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Reviews

Polyhydroxyalkanoate (PHA)/Inorganic Phase Composites for Tissue Engineering Applications

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Polyhydroxyalkanoates are emerging as a class of biodegradable polymers for applications in tissue engineering. Members of the polyhydroxyalkanoates family encompass a wide variety of materials, from hard and brittle materials to soft and elastomeric. Over the years, efforts have been made to extend the group of polyhydroxyalkanoates and to investigate their use in numerous biomedical applications, such as sutures, cardiovascular patches, wound dressings, guided tissue repair/regeneration devices, and tissue engineering scaffolds. Along with the development of polyhydroxyalkanoates, researchers have looked into the possibility of designing composites in combination with inorganic phases to further improve the mechanical properties, rate of degradation, and also impart bioactivity. Poly(3-hydroxybutyrate), poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate), and poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) are some of the polymers which have been studied extensively to fabricate composites in combination with hydroxyapatite, bioactive glass, and glass−ceramic fillers or coatings. This paper reviews international research carried out toward development of polyhydroxyalkanoates/inorganic phase composites in terms of systems investigated, microstructures, properties achieved, and applications, with special focus on tissue engineering scaffolds. A comparison between different composite systems developed in the past few years is presented. The paper also addresses the prospect of potential further development of polyhydroxyalkanoates/inorganic phase composites with optimized microstructure and properties for improved tissue engineering scaffolds.

1. Introduction

Polyhydroxyalkanoates (PHAs) are a group of naturally occurring biodegradable and biocompatible polymers that belong to the aliphatic polyester family as shown in Table 1. PHAs act as storage compounds of carbon and energy that accumulate during imbalanced growth by various microorganisms, i.e., in the presence of an excess of a carbon source and nutrient limiting conditions (e.g., nitrogen, phosphorus).[1] PHAs (general structure shown in Figure 1) are reported to be stored in inclusion bodies within the cytoplasm of the microbial cells.[2,3] Members of the PHA family can exist as homopolymers of hydroxyalkanoic acids, as well as copolymers of two or more hydroxyalkanoic acids. The monomer composition of PHA is variable and can be manipulated by means of the carbon source used and by changing the growth conditions. More than 100 different known types of PHA monomers with different structures have been reported.[4] Depending on the number of carbon atoms in the monomers, PHAs are classified as short-chain-length PHAs (scl-PHA; 3−5 C-atoms) and medium-chain-length PHAs (mcl-PHA; 6 or more C-atoms).[1,3,5]

Polymers of the PHA family are constantly increasing in number due to the continuous discovery of new homopolymers and copolymers. This is in turn resulting in the availability of PHAs with a wide range of chemical structures and an assortment of properties.[2,4,6−8] In the case of copolymers, the systematic change of physical properties with comonomer compositions adds another dimension to the range of variability among these materials. The differences in their properties have also been shown to greatly affect the mode/rate of degradation in aqueous or biological media.[1,8]

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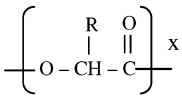
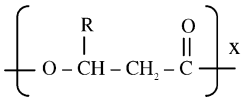
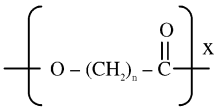
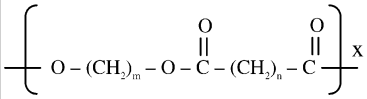
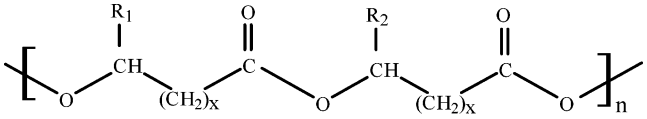
Table 1. Classification of Aliphatic Polyesters into Two Groups with Regard to the Mode of Bonding of Constituent Monomers, i.e., Poly(hydroxyl acid)s and Poly(alkylene dicarboxylate)s[1]

Polyester	Chemical structure	Examples
Poly(-hydroxy acid)		Poly(glycolic acid) PGA Poly(L-lactic acid) PLLA
Poly(-hydroxyalkanoate)		Poly(-hydroxybutyrate) PHB Poly(-hydroxybutyrate-co- -hydroxyvalerate) PHBV
Poly(-hydroxybutyrate)		Poly(-propiolactone) PPL Poly(-caprolactone) PCL
Poly(alkylene dicarboxylate)		Poly(ethylene succinate) PES Poly(butylene succinate) PBS



R_1/R_2 = alkyl groups (C_1-C_{13})

x = 1,2,3,4

n = 100 - 30000

Figure 1. Chemical structure of a generic polyhydroxyalkanoate.

Due to their natural origin and enhanced biocompatibility, i.e., understood in this review as the lack of cytotoxic effects upon implantation, PHAs are attractive materials for biomedical applications, as several previous reviews have summarized.[1,7,8] Commercial PHA products are available from a few companies i.e., TEPHA (U.S.A.), Metabolix (U.S.A.), and P&G (U.S.A.), in the form of sutures, pins, films, screws, and other devices. Due to the variable composition of PHAs, implants made of them can have different physiochemical properties and degrade at a tailored rate in biological media, retaining their mechanical strength for a given short or extended period of time. The disparity in the properties of various PHAs arises because of their chemical composition, either from the length of the pendant groups which extend from the polymer backbones or from the distance between the ester linkages in the polymer backbones.[8]

For the past few decades, polymers of the PHA family are paving the way for the development of new biomedical products. Recent technological advances are extending the range of applicability of the PHAs for novel, previously unconsidered biomedical applications.[8] There have been several recent reports on the application of PHA to manufacture nonwoven fibrous materials, films, sutures, and other products to be used in surgery, transplantology, tissue engineering, and pharmacology.[8,9] Various in vitro and in vivo tests have shown polymers from the PHA family to be compatible with bone and cartilage tissue,[10−12] blood,[13,14] and various cell lines. Moreover, the results of in vivo studies have demonstrated various degrees of biocompatibility and biodegradability in contact with various cell lines (fibroblasts, endothelium cells, isolated hepatocyte) and collagen.[6,10,15] Thus, applications of PHA in medicine are being expanded to include wound management (sutures, skin substitutes, nerve cuffs, surgical meshes, staples, swabs),[8] vascular system devices (heart valves, cardiovascular fabrics, pericardial patches, vascular grafts),[16] orthopedics (scaffolds for cartilage engineering, spinal cages, bone graft substitutes, meniscus regeneration, internal fixation devices), and drug delivery systems.[17] Comprehensive reviews on the subject have been provided by Anderson et al.[18] and Williams et al.[8]

Polymers are easily processed to form complex shapes and structures, yet, in general, they lack bioactive function[19,20] (i.e., strong interfacial bonding of the implant to living bone tissue by means of the formation of a biologically active apatite layer on the implant surface[20−22]) and are often too flexible and weak to meet the mechanical demands in surgery and in the physiologic environment. These are some of the reasons for the combination of biodegradable polymers and bioactive ceramics or glasses in novel composites for tissue engineering and other biomedical applications.[23] Much of the current research is focusing on the development of a variety of bioactive and biodegradable composite materials, both dense and porous systems, with the bioactive inorganic phase incorporated as either filler or coating (or both) into the biodegradable polymer matrix. Composites resulting by addition of inorganic bioactive phases such as hydroxyapatite or bioactive glass, in the form of particles or fibers to biodegradable polymers, are increasingly being considered for use as bone tissue engineering scaffolds.[23] This is mainly due to their improved physical, biological, and mechanical properties, and in particular the capacity they offer in tailoring their structure and degradation rate to the specific need of the implant site.[23−26] A tissue engineering scaffold is a highly porous substrate that serves as an artificial extracellular matrix, providing a template for growth of new natural tissue from isolated cells.[27,28] Along with providing temporary support for the attachment, growth, and proliferation of cells, the scaffold should also maintain its mechanical integrity while providing a foundation upon which new tissue can be regenerated. Comprehensive reviews on tissue engineering scaffolds are available.[27−30]

