Recent advances of medical polyhydroxyalkanoates in musculoskeletal system

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Key Words:
drug delivery; musculoskeletal system; polyhydroxyalkanoate; tissue engineering

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ABSTRACT

Infection and rejection in musculoskeletal trauma often pose challenges for natural healing, prompting the exploration of biomimetic organ and tissue transplantation as a common alternative solution. Polyhydroxyalkanoates (PHAs) are a large family of biopolyesters synthesised in microorganism, demonstrating excellent biocompatibility and controllable biodegradability for tissue remodelling and drug delivery. With different monomer-combination and polymer-types, multi-mechanical properties of PHAs making them have great application prospects in medical devices with stretching, compression, twist in long time, especially in musculoskeletal tissue engineering. This review systematically summarises the applications of PHAs in multiple tissues repair and drug release, encompassing areas such as bone, cartilage, joint, skin, tendons, ligament, cardiovascular tissue, and nervous tissue. It also discusses challenges encountered in their application, including high production costs, potential cytotoxicity, and uncontrollable particle size distribution. In conclusion, PHAs offer a compelling avenue for musculoskeletal system applications, striking a balance between biocompatibility and mechanical performance. However, addressing challenges in their production and application requires further research to unleash their full potential in tackling the complexities of musculoskeletal regeneration.

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Introduction

The musculoskeletal system is the largest human organ system, including the supporting bone, cartilage and joint, skin, tendon and ligament, cardiovascular tissue and nervous tissue, which are the basis of human life activities.[1] As one of the systems most commonly affected by trauma, the ability of the musculoskeletal system to spontaneously repair itself after injury varies greatly. In many cases, healing is incomplete.[2-5] Transplantation is the only possible option for functional recovery, but problems such as infections, complications and even immune rejection occur in both autologous and allogeneic transplantation.[6-8] To solve these transplantation problems, biodegradable biomaterials have been developed. Polyhydroxyalkanoate (PHA) scaffolds are characterised by good biodegradability, biocompatibility and variable mechanical properties, providing a new idea for the repair and regeneration of the musculoskeletal system.[9-11]

PHAs were discovered by Lemogine in 1926 and is kinds of bacteria-synthesised polymers,[12] which can replace non-biodegradable fossil plastics, thereby reducing health hazards and negative environmental impacts.[13] Compared with other biomaterials, PHAs have excellent biodegradability and biocompatibility, which has led to increasing research in the fields of biomedicine, food, cosmetics, and healthcare.[14-17] From a chemical perspective, PHAs are aliphatic polyesters. The nanofibres range in diameter from 50 nm to 500 nm, and can perfectly simulate the collagen fibres required for the

repair process of the musculoskeletal system.[18, 19] The PHAs' family includes short-chain length PHA (scl-PHA; three to five carbon atoms), medium-chain length PHA (mcl-PHA; six to fourteen carbon atoms), and long-chain length PHA (fifteen or more carbon atoms).[20] Scl-PHA consists of 3-hydroxybutyrate (3HB), 4-hydroxybutyrate (4HB), or 3-hydroxyvalerate. Mcl-PHA is the largest category in the PHA family, including monomeric units of 3-hydroxyhexanoate (HHx), 3-hydroxyoctanoate, 3-hydroxydecanoate, 3-hydroxydodecanoate, 3-hydroxytetradecanoate, or even longer-chain comonomer units.[21] Scl-PHA and its copolymers are semi-crystalline polymers with high melting temperature, which are hard, brittle and highly crystalline in nature.[22, 23] Mcl-PHA, on the other hand, is a crystalline polymer that melts at a lower temperature than scl-PHA (in the range of 39–65°C), is inherently elastomer, more flexible, and has a lower crystallinity (25%), and is called true elastomer because of its lower melting point, when the temperature reaches melting temperature, they become viscous and amorphous. Can be better used in health care.[24-27] Currently, six main types of PHA have been widely applied (**Figure 1**). PHAs have different mechanical properties with different molecular weights, alkyl side group lengths, and comonomer unit ratios, making them suitable for tissue engineering with different hardness levels.[20, 28] Currently, multiple methods have been used to prepare PHA scaffolds. The commonly used technologies include solvent casting particle leaching, fibre spinning technology, melt forming, special leaching injection moulding, freeze drying, phase separation, electrospinning, and gas foaming. This article reviews the properties of PHA scaffolds as biomaterials for musculoskeletal repair and regeneration and also summarises the applications of PHAs in bone, cartilage and joint, skin, tendon and ligament, cardiovascular tissue and nervous tissue (**Figure 2**).

