pure PHBV, the resultant scaffold exhibited 52% enhancement on compressive strength and 94% on tensile strength, also good positive viability for cell proliferation, cytocompatibility for cell adhesion, spreading and growth were observed [124].

### Other Biomedical Application

PHBV has also been grafted with maleic anhydride (PHA-g-MA) and then carboxylated multi-walled carbon nanotubes (MWCNTs-COOH) were dispersed to form a composite with enhanced thermal and mechanical properties. The study has shown considerable enhancements in the mechanical and thermal properties of the PHA-g-MA/MWCNTs-COOH composites compared with PHBV, the authors have explained this through the formation of ester carbonyl groups through the reaction between MWCNTs-COOH and the MA groups of PHA-g-MA [125]. The same authors have also done the same grafted copolymer but with hyaluronic (H) instead of MWCNTs, and they have investigated the produced films for biocompatibility, the conclusion was that PHBV/H or PHA-g-MA/H can form potential membranes for biomedical applications [126].

A solvent casting method was used to prepare a ternary composite of PHBV, nano magnesium calcium phosphate and zein. The produced ternary composites showed enhanced hydrophilicity, bioactivity and in vitro degradability that qualify them to the next level for biomedical applications [127].

PHBV functionalized graphite oxide was fabricated via freeze drying method, and then  $\text{Fe}_3\text{O}_4$  nanoparticles have been loaded to form thermally and mechanically stable, bioimaging  $\text{Fe}_3\text{O}_4$ /graphite oxide-grafted-PHBV composites films. The composite films were found to exhibit more efficiency against gram-negative bacteria strains than to gram-positive strains. Also, the tests of adhesion and proliferation of the cells suggested their biocompatibility [128].

In a comparative study, carbon nanofibers and graphene oxide nano sheets have been used in PHBV in order to qualify the effects of these fillers on PHBV in terms of mechanical, thermal, bioactivity, and biocompatibility properties for the purpose of accelerating these materials in biomedical applications. Though both composites have shown promising results to be utilized in biomedical application, it is the composite containing graphene oxide nano sheets that showed higher cell adhesion, proliferative activity against time and antibacterial activity [129].

Curcumin loaded PHBV nanofibers have also been fabricated and suggested in wound-healing applications [130].

Fish scales have been utilized to prepare composites both in PHBV and maleic anhydride grafted PHBV. Cytocompatibility, antimicrobial and antioxidant properties of the two composites demonstrate their potential to be used in biomedical applications [131].

A composite multilayer scaffold based on PHBV and other materials was fabricated and tested on rabbits as a meniscal scaffold that has shown histological and biomechanical results which were comparable to polyurethane scaffolds [132].

Sheet-like cellulose nanocrystal-ZnO nanohybrid was developed by one-step hydrothermal method. The nanohybrids—being antibacterial agents and UV absorbers—were introduced into PHBV by electrospinning. The incorporation of the nanohybrids greatly enhanced PHBV crystallization ability, thermal stability, excellent antimicrobial ratios of *S. aureus* and *E. coli*. Also, nanofibrous composites with 9 wt% cellulose nanocrystal-ZnO have blocked out most of the UV irradiations for both UVA (99.72%) and UVB (99.95%) with high ultraviolet protection factor (UPF) value of 1674.9. The fibrous composites have shown potential to be applied in wound dressings and other functional biomaterials [133].

Magnetic PHBV microspheres of less than 1  $\mu\text{m}$  size have been prepared by incorporating superparamagnetic iron oxide nanoparticle in PHBV via emulsion-solvent extraction/evaporation method. The tests results suggest the suitability of the fabricated microspheres for biomedical applications [134, 135].

Silver nanoparticles have been loaded onto PHBV nanofibers to study in vitro cell compatibility and in vitro antibacterial activity. The PHBV nano fibrous scaffolds with nanoparticles less than 1.0 wt% showed not only good in vitro cell compatibility but also good antibacterial activity. The research suggested that PHBV nano fibrous scaffolds with silver nanoparticles of less than 1.0 wt% have a potential to be used in joint arthroplasty [136].

Also, and for the purpose of wound covering mats, electrospinning process was used to fabricate nanofibrous scaffolds of PHBV with embedded graphene oxide and collagen. Graphene oxide was shown to increase the antibacterial activity against *S. aureus* and *E. coli*, decrease the pore size and hydrophilicity and enhance mechanical strength. On the other side, collagen addition enhanced hydrophilicity and cell proliferation but without affecting porosity and mechanical strength significantly [137].