The original concept of using a bioceramic[22] to reinforce a polymer for biomedical applications was introduced by Bonfield et al.[31] in the early 1980s. Bioceramics are inorganic materials specially developed for use as medical and dental implants, such

as alumina and zirconia, bioactive glasses, glass−ceramics, hydroxyapatite, and resorbable calcium phosphates. Experiments have proven that the mechanical as well as biological performance of bioactive ceramic/polymer composites can be controlled through using different particulate bioceramics and also through varying the amount of bioceramic particles in the composite.[23] Thus, along with the use of PHA on its own, efforts throughout the past few years have been made to form biocomposites using members of the PHA family and addition of inorganic phases for a variety of biomedical applications, with special focus on novel tissue engineering scaffolds.[32−34] In tandem with polymer/ceramic composites, various attempts have also been made to form blends using members of the PHA family and poly(ϵ-caprolactone),[35] PLA,[36] poly(vinyl acetate),[37] and poly(ethylene oxide).[38]

In this review, attention is focused on PHA/inorganic phase composites for biomedical applications, providing an up-to date summary of published research in the field and a comparative study of various systems proposed in recent years in terms of properties, microstructure, and applications, with special focus on tissue engineering scaffolds.

2. PHA/Inorganic Phase Composites

2.1. Poly(3-hydroxybutyrate) [poly(3HB)] and Poly(3HB) Composites. P(3HB) is the simplest and most common member of the PHA family, first discovered by Lemoigne in 1926.[39] Poly(3HB) was the first PHA polymer to have been used in biomedical applications (by J. N. Baptist in 1962[40]). Poly(3HB) isolated from microorganisms is a crystalline material with crystallinity values of 55−80%.[41] However, the molecules within the bacteria are in their amorphous state and exist as water-insoluble inclusions.[2,42] Various extraction methods[43] have been developed to preserve the native state of the polymer, as this can increase the applicability of the polymer for biomedical applications. Poly(3HB) was the first member of the PHA family to be used for composite material fabrication primarily due to its commercial availability and established mechanical, chemical, and degradation properties.[8]

Poly(3HB) crystals usually show a lamellar morphology and form spherulites when crystallized from the melt into bulk materials. Poly(3HB) is a relatively stiff, rigid material and has a tensile strength comparable to that of polypropylene. Although a natural thermoplastic polyester, P(3HB) has mechanical properties comparable to those of synthetically produced degradable polyesters such as polylactides.[44] However, the relatively high brittleness of crystalline poly(3HB) is a disadvantage. Reports have shown that the mechanical properties of poly(3HB) films can be improved by the addition of plasticizers.[45] Poly(3HB) blends with other degradable polymers also lead to higher flexibility and higher elongation at breaking point.[45,46] The properties of poly(3HB) can vary from sample to sample due to different production organisms, extraction techniques, and sample preparation methods used. As a result, the properties of poly(3HB) quoted in the literature vary in a wide range, as indicated in Table 2. The differences in the measured properties (e.g., crystallinity and thermal and mechanical properties) can be attributed to the variation in the molecular weight and polydispersity index of the extracted polymers.[18,47] For example, the monomers of poly(3HB) are polymerized into high-molecular-weight polymers in the range 200 000−3 000 000 Da,[48] but number-average molecular weight values as high as 20 MDa[49] and as low as 13 000 Da[50] have been reported.

Poly(3HB) has been shown to have excellent biocompatibility[52,53] and lack of toxicity toward mouse fibroblast cell lines,[52] chondrocytes,[54] osteoblasts,[55] and gastrointestinal regions of rats.[56] The fact that low-molecular-weight poly(3HB) occurs naturally in human blood, and that poly(3HB) molecules decompose into 3-hydroxybutyric acid, provides further evidence of the high biocompatibility and nontoxicity of this material.[55] However, there have been reports of poly(3HB) inducing some level of inflammatory responses as shown by Löbler et al.[57] and Unverdorben et al.[58] Although the signs of inflammation were not observed macroscopically, the implantation of poly(3HB) into the gastrointestinal tract of rats, for example, provoked a tissue response.[57] A similar conclusion was drawn by Unverdorben et al.[58] who found poly(3HB) stents to induce inflammatory and proliferative reactions when implanted in the iliac arteries of rabbits. However, these reactions were considered acceptable in those studies.[57,58] As mentioned above, the first use of poly(3HB) for biomedical applications dates back to 1962 followed by in vivo degradation results patented in 1965.[59] Korsatko et al.[60] reported the use of poly(3HB) for drug release systems in the 1980s, while the first wound dressing films of poly(3HB) were described by Webb and Adsetts in 1985.[61]

The fragility of poly(3HB) and its poor bioactivity restrict its application in bone-tissue repair. To address this issue, composites were prepared by incorporating bioactive ceramic materials or fillers, as mentioned above. Researchers have tried to form composites in order to render the polymer more suitable for tissue engineering applications by imparting higher strength, increased bioactivity, and altered degradation behavior.[15,33] Hydroxyapatite [$Ca_5(PO_4)_3OH$], which is the principal crystalline constituent of bone[22,62] providing the bone's compression strength, is one of the most extensively used bioactive ceramics to form composites with PHAs. The thermodynamic stability of hydroxyapatite at physiological pH and its ability to actively take part in bone bonding by forming strong chemical bonds with surrounding bone[21,62] make it a suitable bioactive ceramic option for preparing composites. The first attempt to bring these two materials together was performed in 1991 by Doyle et al.[33] More recently, poly(3HB) matrix composites containing hydroxyapatite particles have been prepared using injection molding,[63] salt leaching,[34] as well as compression molding.[64] In their experiment, Doyle et al.[33] demonstrated that addition of hydroxyapatite particles had a direct positive correlation with an increase of the composite elastic modulus. The rate of increase in the modulus value with addition of hydroxyapatite up to 20 vol % was less than that observed for an increase from 20 vol % to 40 vol % of hydroxyapatite. However, the effect of hydroxyapatite on the tensile strength of the composite had an opposite effect; the strength decreased with increasing

Table 2. Properties of Poly(3-hydroxybutyrate)[a]

properties	measurements
melting temperature (°C)	160−177
glass transition temperature (°C)	−4 to +15
tensile strength (MPa)	15−40
tensile modulus (GPa)	1.1−3.5
crystallinity (%)	55−80
elongation at break (%)	1−6
density (gcm^{-3})	1.243
polydispersity index	1.9−2.1
degradation period	>52 weeks
mode of degradation	hydrolytic, bacterial depolymerase
contact angle (°)	66

[a] Several reports have shown the extreme variation of the measured values; hence, a range has been quoted rather than a unique value.[1,32,51,52]

Table 3. Mechanical Strength Data of Poly(3HB) and Poly(3HB) Matrix Composite with 10 Vol % Hydroxyapatite[a]

	poly(3HB)	poly(3HB)/ 10 vol % hydroxyapatite
tensile Young's modulus (MPa)	400	500
tensile strength (MPa)	37	34
compressive elastic modulus (MPa)	317	419
maximum stress (MPa)	21.6	29.9

[a] Data averaged from results of two investigations.[32,34]

hydroxyapatite content at a constant rate. The effect of hydroxyapatite on failure strain had two distinct phases; the failure strain decreased at a rapid rate between 0 and 20 vol % of hydroxyapatite addition followed by a saturation level at hydroxyapatite concentration of >20 vol %. Similar results were found by Wang et al.,[34] who showed the compressive elastic modulus and the maximum stress to increase with the addition of 10 vol % hydroxyapatite. Table 3 shows results obtained by Doyle et al.[33] and Wang et al.[34] using 10 vol % hydroxyapatite. There is consistency in the data, and the effect of the addition of hydroxyapatite on mechanical properties is clear, as found independently by the two investigations.