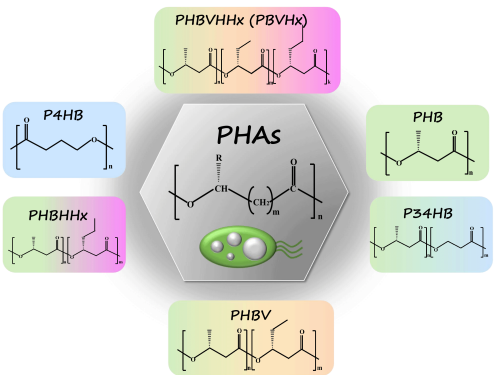

Figure 1. The general structure of PHAs and six commercial PHAs. Created with ChemDraw 2022 and Microsoft PowerPoint 2019. P34HB: poly(3-hydroxybutyrate-co-4-hydroxybutyrate); P4HB: poly(4-hydroxybutyric acid); PHA: polyhydroxyalkanoate; PHB: poly(3-hydroxybutyric acid); PHBHHx: poly(3-hydroxybutyrate-co-3-hydroxyhexanoate); PHBV: poly(3-hydroxybutyrate-co-3-hydroxyvalerate); PHBVHHx (PBVHx): poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid-co-3-hydroxyhexanoic acid trimer).

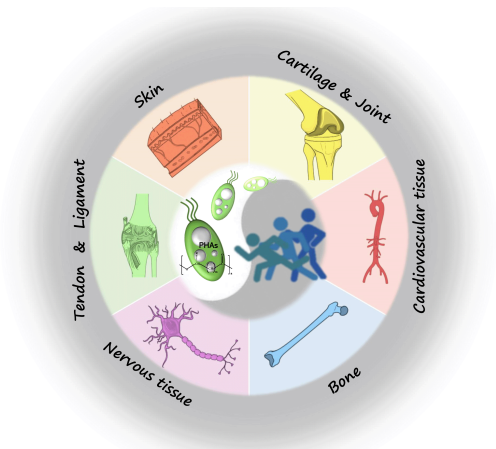

Figure 2. Applications of PHA in the musculoskeletal system. Created with Microsoft PowerPoint 2019. PHA: polyhydroxyalkanoate.

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The Advantages of Polyhydroxyalkanoate for the Musculoskeletal System

Materials used for musculoskeletal tissue engineering must not only have good biocompatibility, support cell growth, guide and organise cells, enable tissue ingrowth, and ultimately degrade into non-toxic products, but also have mechanical and chemical properties of the scaffold.[29, 30] Recent studies have found that some types of PHA have good biocompatibility and can support cell growth and proliferation.[31-34] Furthermore, the tunable mechanical properties of PHA enable it to be widely used as a scaffold in bone tissue engineering.[35] For example, by changing the percentage of HHx, the elastic properties of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) can be adjusted and can be applied to engineered tissues with different hardness requirements: bone, cartilage, nerves and blood vessels.[31]

Biocompatibility

The biocompatibility of the material reflects whether the material will be toxic to the body after implantation; therefore, good biocompatibility is a necessary prerequisite for the successful transplantation of materials for the musculoskeletal system into the human body or animals.[36] In recent years, the biocompatibility of different types of PHA materials has been tested through a variety of studies. Poly(3-hydroxybutyric acid) (PHB), or polyhydroxybutyrate-co-valerate (PHBV) as a commonly used PHA material, can be used to prepare *in vitro* proliferation cell matrix. Chen et al.[31] found that PHB and PHBV promote cell adhesion from various sources, such as fibroblasts, endothelial cells, and isolated hepatocytes. PHBV films proved to have the same properties.[37] In addition, another PHA called poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid-co-3-hydroxyhexanoic acid trimer) (PHBVHHx) can significantly promote the proliferation and adhesion of human umbilical cord mesenchymal stem cells[38] and bone marrow mesenchymal stem cells.[33]