Acid treated MWCNTs were also used in PHBV to produce composites with the crystallization temperature, heat of crystallization, and thermal stability of the composites increase. Consequently, the mechanical properties of the composites have been substantially improved. The

composites become hydrophilic and have no obvious toxicity to the murine fibroblast L929 cells when the content of A-MWCNTs is below 1.5 wt% [138]. Table 1 summarizes the applications of different PHBV-based composites with desired properties.

## Reinforced Materials of PHBV-Based Composites

High-performance materials and biocomposites made of, or reinforced with natural sources are increasing worldwide [139].

**Table 1** Applications of PHBV-based composites with desired properties

PHBV composites	Type of fibers/fillers	Desired properties	Applications	References
PHBV/ZnO	Inorganic	Antibacterial, thermal and optical properties	Packaging	[47]
PHBV/silver and cellulose nanocrystals	Nanohybrids	Biocompatibility, biodegradability, lower immigration rate, high barrier properties	Packaging	[52]
PHBV-grafted multi-walled carbon nanotubes	Natural	Thermal stability and mechanical, optical transparency or opacity barrier, and migration properties	Food packaging	[56]
PHBV/clay vinyl triethoxy silane grafted Sepiolite	Inorganic	Thermal and mechanical properties	Food packaging	[60]
PHBV/wheat straw, corn straw and soy stalk	Natural	Mechanical properties (tensile and storage modulus)		[64]
PHBV/natural rubber	Natural	Mechanical properties (toughness and flexibility)	Food packaging	[68]
PHBV/superparamagnetic iron oxide	Inorganic	Biodegradability, magnetically-guided drug delivery and biocompatibility	Drug delivery	[72, 73]
PHBV/flavonoid Icaritin	Natural	Enhance the bioactivity of orthopedic implants	Drug delivery	[78, 79]
PHBV/cellulose nanocrystal-poly[2-(dimethylamino)ethyl methacrylate]	Natural	Crystallization, hydrophilic and thermal properties	Drug delivery	[77]
PHBV/cerium oxide nanoparticles	Inorganic	Enhance cell proliferation and vascularization	Healing of diabetic wound	[67]
PHBV/hydroxyapatite particles	Inorganic	Proliferation of mesenchymal stem cells	Bone repair	[76]
PHBV/nano diamond and hydroxyapatite	Nanohybrids	Biocompatibility and bioactivity	Bone filling material	[90]
PHBV/hydroxyapatite nanoparticles	Inorganic	Biodegradability, biocompatibility mechanical strength	Tissue engineering	[98, 99, 102–104]
PHBV/ multi-walled carbon nanotubes	Natural	Biocompatibility and osteoinductivity	Tissue engineering	[101]
PHBV/ $\beta$ -Ca <sub>2</sub> SiO <sub>4</sub>	Inorganic	Porosity, cells adhesion and proliferation	Tissue engineering	[105]
PHBV/silk fibroin	Nanohybrids	High hydrophilicity, cell proliferation and cell viability	Tissue engineering	[107]
PHBV/bredigite	Inorganic	Biocompatibility, bioactivity and mechanical properties	Tissue engineering	[108, 109]
PHBV/bovine serum albumin and silver nanoparticles	Nanohybrids	Antibacterial	Joint replacement	[114, 115]
PHBV/PEGylated and acetylated cellulose	Natural	Water uptake and compressive modulus	Tissue engineering	[117]
PHBV/chitosan and chondroitin sulfate	Natural	Mechanical properties and bioactivity	Tissue engineering	[122]
PHBV/poly ( $\epsilon$ -caprolactone) and diatom shell incorporated pullulan	Nanohybrids	Osteocompatibility, cell distribution and cell spreading	Tissue engineering	[83]



## Reinforcement with Natural Fibers

PHBV has been successfully compounded with natural fibers of *Posidonia oceanica* to form biocomposites for which processability by extrusion, mechanical properties and potential biodegradability have been assessed in a natural marine environment. The produced composite with 30 wt% of the natural fibers has shown enhanced mechanical properties and excellent biodegradation profile within less than six months, so the authors suggested that the developed composites can be suitable to manufacture items to be used in marine environments, for example, in natural engineering interventions [140, 141].

Agave fibers were another type of natural materials which were utilized to prepare composites with PHBV, the resultant biocomposites have shown positive results on modulus reinforcements and toughening of this relatively brittle polymer [142]. PHBV was also prepared in a composite with abaca fibers and they have enhanced its biodegradability when evaluated through the soil-burial test [143].