Ni and Wang[64] successfully demonstrated the formation of apatite crystals on the surface of their poly(3HB) composite containing hydroxyapatite after 1−3 days of immersion in simulated body fluid (SBF), which is an acellular fluid designed with a composition equivalent to that of blood plasma.[21,22] The bioactivity of materials for bone tissue engineering applications is usually determined by their ability to induce formation of hydroxyapatite when immersed in simulated body fluid.[21,22] The study of Ni and Wang[64] showed that the quantity of the apatite crystals formed was directly proportional to the amount of hydroxyapatite used in the composite. The storage modulus of poly(3HB)/hydroxyapatite composites was found to increase with increasing percentage of hydroxyapatite.[64] The storage modulus of the composite initially increased with the immersion time in SBF, followed with a decrease in the moduli for prolonged immersion times. The presence of the apatite layer on the composite surface was thought to prohibit the SBF solution from extensively attacking the polymer matrix, thus contributing to the increase of the composite storage modulus.[65] However, after extended periods of immersion in SBF, penetration of SBF into the polymer structure occurs, leading to degradation of the material. Therefore, the addition of hydroxyapatite was confirmed to increase the storage modulus of the composite by inducing the formation of an apatite layer which acts as a barrier, thus retarding the polymer degradation rate. Moreover, the experiments of Doyle et al.[33] showed that the addition of hydroxyapatite helped to retain a higher Young's modulus compared to the polymer on its own, after prolonged immersion in SBF. However, over a four-month period of immersion in SBF, the composite recorded a 44% reduction in its Young's modulus (9 GPa to 5 GPa), whereas the polymer only showed a reduction of 25% (4 GPa to 3 GPa). The authors[33] therefore concluded that the amount of hydroxyapatite has a direct correlation with the level of deterioration of the composite's properties upon immersion in SBF. It is thus thought that the weakening of interfaces within the composite materials is responsible for the increased degradation of the elastic modulus compared to that of the monolithic polymers.

One of the key factors investigated for bone tissue engineering applications is the achievement of bioactive behavior which is related to the formation of hydroxyapatite crystals on the surface of the material upon immersion in simulated body fluid (SBF). The presence of hydroxyapatite particles within the polymer matrix naturally enhances the hydroxyapatite formation ability of the composites. It was also found that having an optimum concentration of hydroxyapatite in the polymer enhances the osteoblast cell attachment and growth on the composite surfaces.[33] In an earlier experiment, it was shown by interfacial shear strength tests of in vivo implanted poly(3HB)/hydroxyapatite composites that both hard as well as soft tissue had attached on the surfaces.[63] Luklinska et al.[66] studied the interface between poly(3HB)/hydroxyapatite composites and bone when implanted in vivo in rabbits. It was found that, after one month of implantation, bone apposition occurred along the whole length of the implant interface. Three months after implantation, bone was found to form an interlocking structure on the exposed hydroxyapatite particles at the interface, followed by dense bone formation after six months of implantation. Various other in vivo experiments have further confirmed the integral role played by hydroxyapatite for integration with bone.[67]

Dynamic mechanical analysis (DMA) experiments carried out by Ni et al.[64] showed that the presence of hydroxyapatite increased the storage modulus in as-fabricated composite, but the material lost its modulus at a faster rate in the biological medium (SBF) in comparison with the neat polymer.[33] The neat polymer retained in general its properties for a longer duration by undergoing slow degradation, as shown by Freier et al.[56] The presence of hydroxyapatite increased the growth of osteoblast and cell proliferation compared to poly(3HB) on its own as found by Wang et al.[34] Experiments by these authors have shown that the presence of hydroxyapatite particles on the surface helps the formation of tenacious bonds with osteoblast cells. Moreover, the presence of hydroxyapatite in poly(3HB) matrixes has been successfully demonstrated to have increased the strength of the composite along with its bioactivity in related investigations.[63,66]

Bioactive glasses, a group of surface-reactive silicate or phosphate glasses with extended applications in medicine and dentistry[20,22] are interesting materials to be combined with polymers for developing composites for tissue engineering.[23,30] Research is presently being carried out in our laboratory to create novel PHA composites containing bioactive glass particles. Initial attempts have been carried out to form poly(3HB)/Bioglass composites. Bioglass (type 45S5) is a commercially available bioactive glass of the composition 46.1 mol % SiO_2, 26.9 mol % CaO, 24.4 mol % Na_2O, and 2.5 mol % P_2O_5, which has FDA approval for biomedical use. This glass undergoes a rapid rate of surface reactions which lead to fast bone tissue bonding via the formation of hyroxycarbonate apatite layers on the glass surface.[23,68] In addition to improving the mechanical properties of the composites,[23] it has also been shown that dissolution products of 45S5 Bioglass can up-regulate diverse families of genes in osteoblast cells[69] and it can contribute to differentiation of human osteoblasts to form new bone. In applications where a high bioactivity of the material is required, Bioglass seems to be a better choice, in comparison to hydroxyapatite, for scaffold development.[30] Different concentrations of Bioglass powder with a mean particle size of $<5\ \mu$m were used to prepare poly(3HB)/Bioglass composite films using the solvent casting technique. Static tensile strength tests on the composite samples confirmed that the presence of low quantities of Bioglass reinforced the composite and increased the Young's modulus (unpublished data). On the other hand, a large quantity of Bioglass addition had a detrimental effect on the modulus and tensile strength of the composite films. Similar

Table 4. Changes Induced in the Properties of Poly(3HB-3HV) by Changing the 3HV Content in the Copolymer[71]

poly(3HB-*co*-3HV) (mol %HV)	crystallinity (%)	T_g[a] (°C)	T_m[b] (°C)	M_w[c]	PDI[d]	UTS[e] (MPa)	EM[f] (GPa)	elongation (%)
0	69	10	170	177000	1.9	67	2.52	2.65
8	58.7	6	153	190000	2	62	2.75	2.25
12	57.8	4	154	250000	4.2	54	0.51	5.42
24	54.7	−6	129	65000	1.7	23	0.47	3.84

[a] Glass transition temperature. [b] Melting temperature. [c] Molecular weight. [d] Polydispersity index. [e] Ultimate tensile strength. [f] Elastic modulus.

results were confirmed by nano-indentation, confirming a sharp increase in the modulus and hardness of the composite film for 20 wt % Bioglass addition.

2.2. Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [poly-(3HB-*co*-3HV)] and Poly(3HB-*co*-3HV) Composites. Poly-(3HB-*co*-3HV) is a copolymer of poly(3HB), and it can exist with various mole percentages of 3-hydroxyvalerate (3HV). Incorporating HV in the structure of poly(3HB) changes the mechanical properties of the polymer. Poly(3HB), being isodimorphic, accommodates the 3HV units into its crystal structure, and hence, addition of 3HV does not have a huge impact on the crystallinity of poly(3HB). However, it can be shown from Table 4 that addition of 3HV makes the polymer more flexible and reduces the strength. This makes poly(3HB-*co*-3HV) easier to process than poly(3HB).[1,51] Due to the commercial availability of poly(3HB-*co*-3HV) with different molar percentages of HV, polymers of this group have been studied extensively to determine their mechanical properties as well as their degradation properties in aqueous medium. The degradation rate of poly(3HB-*co*-3HV) can be controlled by monitoring the copolymer composition. For example, poly(3HB-*co*-3HV) degrades at a much slower rate and loses its properties at a slower pace than poly(L-lactic acid) (PLLA).[36] Signs of possible degradation of poly(3HB-*co*-3HV) can be observed after 53 weeks; in comparison, PLLA begins its degradation in only a few weeks (2 weeks).[36] The amount of 3HV in poly-(3HB-*co*-3HV) is an important parameter, and it has been optimized to achieve the desired properties. Galego et al.[70] showed that increasing the 3HV content results in a decrease of the melting temperature as well as in reduction of the strength and modulus of the polymer. In their study, they found 8 mol % of 3HV to be the optimum addition in terms of mechanical properties. However, there have been reports of a higher value of elongation when the 3HV content increases further.[1] By varying the hydroxyvalerate content of poly(3HB-*co*-3HV), the flexibility, impact strength, and degree of degradation of the final copolymer can be altered.[1,47,51] The less crystalline component (hydroxyvalerate) in the copolymer system was found to exhibit a higher degradation rate than the highly crystalline component (hydroxybutyrate).[71,72] By adjusting the percentage composition of the hydroxyvalerate content in the copolymer, the degradation of P(3HB-*co*-3HV) can be tuned to achieve the desired rates. Addition of 3HV in poly(3HB) also results in lowering the water contact angle and thus in the increase of hydrophilicity, which will affect the rate of degradation.[12] Moreover, various in vivo experiments have further confirmed the biocompatibility of the incorporation of 3HV into the copolymer poly(3HB-*co*-3HV) as described in the following paragraph.