However, different types of PHA have different biocompatibilities. A study found that although both PHB and PHBV can promote cell proliferation and adhesion, PHBV has better biocompatibility.[39] Another study with inoculating rabbit bone marrow cells on the PHBHHx triad scaffold found that its cell adhesion proliferation ability was significantly better than that of PHB, proving that PHBHHx also has better biocompatibility than PHB.[40] In addition, some studies have shown that the biocompatibility of PHB and PHBV can be improved by surface modification. Tesema et al.[41] used collagen to physically and chemically fix the surface of PHBV membrane and found that PHBV could better promote cell proliferation. Chen et al.[42] improved the cytocompatibility by ion implantation into the posterior membrane. Wu et al.[43] modified the surface of the PHB using gelatin and implanted adrenal cortical cells into the modified PHB. The study has shown that the modified PHB is prone to cell proliferation.[43] Yu et al.[44] used hyaluronic acid (HA) to modify the surface properties of PHA films. They found that HA coated on PHA membranes can improve the metabolic activity of mesenchymal stem cells.[44] All of the above studies indicate that PHA has good biocompatibility and can be used in the musculoskeletal system.

Biodegradability

PHAs are a type of biopolymer made of polyesters with many different hydroxycarboxylic acid molecules,[45] which can be degraded into monomers or oligomers. Whether the degradation products are toxic is closely related to whether PHA can be used in tissue engineering.[36] Fortunately, among the degradation products of PHA, 3HB and 4HB are natural metabolites in the human body. 3HB is a component of the blood, and 4HB is widely distributed in all major organs of the body.[30] In addition, another study has found that PHA must be degraded at an appropriate rate to prevent inflammation and rejection of the implant material.[40] The biodegradability of five different types of PHA was found the biodegradability of HA is related to its chemical composition.[46] Qu et al.[47] found the degradation rate of PHB < PHBHHx < polylactic acid (PLA) by studying the *in vivo* tissue reaction and biodegradation of PHBHHx, PLA, PHB, PHBHHx (X), and poly(ethylene glycol) (PEG) (E) blends.

Multiple mechanical properties

The biomaterials used for musculoskeletal system recovery require a certain tensile strength, elongation at break and toughness.[48, 49] The reconstruction of bones, cartilage and joints in the musculoskeletal system requires a certain degree of hardness, while the recovery of muscles, tendons and ligaments requires a certain degree of toughness. Since the mechanical strength of PHA scaffolds is determined by the monomer composition of PHA, the chain length, and the distance between the ester bond and the R base, they can be widely used in the musculoskeletal system.[50, 51] As an important member of scl-PHA, PHB has superior thermoplastic properties, but its mechanical properties are poor and can be used to induce osteogenic processes.[52] On the contrary, another type of scl-PHA, poly(4-hydroxybutyric acid), has strong ductility, tensile strength, and elasticity. In summary, scl-PHA is relatively hard and brittle, lacks toughness, and can be used for bone tissue reconstruction.[30] Mcl-PHA is different from scl-PHA in that it has good flexibility and elasticity and can be used as a semi-crystalline biomaterial. As the side chain length further increases, they become stickier.[53] Scl-PHA-mcl-PHA copolymer like PHBHHx can combine the benefits of both to better support the musculoskeletal system recovery. In order to obtain copolymers with more suitable mechanical properties of PHA, researchers have made many efforts to make PHA can be applied to a greater extent.[54, 55]

Applications of Tissue-Engineered Polyhydroxyalkanoate Scaffolds in the Musculoskeletal System

As a biomaterial with good performance, various PHA types and manufacturing technologies can be used for a variety of biomedical applications, including the musculoskeletal system.[56] When applied to the musculoskeletal system, PHA should not only have biocompatibility, biological activity, and good mechanical properties but also have bone conductivity and even bone sensitivity to promote the growth of new tissue.[57] Due to its biological characteristics, it helps to maintain the mechanical integrity, strength, and toughness

of the musculoskeletal system. In addition, it actively participates in various important biological processes such as cell adhesion, migration, proliferation, differentiation, and neuroangiogenesis and plays an important role in regulating inflammation, wound healing, and tissue repair, such as promoting the differentiation and growth of osteoblasts and chondrocytes and inhibiting the proliferation of bacteria such as *Escherichia coli*.[58-62] The specific applications are listed in **Table 1**.[36, 45, 63-97]