Both mercerization (i.e., alkali-treatment) and silane-treatment, separately and together, were performed on the coir fibers. This pretreatment has been performed for the purpose of enhancing the interfacial adhesion between the coir fiber and the PHBV polymer matrix and hence increasing the effectiveness of coir fibers as reinforcement. The composites were prepared by microcellular and conventional injection-molding processes. The addition of coir fibers has improved mechanical properties such as strain-at-break and specific toughness. Also, PHBV treated fibers composites showed high degree of crystallinity compared to the untreated fibers composites [144]. Utilizing twin-screw extrusion and injection process, PHBV has been reinforced with varying amounts of curauá fibers, the composites had slightly enhanced mechanical properties though there was poor adhesion between the matrix and fiber [145]. Unidirectional Alfa fibers were investigated as mechanical reinforcements to PHBV. The modeling study was performed to compare real life mechanical properties to that expected by modeling [146].

Sisal fibers and clay particles were used in composites with PHBV for the purpose of enhancing mechanical and thermal properties which were slightly enhanced for some selected compositions [147]. Green biocomposites based on flax fibers treated with alginic acid and PHBV were investigated for biodegradability under composting conditions as per ASTM D5338. The study concludes that introducing flax fibers (treated and untreated) into PHBV offers enhanced biodegradation to the green composites [148].

In another study PHBV was reinforced with natural fiber textile, and the mechanical properties in addition to creep behavior have been studied. The study also showed that the reinforced bio-based composites under investigation

have similar mechanical properties to many conventional construction materials [149]. Biodegradable films based on acetylated chitin nanocrystals and PHBV matrix were evaluated in terms of mechanical and thermal properties.

Both Young's modulus and tensile strength have improved by 67 and 44%, respectively, when 5% of the acetylated nanocrystals were incorporated into the PHBV matrix [150].

## Reinforcement with Agricultural Waste

For the purpose of producing biocomposites of PHBV with improved biodegradability, and to utilize agricultural waste materials, different types of olive pomace have been used as fillers to form biocomposites that were investigated for biodegradability compared to neat PHBV. All types of the pomace have contributed positively to enhance the biodegradation of the PHBV. In fact, less than 90 days were sufficient for all types of the prepared composites to completely biodegrade in a standardized soil environment, while it took neat PHBV 123 days to achieve 91% degradation under the same conditions [151]. Also, biocomposites with added value were fabricated from PHBV using three types of agricultural fibers which are considered as regionally significant food crop residues, namely solid stem wheat, hollow stem wheat and barley [152]. Extruded composites based on PHBV, oil palm empty fruit bunch and a fertilizer were prepared as biodegradable plastic fertilizer with slow release [153]. Spent coffee bean powder was also used as filler in PHBV matrix to form green biocomposites by simple solution casting, the authors claim that those had comparable tensile and thermal properties like polypropylene and polyethylene and hence they are suggesting these composites as a better alternative due to their biodegradability and sustainability [154]. Olive husk flour (20 wt%) has been used after being surface-modified by trimethoxyoctadecylsilane to be incorporated into PHBV to form biocomposites that show higher thermal stability, crystalline index and better tensile properties [155].

## Reinforcement with Wood-Derived Materials

Green composites fabricated from maple wood fiber and PHBV by extrusion–injection molding process have shown enhanced tensile and flexural modulus compared to neat PHBV. The heat deflection temperature was increased while coefficient of linear thermal expansion was reduced [156]. In another extrusion injection molding process, a natural composite was fabricated based on the lignocellulosic filler oak wood flour and PHBV. The fiber–matrix compatibilization techniques have been investigated [157]. For the purpose of developing knowledge on the linkage between mechanical properties and the micro scale structure, and to understand the long-term indoor stability and material properties of the

biocomposite, composites of pine wood flour and PHBV modified by boron nitride (BN), and an inorganic filler, talc, were prepared [158]. Also, composites that are composed of wood and PHBV have been fabricated with different extrusion processing parameters and conditions in order to investigate the effect of processing conditions on the tensile strength and mechanical performance of the prepared composite [159, 160]. In another research, hybrid composite of PHBV with natural wood fibers and talc were fabricated via the extrusion–injection molding, improvement on mechanical and thermal properties were noticed in a synergistic fashion [161].