Poly(3HB-*co*-3HV) scaffolds have been developed for bone regeneration, and they have been tested in contact with rat osteoblasts, which retain their characteristic morphology growing on poly(3HB-*co*-3HV) matrixes.[73] Rivard et al.[74] demonstrated that P(3HB-*co*-3HV) sustained a fibroblast proliferation rate similar to that observed in collagen sponges. In addition, polyP(3HB-*co*-3HV) materials maintained their integrity during the whole culture period (0−35 days), while the collagen foams contracted substantially. Also, it was found that the total protein production on poly(3HB-*co*-3HV) foams was twice that on collagen foams. In separate investigations, the strength of the in vivo interfaces developed between cortical bone and polymer implants has been reported to be higher for poly(3HB) than for polyethylene.[75] Kumarasuriyar et al.[76] further verified the possibility of using poly(3HB-*co*-3HV) as an appropriate substrate to augment bone regeneration by showing that the attachment of preosteoblast-like cell lines on solvent cast poly(3HB-*co*-3HV) films was comparable to that on various matrix proteins. Chaput et al.[77] have studied cytotoxicity of poly(3HB-*co*-3HV) on fibroblasts and found that, when the portion of hydroxyvalerate in the polymer increased from 7 to 22 mol %, a slight cytotoxic effect was recorded.

Among the polymers from the PHA family, poly(3HB-*co*-3HV) is the most extensively studied and used for composite development. Experiments have been carried out to form poly(3HB-*co*-3HV) composites using tricalcium phosphate (TCP),[19] hydroxyapatite,[15,70] wollastonite (a naturally occurring calcium silicate ($CaSiO_3$), which is used as filler in polymers to form composites[21,32]), and sol−gel bioactive glass[78] as fillers. Typical volume fractions of inclusions have been 0−30 vol %. Techniques such as compression molding and particulate leaching have been used to prepare porous poly(3HB-*co*-3HV) composite scaffolds, and homogeneous distribution of the bioceramic particles in the poly(3HB-*co*-3HV) matrix has been reported.[55,63] The presence of a homogeneous distribution of bioceramic particles within the polymer matrix is of fundamental importance in order to develop a continuous biological apatite structure on the composite surface and thus to improve the bioactivity of the composite.[23,64] Chen et al.[19] have carried out experiments to understand the effect of addition of hydroxyapatite and TCP on the composite's effective properties. In their experiments, they observed that the presence of hydroxyapatite has more influence on the mechanical properties of poly(3HB-*co*-3HV) scaffolds than the presence of TCP.[19] This was demonstrated in their findings showing that the addition of hydroxyapatite particles reduced the degradation temperature and the crystallinity of poly(3HB-*co*-3HV) to a longer extent than that achieved by TCP particle additions. However, the presence of TCP did not affect the melting temperature, whereas the melting temperature of the composite increased with increasing content of hydroxyapatite, as summarized in Table 5.

Increasing the volume percent of hydroxyapatite and TCP increased the microhardness and Young's modulus of the composites. The incorporation of hydroxyapatite had a greater impact on the microhardness than TCP additions. Chen et al.[19] also demonstrated that addition of hydroxyapatite or TCP to poly(3HB-*co*-3HV) increases the Young's modulus and dynamic modulus as compared to poly(3HB-*co*-3HV) copolymer itself. A similar result was recorded by Li et al.[32] who showed that the compressive strength of a composite poly(3HB-*co*-3HV)-

Table 5. Changes Brought out by the Addition of Various Vol % of Hydroxyapatite (HA) and Tricalcium Phosphate (TCP) on the Properties of Poly(3HB-*co*-3HV) Composites[19]

	melting temperature		crystallinity of PHBV		microhardness (VHN)		degradation temperature	
bioceramic content	HA/ poly-(3HB-*co*-3HV)	TCP/ poly-(3HB-*co*-3HV)	HA/ poly-(3HB-*co*-3HV)	TCP/ poly-(3HB-*co*-3HV)	HA/ poly-(3HB-*co*-3HV)	TCP/ poly-(3HB-*co*-3HV)	HA/ poly-(3HB-*co*-3HV)	TCP/ poly-(3HB-*co*-3HV)
0 vol %	137.4 °C	137.4 °C	47.3%	47.3%	8.56	8.56	279.6 °C	279.6 °C
10 vol %	144.1 °C	137.4 °C	44.6%	44.5%	10.12	9.26	280.2 °C	277.3 °C
20 vol %	144.7 °C	136.7 °C	41.0%	42.6%	13.189	9.54	266.9 °C	274.9 °C
30 vol %	142.7 °C	136.7 °C	39.0%	40.9%	15.73	10.18	252.2 °C	269.7 °C

based scaffold increased from 0.16 MPa to 0.28 MPa by increasing the content of wollastonite ($CaSiO_3$) from 0 to 40 wt %. However a study carried out by Galego et al.[70] showed that increasing the hydroxyapatite content above 40 wt % resulted in a sharp decrease in the elastic modulus of the composite. This was explained by considering that hydroxyapatite particles did not have adequate chemical interaction with the polymer, compared to them acting as sites of defects from which cracks could propagate. However, as shown by Boeree et al.,[79] these flaws should not affect the ultimate compressive stress, and hence, the compressive strength of the poly(3HB-*co*-3HV)/hydroxyapatite composites has been found to be in a direct correlation with the concentration of hydroxyapatite.[79] The results reported in the literature therefore demonstrate that a large amount of hydroxyapatite inclusions can have a profound detrimental effect on the stiffness and strength of the composites, and that there is an optimal concentration of hydroxyapatite that should be added. Recent experiments by Li et al.[80] attempted the development of composite scaffolds of poly(3HB-*co*-3HV) and sol−gel-derived bioactive glass (BG). The concentration of bioactive glass inclusion was kept relatively low. Addition of 10 wt % and 20 wt % of bioactive glass caused an increase in the compressive yield strength of the scaffolds.

In an earlier study,[63] the mechanical strength of poly(3HB-*co*-3HV)-based composites prepared using different manufacturing techniques was investigated. Poly(3HB-*co*-3HV) (7 mol % 3HV) was used to fabricate composites with various concentrations of hydroxyapatite, using injection molding, hot compression molding, and wet compression molding. It was found that for injection molded and compression molded samples the ultimate tensile strength and the strain to failure had an inverse relation to the concentration of hydroxyapatite, and a slight increase was recorded in the compressive stress. At similar polymer/hydroxyapatite mix ratios, injection-molded composites had significantly greater tensile and compressive strength than the compression-molded samples. It was also demonstrated that injection molding resulted in a full melting of the polymer, and as a consequence, hydroxyapatite crystals were well-dispersed in the polymer matrix. On the contrary, for compression molding only partial melting of the polymer was observed, and the samples exhibited a porous structure and inhomogeneous distribution of hydroxyapatite inclusions. It was further concluded in their study that the pore size distribution obtained by compression molding was uncontrolled, and the average pore size was too small for bone tissue in-growth. Moreover, there was no improvement in mechanical properties in comparison to injection-molded samples.[63]