Bone

Although significant progress has been made in the field of regenerative medicine, bone tissue engineering repair and regeneration still faces major challenges given the peculiarities of bone tissue engineering.[66] Many studies have shown that PHA, as a biomaterial with good properties, can promote the adhesion, proliferation, and differentiation of mesenchymal stem cells and bone cells, can inhibit bacteria to some extent, and has excellent mechanical properties compared with other biological materials. Its complexes are often used in bone tissues. In addition, scaffolds for bone tissue repair also have appropriate porous structures and microenvironments that promote cell adhesion and growth, mimicking the microstructure and function of natural bone.[98, 99] For example, the highly porous microspheres prepared by Wei et al.[100] using PHA not only improved differentiation of human bone marrow mesenchymal stem cells, but also support stronger osteoblast regeneration. Codreanu et al.[63] investigated the restorative ability of bacterial cellulose (BC)-modified polyhydroxychain alkanoate (PHB/BC) stents obtained using salt roomsion technology in skull fractures of mice and showed that PHB/BC stents can promote osteoblast differentiation in mice. Nishizuka et al.[67] developed a technology for intramedullary fixation of long bone fractures made of biodegradable materials. In animal experiments, there was no fracture displacement in the intramedullary-fixation with biodegradable materials group (poly(L-lactide) + calcium phosphate cement + PHA), even when the rabbits were fully loaded. It is shown that this type of biological scaffold can be used to strengthen and stabilise fractures of long bones.[67] On the other hand, PHA scaffolds can promote cell proliferation and differentiation. Research shows that PHBHHx scaffold has adequate osteoblast attachment and proliferation roughness compared with PHB and PLA, which is suitable for myelocyte attachment, proliferation and differentiation.[32] Pecorini et al.[64] found that the PHBV-poly (D, L-lacrolyde-co-glycol ester) hybrid scaffold prepared by the scaffold can promote the colonisation and differentiation of rat osteoblasts into osteoblastic phenotypes. Two studies also confirmed that when PHA (polyhydroxyoctanoate and poly(3-hydrobutyric acid)) is mixed with tricalcium phosphate, it can promote cell proliferation, maintain high cell activity, and nourish surrounding tissues.[68, 71] In addition, the materials implanted during bone regeneration should also have certain antibacterial properties. Marcello et al.[36] found that the composite sample prepared from PHA and a new antibacterial HA containing strontium selenide could inhibit the bacterial cell activity of *Staphylococcus aureus* 6538P and *Escherichia coli* 8739 with good antibacterial performance through *in vitro* antibacterial tests. Experiments show that P(3HO-co-3HD-co-3HDD) composite membrane loaded with different contents of Se-Sr-HA, the bactericidal effect on *Staphylococcus aureus* 6538P is not the same: Composite membranes containing 10 wt% Se-Sr-HA resulted in an average 90% reduction in cell numbers, but bacterial cells were reduced by an average of 96% when the Se-Sr-HA content was 30 wt%.[36] Chotchindakun et al.[69] used the emission solvent extraction/evaporation method to incorporate mesoporous bioactive glass nanoparticles into PHBV, while cinnamonaldehyde was loaded in mesoporous bioactive glass nanoparticles. The microspheres loaded with cinnamonaldehyde showed that the activity of *Staphylococcus aureus* and *Escherichia coli* could be significantly inhibited in the first 3 hours, and cinnamonaldehyde release behaviour lasted for 7 days. The research results suggest that the system represents an alternative model for antibacterial biomaterials and can be used for potential applications in bone tissue engineering.[69] In addition, Zhao et al.[101] simulated the preparation of an intracellular growth factor release system based on PLA and PHA nanoparticles under microgravity conditions, and the results proved that the mixed nanoparticles can be used as a reliable and stable medium and long term osteogenic differentiation in future space medicine.[101]