### Reinforcement with Cellulose-Derived Materials

PHBV was compounded with man-made cellulose, jute and abaca fibers, the incorporation of fibers increased the tensile stiffness and strength significantly; enhancements in impact and tensile strength by the man-made cellulose fibers have been achieved [162]. Cellulose nanowhiskers were aligned in PHBV matrix utilizing an external electric field. The aligned composites showed substantial mechanical anisotropy [163]. PHBV has been grafted onto cellulose nanocrystals using toluene isocyanate as a coupling agent. Compared to neat PHBV, the decomposition temperature increased by 48.5 °C and the crystallinity has slightly decreased, also compared to cellulose nanocrystals contact angle of 30°, the formed graft has a contact angle of 58° indicating the expected decreased hydrophilicity [164]. While greater thermal degradation and inhibition of the foaming behavior have resulted from the incorporation of nanofibrillated cellulose fiber into the PHBV, the tensile modulus of the nanocomposite almost doubled and the storage modulus above the glass transition temperature has increased [165]. PHBV has also used in a ternary composite with PLA and cellulose in order to have 100% biodegradability, the effect of ball-milling the cellulose pulp fibers was investigated [166]. Recently, nanocomposites of PHBV with cellulose nanocrystals (CNCs) have been prepared and processed in different methodologies. The tensile strength improvement (by 13%) was observed only in the case of the peroxide-aided extrusion [167].

In a recent research, cellulose nanocrystals and cellulose nanofibers were both used as hybrid fillers in PHBV and prepared by melt compounding, different mixtures have been prepared and investigated in terms of thermal, mechanical and crystallization properties [168].

### Reinforcement with Clay and Silicate Minerals

PHBV has been prepared in a composite with organoclays nanoparticles by twin-screw extrusion, then the mixture was processed by a continuous extrusion process that was assisted by continuous supercritical carbon dioxide

(sc-CO<sub>2</sub>). This has resulted in nanobiocomposite foams with higher porosity up to 50% and better homogeneity [169].

Nanocomposite of organophilic montmorillonite with PHBV was synthesized and found to have reduced PHBV crystallinity, melting point and biodegradability, while increased the processing temperature as the organophilic montmorillonite increased [170].

Utilizing twin screw extrusion process, hydrophilic fumed silica nanoparticles has been composited with PHBV in order to enhance its thermal properties. Introducing silica nanoparticles into the PHBV with 5% ratio has increased the onset and inflection temperature of its thermal degradation while it has either minimal or negative influence on the mechanical properties [171].

Natural and organosilanes-modified halloysite nanotube fillers were used with 3 wt% to fabricate nanocomposites with PHBV by melt processing in a twin screw co-rotating extruder. Lower viscosity and lower degradation temperatures of the biopolymer have resulted in the presence of aminosilane-modified halloysite nanotube [172].

Another two types of nanoparticles were used to synthesize composites of PHBV, these are tubular like clay, halloysite (HNT), and organomodified montmorillonite cloisite 30B (C-30B), that resulted in an increase in Young's modulus, a higher melting point only in case of halloysite, and a decrease of impact strength and strain at break in the case of montmorillonite and cloisite 30B [173, 174]. In another study, morphology, thermal and barrier properties of PHBV-organomodified montmorillonite prepared by melt intercalation were investigated [175]. In another very similar study the clays montmorillonite and halloysite nanoparticles were modified with (3-aminopropyl) triethoxysilane. The study concluded that both PHBV nanocomposites utilizing unmodified or modified montmorillonite have similar or improved mechanical and thermal properties, while it wasn't the case when organically modified halloysite nanofiller was used where it presented a general decrease in the properties compared to neat PHBV [176].

### Other PHBV Composites and Applications

In a study to immobilize the enzyme acetylcholinesterase on a biopolymer, and among the three tested biopolymers [i.e. polylactic acid (PLA), polycaprolactone (PCL), and PHBV], PHBV was selected as the best support to immobilize the enzyme by cross-linking method. The ultimate purpose was to fabricate a biosystem that can detect organophosphate compounds based on optical bioassay as these pesticides inhibit the immobilized enzyme, and this in turn can be detected by Ellman colorimetric method. The developed bioassay has shown promising results in detecting

organophosphate compounds down to detection limit of 10 part per billion, ppb [177].

A book-shaped triboelectric nanogenerator was fabricated by electrospinning fibers of PHBV and polyvinylidene fluoride, the purpose was to furnish a nanodevice that can harvest energy from various ambient mechanical motions and vibrations.

Being a direct power source, it was used to drive small electronics such as LED bulbs [178]. Also, cellulose nano crystals have been incorporated into a matrix of PHBV that was grafted with glycidyl methacrylate but the resultant nanocomposite has little increase in strength properties [179].

For the purpose of developing a delivery system for agricultural applications, PHBV has been fabricated as cylindrical pellets by extrusion to encapsulate dicyandiamide (a stabilizer for nitrogen fertilizers) and it is shown that PHBV could make a long-term slow release system for the nitrification inhibitor through an effective biodegradable matrix [180]. In another research, biochar has been used to accelerate the biodegradation of composites of PHBV with silver nanoparticles [181].