A detailed in vitro analysis has been carried out by Li et al. using wollastonite[32] and bioactive glass[80] as additions in poly(3HB-*co*-3HV). They investigated the change of ion concentration, pH, and also the change in calcium and phosphorus concentration upon immersion of the composites in SBF over certain periods of time. In their experiments, they found that the presence of wollastonite helps in neutralizing the acidic byproducts and aids in stabilizing the pH, compared to poly(3HB-*co*-3HV) on its own, whose pH gradually decreased over a period of time. Adding wollastonite in the range 0−40 wt % resulted in a decrease of the water contact angle from 66° to 16°. This effect indicates the increased hydrophilicity of the composite and its ability to alter the rate of hydrolytic degradation. Similar results were confirmed in poly(3HB-*co*-3HV)/bioactive glass composites, wherein the water contact angle reduced from 65° to 32° upon addition of 0−20 wt % of bioactive glass.[80] Addition of 20 wt % of bioactive glass resulted in higher water absorption than in composites with 10 wt % bioactive glass and pure poly(3HB-*co*-3HV). Bioactivity of poly(3HB-*co*-3HV)/bioactive glass composites was further verified, by confirming the formation of nanosized carbonated apatite crystals on composite surfaces after only 3 days of immersion in SBF, and further confirmed using EDS spectrum analysis. The weight loss of the composite was reported to increase with the addition of bioactive glass. However, the reduction of weight is not necessarily a direct indication of polymer degradation, and it can arise also because of the dissolution of bioactive glass particles. The weight average molecular weight (M_w) measurement was carried out in the study of Li et al.,[80] and it was found that the M_w of the polymer on its own decreased more than that of the composite upon immersion in SBF for 9 weeks. This finding can be related to the fact that the presence of bioactive glass particles helps in stabilizing the pH at a standard level, when compared to the continuously decreasing pH of the polymer on its own.

In vivo tests into the tibias of rabbits were performed using cylindrical specimens of poly(3HB-*co*-3HV)/hydroxyapatite (40 vol %) composites to study the morphological changes induced by the addition of hydroxyapatite.[15] Optical microscopy revealed a well-developed lamellar bone structure around the implant surface after 1 month of implantation, and this was reported to be preserved after 3 and 6 months of implantation. It was also reported that new bone formed at the interface followed the shape of the implant surface, and marrow cells were observed within the bone structure. For the poly(3HB-*co*-3HV)/hydroxyapatite (40 vol %) composite, bone formation was an ongoing process even after 6 months of implantation. Along with the ability of hydroxyapatite to bond with the surrounding tissue, the poly(3HB-*co*-3HV) matrix provided adequate support for cell growth, and it was tolerant toward new tissue formation.[15]

It can be concluded that the tailored addition of bioceramic particles in poly(3HB-*co*-3HV) matrixes enhances the applicability of the polymer by increasing its mechanical competence and bioactivity. It can be further deduced from the above analyzed results that the changes observed in the crystallinity percentage and water contact angle caused by the inorganic particulate content may also alter the degradation rate compared to unfilled poly(3HB-*co*-3HV), as both crystallinity and contact angle have been shown to affect the degradation rate of polymers.[81]

Table 6. Thermal and Mechanical Properties of Solution Cast Films of Poly(3HB-*co*-3HHx)[83]

poly(3HB-*co*-3HHx) (mol % HHx)	T_g (°C)	T_m (°C)	ΔH_m (J g^{-1})	tensile strength (MPa)	elongation to break (%)
0	4	177	97	43	5
10	−1	127	77	21	400
15	0	115	54	23	760
17	−2	120	34	20	850

2.3. Poly(3-hydroxybutyrate-*co*-3hydroxyhexanoate) [poly-(3HB-*co*-3HHx)] and Poly(3HB-*co*-3HHx) Composites. Poly-(3HB-*co*-3HHx) is a polymer from the same PHA family as poly(3HB) and poly(3HB-*co*-3HV), and it represents a recent addition to the PHA group of polymers for biomedical applications. The introduction of a 3-hydroxyhexanoate (3HHx) comonomer into the polymer backbone of poly(3HB) significantly increases the flexibility and changes the degradation kinetics of the polymer accompanied by a reduction in polymer stiffness.[51,82−85] Doi et al.[82] have shown in their earlier experiments that the crystallinity of poly(3HB-*co*-3HHx) decreases steeply with increasing fraction of 3HHx (Table 6) demonstrating that the 3HHx units are excluded from the poly(3HB) crystalline phase.[1,82] Large-scale production of poly(3HB-*co*-3HHx) has been carried out by Chen et al.[83] The presence of a given 3HHx fraction in the polymer results in an increase in the elongation to failure and a decrease in the tensile strength, i.e., it makes the polymer softer and more flexible. Poly(3HB-*co*-3HHx) was reported to possess similar mechanical properties to low-density polyethylene (LDPE).[82] In particular, P(3HB-*co*-3HHx) containing 10−17 mol % of 3HHx fractions possesses higher elongation to break up to 850%, which is much better than poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3HV)] containing 20 mol % of 3HV. Apart from the changes in the mechanical properties, the most remarkable transformation poly(3HB-*co*-3HHx) brings along is its ability to undergo enzymatic degradation by lipase,[1,82] which is not seen in poly(3HB) or poly(3HB-*co*-3HV). This property should make poly(3HB-*co*-3HHx) a suitable choice for several biomedical applications, since it adds a further variable which can be used to tailor the polymer degradation.

Prior experiments have shown that poly(3HB-*co*-3HHx)-based materials have good biocompatibility for chondrocyte,[14] nerve cells,[13] osteoblast, and fibroblast cells.[84,85] Poly(3HB-*co*-3HHx) has much better elongation properties than poly(3HB) and supports cell growth.[83,85,86] Yang et al.[52] showed that cell growth on untreated poly(3HB-*co*-3HHx) was better than that on poly(3HB) and PLA films. Surface treatment of poly(3HB-*co*-3HHx) with lipase showed an increased biocompatibility.[52,84] Further experiments[86] suggested that poly(3HB-*co*-HHx) exhibited a better ability to support the growth of fibroblast and osteoblast cells compared to poly(3HB) and PLA. These are important contributions to understand the effect of 3HHx inclusion on the properties of poly(3HB) copolymers, and it also adds value to the existing array of properties offered by the PHA family of biomedical-grade polymers.

The attempt to create the first polymer/ceramic composites from poly(3HB-*co*-3HHx) (12% HHx), using 10 vol % hydroxyapatite, was performed recently by Wang et al.[34] Porous composite scaffolds were prepared using the salt leaching technique, which is by far the easiest and most common way to make porous scaffolds. It was found that addition of 10 vol % of hydroxyapatite particles decreased the compressive elastic modulus from 0.173 to 0.068 GPa, but there was no effect on the maximum compressive stress (0.0079 GPa) sustained by the composite.

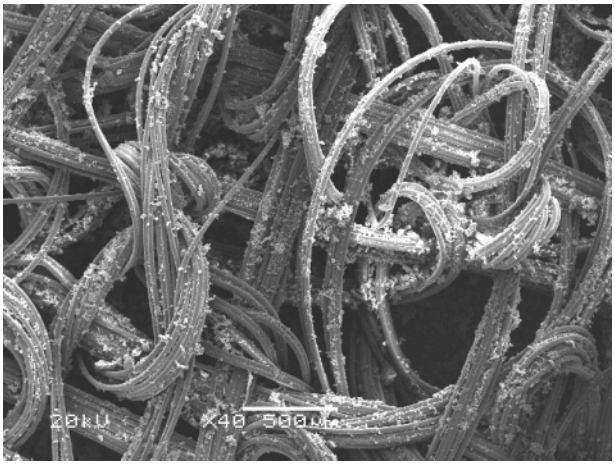


Figure 2. SEM image showing a poly(3HB) mesh coated with Bioglass particles using a 40 wt % aqueous slurry and immersion time of 10 min.[87]

In their study, however, Wang et al.[34] found out that addition of hydroxyapatite to the poly(3HB-*co*-3HHx) matrix has a negative effect on osteoblast cell proliferation, which was confirmed by using MTT assay and alkaline phosphatase activity assay. Surface examination of poly(3HB-*co*-3HHx)/hydroxyapatite sample showed aggregations of hydroxyapatite particles. Such aggregations can lead not only to a decrease in mechanical strength but also to a reduction in the effectiveness of contact formation with osteoblast cells. Thus, the experiments carried out by Wang et al.[34] showed that the presence of hydroxyapatite in poly(3HB-*co*-3HHx)/ hydroxyapatite composites does not have a positive influence over the cell proliferation behavior and on the measured mechanical properties compared to poly(3HB-*co*-3HHx) on its own. There has not been any previous attempt to incorporate bioactive glass particles into poly(3HB-*co*-3HHx) matrixes; this remains an area for further research efforts.