Cartilage and joint

At the heart of the musculoskeletal system, articular cartilage plays a role in connecting bones, absorbing mechanical loads and providing lubrication. Articular cartilage is usually understood to be an elastic connective tissue without blood vessels, nerves, or lymphatic vessels. Its damage can be caused by various causes, and the internal repair capacity of the tissue is very limited.[102, 103] When defects in articular cartilage cannot be repaired, it often leads to degenerative joint diseases, such as arthritis. Unfortunately, current clinical interventions have shown little effectiveness in treating such diseases, while cartilage tissue engineering offers new strategies and directions.[104] Considering that PHAs have excellent properties in cartilage tissue engineering, they can be widely used as biomaterials in cartilage tissue engineering. Ching et al.[70] found that nanofibre PHB/poly(3-hydroxycaprylic acid) scaffolds matched the collagen fibres and stiffness of natural cartilage and could be a good material for cartilage repair. Research has shown that the addition of halloysite nanotubes to scaffolds (such as PHB-chitosan and PHB-starch) can improve the performance of the scaffold: the tensile strength is improved, the hydrophilicity of the material is also improved, and the scaffold with halloysite nanotubes supports cell growth and attachment, and cell activity is also increased. It is expected to find application in cartilage tissue engineering.[73, 105] Studies have shown that polyhydroxyalkanoate granule-binding protein (PhaP) or phasin is a heat-stable amphiphilic protein located on the surface of microbial stored polyhydroxyalkanoate particles, which can be used as a natural environmentally friendly surfactant for food, cosmetics and pharmaceuticals.[106] By coating the PHBHHx membrane onto the surface of Arg-Gly-Asp (RGD) peptide-fused PhaP, Li et al.[74] found that PHAP-RGD coating can promote the proliferation and cartilage differentiation of

Table 1. Applications of PHAs in the musculoskeletal system

Application	Material type	Fabrication method	Function	Reference
Bone	PHB/BC	Salt leaching technique	Promote bone formation in critical size calvarial defects in mice	63
	PHAs/Se-Sr-HA	Solvent casting	A high reduction of the number of *Staphylococcus aureus* 6538P and *Escherichia coli* 8739 bacterial cells	36
	PHBV/PLGA	Computer-aided wet-spinning	Support murine preosteoblast cell colonisation and differentiation towards an osteoblastic phenotype	64
	TCP/PHO	Soaking and drying	Enhance the wettability towards more cell-friendly material, enhance the durability of the composites (stress-strain characteristics)	65
	P(3HO-co-3HHX)/HA	Solvent casting-particulate leaching	Allow migration and proliferation of osteoblasts and mesenchymal cells as well as vascularisation	66
	IM-BM(PLLA+CPC+PHA)	Electrospinning	Reinforce and stabilise incomplete fractures with both mechanical testing and an animal experiment	67
	βTCP/P(3HB)	Polyurethane sponge replica method followed by polymer infiltration	Provide cell-friendly environment, ensure high cell viability, and reduce surface hydrophobicity	68
	PHBV/MBGN/CIN	Emulsion solvent extraction/ evaporation	High biological activity and antibacterial performance applied simultaneously in bone tissue engineering	69
Cartilage and joint	Poly(3-hydroxybutyrate)/ poly(3-hydroxyoctanoate)	Electrospinning	Allow to produce a cartilage repair kit for clinical use to reduce the risk of developing secondary osteoarthritis	70
	PLCL/PHBV	Emulsion solvent evaporation	Enhance the compressive modulus of PLCL scaffolds, but could also serve as scaffolding structures for cartilaginous tissue formation	71
	PHB-CS/HNT	Electrospinning	Demonstrate a significant increase in cell viability of chondrocytes	72
	PHB-starch/HNTs	Electrospinning	Improve the tensile strength, support cell growth and attachment without any toxicity for biomedical applications	73
	PhaP-RGD/PHBHHx	Solvent evaporation	The biomaterial films of PHBHHx modified with PhaP-RGD fusion protein can promote its biocompatibility with chondrocytes	72
			Promote the proliferation and chondrogenic differentiation of human umbilical-cord-derived mesenchymal stem cells seeded on PHBHHx films	74
			Lead to more homogeneous cell spreading, better cell adhesion, proliferation and chondrogenic differentiation in the scaffolds	75
Skin	PHB/PHB-HV	Electrospinning	Improve vascularisation of engineered bone tissue	76
	PHB/CA	Electrospinning	Improve cell proliferation	77
	PHB/HEAA	Grafting	Promote the proliferation of human fibroblasts	78
	P(3HB-co-4HB)	Freeze-drying	Promote the adhesion of mouse fibroblasts	79
	PHB	Electrospinning	Support the growth of normal human dermal fibroblasts and keratinocytes	80
			Promote the healing of diabetic wounds	81, 82
	PHBA/CA	Electrospinning	Improve fibroblast adhesion and growth	83
Tendon and ligament	PHB/PHBV/PHUE/ PHOUE	Electrospinning	Promote cell adhesion and proliferation	84
	PHBHHX	Electrospinning	Promote tendon repair *in vivo*, which is conducive to restoring weight-bearing and motor function	45