Extruded cast PHBV films mechanical and barrier properties have been studied after incorporating different plasticizers [182]. The study has shown that polyethylene glycol and triethyl citrate gave promising results on the enhancement of mechanical, thermal and permeative properties. In another research, carbon Fibers have been introduced into PHBV and the biocomposite have been extruded before injection moulding, and this has highly enhanced its mechanical and thermal properties [183].

Nanocomposites of PHBV/cellulose nanocrystal–graphene oxide nanohybrids were prepared through a simple solution casting method. The synergistic effect of cellulose nanocrystal and graphene oxide nanohybrids obtained by chemical grafting (nanocrystal–graphene oxide, covalent bonds) and physical blending (nanocrystal–graphene oxide, noncovalent bonds) on the physicochemical properties of PHBV nanocomposites was evaluated and the results compared with a single component nanofiller (nanocrystal or graphene oxide) in binary nanocomposites. Ternary nanocomposites—compared to bare PHBV and binary nanocomposites—displayed the highest thermal stability and mechanical properties. The ternary nanocomposites with 1 wt% covalent bonded nanocrystal–graphene oxide exhibited good antibacterial activity, excellent barrier properties and lower migration level [184].

A nanofibrous film from PHBV was fabricated by electrospinning and composited with hydroxyapatite which resulted in an extremely hydrophilic surface and enhanced biodegradability [185]. Also, carbon nanofibers and nanotubes have been used to enhance the thermal, conductivity, mechanical and gas barrier properties of PHBV [186].

Additionally, electrospinning method was used to produce nanofibers based on a composite of zinc oxide nanoparticles in PHBV, and it was shown that the addition of zinc oxide nanoparticles had decreased crystallinity and crystallization rate of the polymer [187].

PHBV was modified to have acyl chloride end groups and then grafted to the surface of chitin nanocrystals, as a result, the melting point of PHBV was increased and the crystallization was suppressed [188]. In another study, the biodegradability of PHBV hasn't changed much, though the mechanical properties has indeed improved by reinforcing the polymer with up to 5% organo-modified Mg–Al layered double hydroxide [189]. Also, hydroxyapatite crystals, both surface-treated and untreated—were incorporated into PHBV via melt extrusion process, the micromechanical models were used to correlate with experimental values [190].

A comparative study between cellulose nanoparticles and aluminum oxide as nano reinforcement fillers in PHBV matrix has been conducted, the study concludes that cellulose nanocrystals are better nano reinforcements than aluminum oxide nanoparticles [54]. Another research showed that boron nitride nanoparticles were incorporated into PHBV to form nanocomposites which have been investigated in terms of oxygen-barrier performance as a function of nanoparticle content and temperature. Oxygen-barrier properties have decreased with nanoparticles addition and increased with temperature, also the thermal stability of the composites has increased compared to neat PHBV [191].

Composite fibers of PHBV and tungsten disulfide were fabricated by melt reactive processing with dicumyl peroxide used as initiator. The fibers showed improved mechanical properties, a rapid crystallization rate and a higher nucleation temperature. Also, the tensile strength and elongation at break of the fibers were up to 189.8 MPa and 46.5%, respectively [192]. Ethylene-*co*-vinyl acetate was blended with PHBV, and [57] fullerene (C<sub>60</sub>) was added as a filler with small amounts to form a nanocomposite with improved thermal properties [193].

## Conclusion

PHAs are considered as the most readily biodegradable polymer, among many in literature, in both aerobic and anaerobic environments. PHBV is one of the most studied members of the PHAs family, with numerous applications. In this review PHBV based composites were well studied and found more suitable than neat PHBV as biomaterials for various applications. The limitations in PHBV properties that restricted its wide utilization have been overcome by the incorporation of many types of fillers, (i) natural fibers, (ii) agricultural waste, (iii) wood-derived materials, (iv) cellulose-derived

materials, (v) clay, silicate minerals and others. The incorporation of bio-origin fibers and fillers can accelerate the rate of biodegradation based on the PHBV composition of fibers, while it isn't always the case for inorganic fillers. The produced composites have also opened new horizons and possibilities for the development of materials that highly add and contribute to the circular economy concept, and will definitely give a helping hand to the world in its fight against environmental challenges and global warming issues. Most of the developed new biocomposites have had fillers of bio-origin resulting in totally biodegradable materials. Though there has been huge research published in PHBV composite synthesis, there is still a big room for the introduction of new biocomposites especially with biodegradable and bio-compatible metal–organic frameworks as there is very little research published in this field.

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