3. Bioactive Inorganic Coatings on PHA Polymers

The deposition of Bioglass particles on poly(3HB) meshes and fibers by the slurry dipping technique was investigated by Olson-Claire et al.[87] with the aim of developing bioactive coatings for tissue engineering scaffolds. Bioglass particles of average size of <5 μm were used to produce homogeneous coatings on engineered poly(3HB) meshes generated by means of an embroidery textile technology. Their work showed that the coating thickness and homogeneity of the Bioglass coatings could be controlled by varying the concentration of Bioglass particles in the aqueous slurry used for slurry dipping. A typical microstructure of a poly(3HB) mesh coated with Bioglass particles is shown in Figure 2. The technique used was able to achieve homogeneous distribution of Bioglass particles on individual poly(3HB) fibers, forming a relatively thick layer overall on the mesh surfaces and homogeneously covering individual fibers. The acellular in vitro bioactivity of Bioglass-coated poly(3HB) meshes was demonstrated by the presence of hydroxyapatite crystals after 3 days of immersion of samples in SBF as confirmed by SEM and XRD results.[87] The amount of hydroxyapatite crystals was shown to increase with incubation time in SBF. The more rapid hydroxyapatite crystal growth on meshes coated with Bioglass confirmed that nucleation sites were proportional to the Bioglass content, as also found in other studies.[24,25] Specifically, the soluble silica layer formed upon

Table 7. Some of the Biomedical Applications Involving PHAs and PHA/Inorganic Phase Composites

applications	material	refs
	Patches	
gastrointestinal	poly(3HB)	43, 56
right ventrical, pulmonary artery	poly(3HB)	90
	Nutritional/Therapeutic Applications	
	poly(4HB)	91
	Orthopedic	
femur	poly(3HB-*co*-3HV)/hydroxyapatite	63
bone analogue material	poly(3HB)/hydroxyapatite	33, 66
	poly(3HB-*co*-3HV)/hydroxyapatite	15
cortico-cancelous bone graft	poly(3HB-*co*-3HV)/hydroxyapatite	79
bone reconstruction	poly(3HB-*co*-3HHx)/hydroxyapatite	34
	Tissue Scaffolds	
muscle	poly(3HB-*co*-3HV)/bioactive glass	78
bone cell proliferation	poly(3HB-*co*-3HV)	12, 73, 76
cartilage generation	blend of poly(hydroxybutyrate-*co*-3-hydroxyhexanoate)/poly(3HB)	54
bone tissue regeneration	poly(3HB-*co*-3HHx)	84, 85, 86, 55, 73
	poly(3HB-*co*-3HV)	
	Drug Release	
Tetracycline	poly(3HB-*co*-3HV)	17
Sulperazone, Gentamicin	poly(3HB-*co*-3HV)	92
	poly(3HB-*co*-3HV)/wollastonite	93
	Sutures	
	poly(3HB-*co*-3HV)	6
	Conduits	
	poly(3HB)	94
	Nerve Regeneration	
	poly(3HB-*co*-3HV)	94
	poly(3HB)	95
	Stents	
	poly(3HB)	42
	Wound Healing	
	poly(3HB-*co*-3HV)	96
	Cardiovascular Applications	
	poly(4HB)	7, 16
	PHO	16, 88

immersion in SBF due to dissolution of Bioglass[22] was shown to act as a heterogeneous nucleation site for hydroxyapatite crystals. There has been no further work dealing with bioactive inorganic coatings on PHAs, and further research is expected to be carried out in this new research field.

4. Future Directions

The use of bioceramics for fabrication of composites in combination with biodegradable polymers for biomedical applications has a long and established history, as described in previous reviews.[23,30] However, for the case of PHAs, this approach still has to be developed and studied extensively, particularly for tissue engineering applications. From the limited experiments and research carried out on PHA-based composites, reviewed in this report, it is clear that PHAs are paving their way through on becoming one of the biomaterials of choice for tissue engineering scaffold developments. The available results reveal however that the effect of bioceramic additions on material properties is not the same for every polymer in the PHA family.

A summary of biomedical applications for PHAs and PHA composites is presented in Table 7. It is clear that due, to the present huge interest in the tissue engineering field, the scope for research and development and the application fields of polyhydroxyalkanoates and their composites are ever increasing. As a result of this, the following two important aspects are identified for future research in this area.

1. First, it is recognized that the involvement of polymers of the PHA family in tissue engineering strategies will increase following the optimization of copolymer constituents. There are more than 100 different reported PHAs; however, the ones investigated for composite production make up only a handful. Published reports on new types of PHA highlight the potential of several of these polymers to be explored for tissue engineering. The mcl-PHA, poly(3-hydroxyoctanoate-*co*-3-hydroxyhexanoate), for example, shares the same backbone as poly(3HB), but in contrast, it is a highly flexible thermoplastic elastomer with properties comparable to those of commercially produced materials such as poly(lactide-*co*-glycolide).[88] Recently, there has been considerable interest in developing poly(4-hydroxybutyrate) [poly(4HB)] and copolymers of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) as materials for cardiovascular applications because of their high degree of flexibility (tensile modulus, 70 MPa; elongation at break, 1000%) in comparison to other synthetic absorbable polymers such as PGA and PLLA.[7] Poly(4HB) is, in fact, a polymer of significant potential in the

biomedical field,[91] which has not been considered to produce composites for tissue engineering as yet. Combinations of scl−mcl PHAs have been shown to have superior material properties compared with those of PHAs consisting of scl or mcl monomers only.[82] Along with copolymers, reports have been published on the discovery of oligomers[89] (poly(3HB-*co*-3HV-*co*-4HB)) of the PHA family, which should be investigated with the aim to enhance and tailor the mechanical and degradation properties of the composites for tissue engineering applications. These polymers will have properties more favorable than poly(3HB) or poly(3HB-*co*-3HV), and they might be more compatible in physiological conditions. There are no reports yet on the combination of these materials with bioceramics. Experimental work reviewed here has successfully demonstrated the viability of using bioceramics in PHA matrixes. It can be anticipated that the availability (large-scale production) of diverse members of the PHA family and the optimization of copolymers will lead to an increase of their use in combination with bioceramics for production of organic/inorganic composites with improved mechanical and biological behavior for tissue engineering and regenerative medicine applications.

2. Second, it will be important to understand the specific interaction of these polymers with bioceramics since the interface between the polymer and the ceramic inclusions can be vital to determine the mechanical behavior of the composite when implanted in vivo. Although the presence of bioceramics such as hydroxyapatite, sol−gel bioactive glass, tricalcium phosphate, or wollastonite usually increases the mechanical properties of poly(3HB) and poly(3HB-*co*-3HV) along with increasing their bioactivity, for poly(3HB-*co*-3HHx), the addition of hydroxyapatite has been shown to have a detrimental effect on its mechanical and cell-adhesion properties. Therefore, it will be important to understand the effect of addition of these various bioceramics on the mechanical and structural properties of the PHAs. Moreover, knowledge about the influence of bioactive ceramic addition on the degradation rate of the composite, which has been addressed only to a limited extent so far, will aid in tailoring the degradation rate of the composites for optimized tissue engineering scaffolds.