Table 1. Continued

Application	Material type	Fabrication method	Function	Reference
		Make thin films	Promote adhesion and migration of mesenchymal stem cells and tendon cells	85
	PHA	Mesh-augmented single-row RCRs and nonaugmented RCRs	Improve the initial biomechanical repair strength of tears at risk of rupture	86
Cardiovascular	PHA	Electrospinning	Promote the fusion of scaffolds with cells	87
	PHB	Electrospinning	Promote the adhesion and growth of cardiomyocytes and cardiac fibroblasts	88
	PHBHHx/SF	Make thin films	Promote cell adhesion and proliferation	89
	P(3HO)	Cardiac patches	Promote the adhesion and proliferation of neonatal ventricular rat muscle cells	90
Nervous	PHB	Electrospinning	Have high biocompatibility with human mesenchymal stem cells	91
			Promotes the adhesion and differentiation of embryonic cells into nerve cells	92
			Support the survival and regeneration of neurons after spinal cord injury	93
	PHB/PHBV	Electrospinning	Promote the interaction between Schwann cells and scaffolds	93
			Triggers the activity of Schwann cells	94
	PCL-PHB	Electrospinning	Pluripotent stem cells were induced to differentiate into neurons	95
	PHBHHx	Porous nerve conduit	Have good nerve regeneration ability, which can promote the rapid functional recovery of damaged nerves	96
		Electrospinning	Promote the differentiation of neural stem cells into neurons	97

Note: CA: cellulose; CIN: cinnamaldehyde; CPC: calcium phosphate cement; HA: hyaluronic acid; HEAA: N-hydroxyethyl acrylamide; HNTs: halloysite nanotubes; HV: 3-hydroxyvalerate; IM-BM: intramedullary-fixation with biodegradable materials; MBGN: mesoporous bioactive glass nanoparticles; P(3HB): poly(3-hydrobutyric acid); P(3HB-co-4HB): poly(3-hydrobutyric acid co-4-hydrobutyric acid); P(3HO): poly(3-hydroxyoctanoate); P(3HO-co-3HHX): poly(3-hydroxyoctanoate-co-3-hydroxyhexanoate); PCL: poly(ε-caprolactone); PhaP: polyhydroxyalkanoate granule-binding protein; PHA: polyhydroxyalkanoate; PHB: polyhydroxybutyrate; PHB/BC: polyhydroxychain alkanoate; PHB-CS: PHB-chitosan; PHBHHx: poly (D, L-lacrolyde co glycol ester); PHBV: polyhydroxy-butyrate-co-valerate; PHO: polyhydroxyoctanoate; PHOUE: poly([R]-3-hydroxy-omega-undecenoate-co-3-hydroxy-omega-nonenoate-co-3-hydroxyoctanoate-co-3-hydroxy-omega-heptenoate-co-3-hydroxyhexanoate); PLCL: poly L-lactide co-ε-caprolactone; PLGA: poly (3-hydroxybutyric acid); PLLA: poly(L-lactide); RGD: Arg-Gly-Asp; SF: silk fibroin; TCP: tricalcium phosphate.

human umbilical cord blood-derived mesenchymal stem cells inoculated on the PHBHHx membrane. Despite their similar surface topography, it is worth noting that the coated film can reduce the water contact Angle from 98.69° to 10.63°.[74] In addition, You et al.[75] found that PHBHHx scaffolds coated with PhaP-RGD can promote the proliferation and cartilage differentiation of human bone marrow mesenchymal stem cells. Additionally, RGD also had a positive influence on the extracellular matrix: increased expression of chondrocyte-specific genes and increased content of cartilage-specific extracellular substances.[75] Meanwhile, PHBHHx biomaterial membrane modified with PhaP-RGD fusion protein can also promote its biocompatibility with chondrocytes.[72] Not only are porous scaffolds widely used as biodegradable biomaterials, but microspheres can also serve as potential candidates for cartilage tissue engineering. Li et al.[71] found that mixing PHBV microspheres with poly L-lactide co-ε-caprolactone porous scaffolds can not only improve the compression modulus of poly L-lactide-co-caprolactone scaffolds but also serve as scaffolds formed by cartilage tissue.