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Abbreviations

PHA	Polyhydroxyalkanoate
Poly(3HB)	Poly(3-hydroxybutyrate)
Poly(4HB)	Poly(4-hydroxybutyrate)
PHO	Poly(β-hydroxyoctanoate)
Poly(3HB-*co*-3HV)	Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)
Poly(3HB-*co*-3HHx)	Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate)
BG	Bioactive glass
TCP	Tricalcium phosphate
XRD	X-ray diffraction
SEM	Scanning electron microscopy
SBF	Simulated body fluid
PLA	Poly(lactic acid)
EDS	Energy dispersive spectrum
3HV	3-hydroxyvalerate
PDLLA	Poly(DL-lactic acid)
PLLA	Poly(L-lactic acid)
PGA	Poly(glycolic acid)
Poly(3HB-*co*-3HV-*co*-4HB)	Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate-*co*-4-hydroxybutyrate)
scl-PHA	short-chain-length polyhydroxyalkanoate
mcl-PHA	medium-chain-length polyhydroxyalkanoate

References and Notes

(1) Doi, Y.; Steinbüchel A In *Biopolymers*; Doi, Y., Steinbuchel, A., Eds; Wiley-VCH: Weinheim, 2002; Vol. 4 Polyesters III, Chapter 8.
(2) Williamson, D. H.; Wilkinson, J. F. *J. Gen. Microbiol.* **1958**, *19*, 198.
(3) Steinbüchel, A.; Valentin, H. E. *FEMS Microbiol. Lett.* **1995**, *128*, 219.
(4) Steinbüchel, A. *Macromol. Biosci.* **2001**, *1*, 1.
(5) Lee, S. Y. *Biotechnol. Bioeng.* **1996a**, *49*, 1.
(6) Shishatskaya, E. I.; Volova, T. G.; Puzyr, A. P.; Mogilnaya, O. A. *J. Mater. Sci.: Mater. Med.* **2004**, *15*, 719.
(7) Martin, D. P.; Williams, S. F. *Biochem. Eng. J.* **2003**, *16*, 97.
(8) Williams, S. F.; Martin, D. P.; Horowitz, D. M.; Peoples, O. P. *Int. J. Biol. Macromol.* **1999**, *25*, 111,.
(9) Williams, S. F.; Martin, D. P. Applications of PHAs in medicine and pharmacy. In *Biopolymers*; Doi, Y., Steinbüchel, A., Eds.; Wiley-VCH: Weinheim, 2002; Vol. 4 Polyesters III.
(10) Shishatskaya, E. I.; Volova, T. G. *J. Mater. Sci.: Mater. Med.* **2004**, *15*, 915.
(11) Gogowleski, S.; Jovanovic, M.; Perren, S. M.; Dillon, J. G.; Hughes, M. K. *J. Biomed. Mater. Res.* **1993**, *27*, 1135.
(12) Tesema, Y.; Raghavan, D.; Stubbs, J. *J. Appl. Polym. Sci.* **2004**, *93*, 2445.
(13) Yang, Y.; Li, X.; Li, G.; Zhao, N.; Zhang, X. *J Biomed. Eng. (Chengdu, China)* **2002**, *19*, 25.
(14) Deng, Y.; Lin, X. S.; Zheng, Z.; Deng, J. G.; Chen, J. C.; Ma, H.; Chen, G. Q. *Biomaterials* **2003**, *24*, 4273.
(15) Luklinska, Z. B.; Schluckwerder, H. *J. Microsc. (Oxford, UK)* **2003**, *211*, 121.
(16) Hoerstrup, S. R.; Sperling, J. S.; Martin, D. P.; Daebritz, S.; Mayer, J. E.; Vacanti, J. P. *ASAIO J.* **2000**, *46*, 107.
(17) Sendil, D.; Gursel, I.; Wise, D. L.; Hasirci, V. *J. Controlled Release* **1999**, *59*, 207.
(18) Anderson, A. J.; Dawes, E. A. *Microbiol. Rev.* **1990**, *54*, 450.
(19) Chen, L. J.; Wang, M. *Biomaterials* **2002**, *23*, 2631.
(20) Jones, J. R.; Hench, L. L. *Mater. Sci. Technol.* **2001**, *17*, 891.
(21) Kokubo, T.; Kim, H. M.; Kawashita, M. *Biomaterials.* **2003**, *24*, 2161.
(22) Hench, L. L. *J Am. Ceram. Soc.* **1998**, *81*, 1705.
(23) Boccaccini, A. R.; Blaker, J. J. *Expert Rev. Med. Dev.* **2005**, *2*, 303.
(24) Zhang, K.; Wang, Y.; Hillmyer, M. A.; Francis, L. F. *Biomaterials* **2004**, *25*, 2489.
(25) Boccaccini, A. R.; Stamboulis, A. G.; Rashid, A.; Roether, J. A. *J. Biomed. Mater. Res., Part B* **2003**, *67*, 618.
(26) Thomson, R. C.; Yaszemski, M. J.; Powers, J. M.; Mikos, A. G. *Biomaterials* **1998**, *19*, 1935.
(27) Yang, S.; Leong, K. F.; Du, Z.; Chua, C. K. *Tissue Eng.* **2001**, *7*, 679.
(28) Liu, X.; Ma, P. X. *Ann. Biomed. Eng.* **2004**, *32*, 477.
(29) Tsang, V. L.; Bhatia, S. N. *Adv. Drug Delivery Rev.* **2004**, *56*, 1635.
(30) Rezwan, K.; Chen, Q. Z.; Blaker, J. J.; Boccaccini, A. R. *Biomaterials.* **2006**, *27*, 3413.
(31) Bonfield, W.; Grynpas, M. D.; Trully, A. E.; Bowman, J.; Abram, J. *Biomaterials* **1981**, *2*, 185.
(32) Li, H.; Chang, J. *Biomaterials* **2004**, *25*, 5473.
(33) Doyle, C.; Tanner, E. T.; Bonfield, W. *Biomaterials* **1991**, *12*, 841.
(34) WangY.-W.; Wu, O.; Chen, J.; Chen, G.-Q. *Biomaterials* **2005**, *26*, 899.
(35) Kumagi, Y.; Doi, Y. *Polym. Degrad. Stab.* **1992**, *36*, 241.
(36) Ferreira, B. M. P.; Zavaglia, C. A. C.; Duek, A. R. *J. Mater. Res.* **2001**, *4*, 34.
(37) An, Y.; Dong, L.; Xing, P.; Zhuang, Y.; Mo, Z.; Feng, Z. *Eur. Polym. J.* **1997**, *33*, 1449.
(38) You, J. W.; Chiu, H.; Don, T.-M. *Polymer* **2003**, *44*, 4355.
(39) Lemoigne, M. *Bull. Soc. Chem. Biol.* **1926**, *8*, 770.
(40) Baptist, J. N. U.S. Patent 3,044,942, 1962.
(41) Holmes, P. A. In *Developments in crystalline polymers*; Bassett, D. C., Ed.; Elsevier: London, 1988; Vol. 2, pp 1−65.

(42) Kawaguchi, Y.; Doi, Y. *FEMS Microbiol. Lett.* **1990**, *79*, 151.
(43) Hahn, S. K.; Chang, Y. K.; Lee, S. Y. *Appl. Environ. Microbiol.* **1995**, *61*, 34.
(44) Engelberg, I.; Kohn, J. *Biomaterials* **1991**, *12*, 292.
(45) El-Hadi A.; Schnabel, R.; Straube, E.; Müller, G.; Henning, S. *Polym. Test.* **2002**, *21*, 665.
(46) Chen, G. Q.; Wu, Q. *Biomaterials* **2005**, *26*, 6565.
(47) Sudesh, K.; Abe, H.; Doi, Y. *Prog. Polym. Sci.* **2000**, *25*, 1503.
(48) Byrom, D. In *Plastics from Microbes: Microbial synthesis of polymers and polymer precursors*; Mobley, D. P., Ed.; Hanser: Munich, 1994; pp 5−33.
(49) *49.* Kusaka, S.; Abe, H.; Lee, S. Y.; Doi, Y. *Appl. Microbiol. Biotechnol.* **1997**, *47*, 140.
(50) Reusch, R. N.; Sadoff, H. L. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4176.
(51) Gassner, F.; Owen, A. J. *Polym. Int.* **1996**, *39*, 215.
(52) Yang, X.; Zhao, K.; Chen, G. Q. *Biomaterials* **2002**, *23*, 1391.
(53) Saito, T.; Tomita, K.; Juni, K.; Ooba, K. *Biomaterials* **1991**, *12*, 309.
(54) Zhao, K.; Deng, Y.; Chen, J. C.; Chen, G.-Q. *Biomaterials* **2003**, *24*, 1041.
(55) Torun, K. G.; Kenar, H.; Hasirci, N.; Hasirci, V. *Biomaterials* **2003**, *24*, 1949.
(56) Freier, T.; Kunze, C.; Nischan, C.; Kramer, S.; Sternberg, K.; Sass, M.; Hopt, U. T.; Schmitz, K. P. *Biomaterials* **2002**, *23*, 2649.
(57) Löbler, M.; Sass, M.; Kunze, C.; Klaus-Peter, S.; Hopt, U. T. *J. Biomed. Mater. Res.* **2002**, *61*, 165.
(58) Unverdorben, M.; Spielberger, A.; Schywalsky, M.; Labahn, D.; Hartwig, S.; Schneider, M.; Lootz, D.; Behrend, D.; Schmitz, K.; Degenhardt, R.; Schaldach, M.; Vallbracht, C. *Cardiovasc. Intervent. Radiol.* **2002**, *25*, 127.
(59) Baptist, J. N.; Ziegler, J. B. U.S. Patent 3,225,766, 1965.
(60) Korsatko, W.; Wabnegg, B.; Tillian, H. M.; Braunegg, G.; Lafferty, R. M. *Pharm. Ind.* **1983**, *45*, 1004.
(61) Webb, Adsetts, J. R. Br. Patent 8,523,824, 1985.
(62) Wahl, D. A.; Czernuszka, J. T. *Eur. Cells Mater.* **2006**, *11*, 43.
(63) Knowles, J. C.; Hastings, G. W.; Ohta, H.; Niwa, S.; Boeree, N. *Biomaterials* **1992**, *13*, 491.
(64) Ni, J.; Wang, M. *Mater. Sci. Eng., C* **2002**, *20*, 101.
(65) Zhang, R.; Ma, P. X. *J. Biomed. Mater. Res.* **1999**, *45*, 285.
(66) Luklinska, Z. B.; Bonfield, W. *J. Mater. Sci.: Mater. Med.* **1997**, *8*, 379.
(67) Shepperd, J. A. N.; Apthorp, H. *J. Bone Jt. Surg., Br. Vol.* **2005**, *87B*, 1046.
(68) Hench, L. L. *J. Am. Ceram. Soc.* **1991**, *74*, 1487.
(69) Xynos, I. D.; Edgar, A. J.; Buttery, L. D. K.; Hench, L. L.; Polak, M. *J. Biomed. Mater. Res.* **2001**, *55*, 151.
(70) Galego, N.; Rozsa, C.; Sanchez, R.; Fung, J.; Vazquez, A.; Tomas, J. S. *Polym. Test.* **2000**, *19*, 485.
(71) Holland, S. J. A. M.; Yasin, J. M.; Tighe, B. J. *Biomaterials* **1987**, *8*, 289.
(72) Hasirci, V. Biodegradable biomedical polymers: A review of degradation of and in vivo response to polylactides and polyhydroxyalkanoates. In *Biomaterials and Bioengineering Handbook*; Wise, D. L., Ed.; Marcel Dekker: New York, 2000; pp 141−155.
(73) Torun, K. G.; Korkusuz, F.; Korkusuz, P.; Purali, N.; Özkul, A.; Hasirci, V. *Biomaterials* **2003**, *24*, 4999.
(74) Rivard, C. H.; Chaput, C.; Rhalmi, S.; Selmani, A. *Ann. Chir.* **1996**, *50*, 9.
(75) Tanner, K. E.; Doyle, C.; Bonfield, W. In *Advances in Biomaterials*; Heimke, G., Soltesz, U., Lee, A. J. C., Eds.; Elsevier Science Publisher BV: Amsterdam, 1990; Vol. 9, pp 149−154.
(76) Kumarasuriyar, A.; Jackson, R. A.; Grøndahl, L.; Trau, M.; Nurcombe, V.; Cool, S. M. *Tissue Eng.* **2005**, *11*, 1281.
(77) Chaput, C.; Yahia, L.; Selmani, A.; Rivard, C.; Mater, C. *Res. Soc. Symp. Proc.* **1995**, *394*, 111.
(78) Wu, G.; Wang, Y.; Zheng, Y.; Chen, Z. *Key Eng. Mater.* **2005**, *288*−*289*, 87.
(79) Boeree, N. R.; Dove, J.; Cooper, J. J.; Knowles, J.; Hastings, G. W. *Biomaterials* **1993**, *14*, 793.
(80) Li, H.; Du, R.; Chang, J. *J. Biomater. Appl.* **2005**, *20*, 137.
(81) Iannace, S.; Maffezzoli, A.; Leo, G.; Nicolais, L. *Polymer* **2001**, *42*, 3799.
(82) Doi, Y.; Kitamura, S.; Abe, H. *Macromolecules* **1995**, *28*, 4822.
(83) Chen, G. Q.; Zhang, G.; Park, S. J.; Lee, S. Y. *Appl. Microbiol. Biotechnol.* **2001**, *57*, 50.
(84) Kai, Z.; Ying, D.; Chen, G. Q. *Biochem. Eng. J.* **2003**, *16*, 115.
(85) Wang, Y.-W.; Wu, Q.; Chen, G. Q. *Biomaterials* **2004**, *25*, 669.
(86) Wang, Y.-W.; Yang, F.; Wu, Q.; Cheng, Y.-c.; Yu, P. H. F.; Chen, J.; Chen, G. Q. *Biomaterials* **2005**, *26*, 755.
(87) Olsen-Claire, J.; Blaker, J. J.; Roether, J. A.; Boccaccini, A. R.; Schmack, G.; Gliesche, K. *Mat-wiss. Werkstofftech.* In press.
(88) Sodian, R.; Hoerstrup, S. P.; Sperling, J. S.; Daebritz, S.; Martin, D. P.; Moran, A. M.; Kim, B. S.; Schoen, F. J.; Vacanti, J. P.; Mayer, J. E., Jr. *Circulation* **2000**, *102*, 22III.
(89) Madden, L. A.; Anderson, A. J.; Asrar, J.; Berger, P.; Garrett, P. *Polymer* **2000**, *41*, 3499.
(90) Malm, T.; Bowald, S.; Bylock, A.; Busch, C.; Saldeen, T. *Eur. Surg. Res.* **1994**, *26*, 298.
(91) Martin, D. P.; Peoples, O. P.; Williams, S. F.; Zhong, L. H. U.S. Pat. Appl. 359086, 1999.
(92) Gursel, I.; Yagmurlu, F.; Korkusuz, F.; Hasirci, V. *J. Microencapsulation* **2002**, *19*, 153.
(93) Li, H.; Chang, J. *J. Controlled Release* **2005**, *107*, 463.
(94) Mosahebi, A.; Fuller, P.; Wiberg, M.; Terenghi, G. *Exp. Neurol.* **2002**, *173*, 213.
(95) Nokikov, L. N.; Novikova, L. N.; Mosahebi, A.; Wiberg, M.; Terenghi, G.; Kellerth, J. O. *Biomaterials* **2002**, *23*, 3369.
(96) Leenstra, T. S.; Kuijpers-Jagtam, A. M.; Maltha, J. C. *J. Mater. Sci.: Mater. Med.* **1998**, *9*, 249.

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