Skin

Because skin wound healing is an extremely complex process, high standards apply to biomaterials used for skin tissue engineering.[107, 108] PHAs not only have good biocompatibility, but can also support cell growth and are often used in skin tissue engineering. PHB is the most common one in PHAs. Zonari et al.[76] found that electrospun PHB/PHB-3-hydroxyvalerate fibre sheets can be used in combination with endothelial differentiated cells to promote vascularisation of bone tissue. Zhijiang et al.[77] found that PHB/cellulose hybrid nanofibre scaffolds have biological activity, can promote cell proliferation, and can be used as wound dressings or tissue engineering scaffolds. Ochoa-Segundo et al.[78] found that the prepared N-hydroxyethyl acrylamide monomer and PHB graft copolymer had adequate degradation and porosity, improved

mechanical properties, and could promote human fibroblast culture, which could be widely used in skin tissue engineering. The biocomposite scaffold prepared by Kanimozhi et al.[79] with poly (3-hydrobutyric acid co-4-hydrobutyric acid) and BC as raw materials can promote the adhesion of mouse fibroblasts and can be used for wound repair or tissue engineering scaffolds. The PHB-core-coated coaxial electrospun fibre prepared by Nagiah et al.[80] has good stretch properties in skin regeneration and can support the growth of normal human dermal fibroblasts and keratinocytes, indicating its potential as a scaffold for skin regeneration. In addition, studies have shown that PHB can promote the healing of diabetic wounds, so PHAs may play a role in the regeneration of diabetic wound tissue.[81, 82] PHBV is another polymer of PHA, and studies have shown that PHBV/chitosan (4:1) scaffolds have an excellent ability to improve the adhesion and growth of fibroblasts and can better adapt to the wound healing process *in vivo*.[83, 109]

Tendon and ligament

In tendon and ligament tissue engineering, it is required that the biomaterials used not only have good mechanical properties but also that the degradation rate of polymers cannot be too fast, and the members of PHA just meet the above conditions. Rathbone et al.[84] used L929 mouse fibroblasts to study the biocompatibility of PHAs. They found that the membranes prepared by PHB and PHBV not only had good biocompatibility but could effectively promote cell adhesion and proliferation and could also degrade slowly due to surface erosion. Therefore, PHA can be widely used in tendon and ligament tissue engineering.[84]

Since tendon injury is not easy to repair and all repair methods may have potential problems (such as foreign body reactions) and fracture again, it is required that materials used for tendon tissue engineering can simulate the structure and mechanical properties of natural tendon tissue.[45, 110-116] Webb et al.[45] found that the PHBHHx stent can promote the repair of the Achilles tendon in rats, which is conducive to the recovery of weight-bearing and motor function. Lomas et al.[85] found that PHBHHx may be a suitable polymer for cell/polymer replacement strategies in future tendon repair. Tashjian et al.[86] suggested that PHA could be used to improve the initial biomechanical repair strength of tears at risk of rupture.

Cardiovascular tissue

Because the musculoskeletal system is rich in blood vessels, these blood vessels can effectively maintain the homeostasis of the musculoskeletal system and effectively promote bone formation to a certain extent.[117] Therefore, the vascular repair is particularly important of the musculoskeletal system. Considering that PHA has good angiogenesis, biocompatibility and biodegradability and can prevent adverse remodelling of defective tissues, PHA has been successfully used in vascular tissue engineering.[88, 109] The regenerative filament protein silk fibroin and PHBHHx porous scaffold prepared by Sun et al.[89] can promote cell adhesion and proliferation. Therefore, the silk fibroin-modified PHBHHx material could be a potential material for vascular tissue engineering.[89] Bagdadi et al.[90] used a novel functional material, poly (3-hydroxyoctane ester), a mcl-PHA, to create engineered structures with improved mechanical properties, and they found that the polymer enhances the adhesion and proliferation of neonatal ventricular rat myocytes. The mechanical properties of the final patch were similar to those of the myocardium.[90] The above studies show that PHA can be widely used in musculoskeletal vascular repair.

Nervous tissue

Nerve damage can block the connection between the neuroregulatory brain and muscles, ultimately affecting motor function and even leading to permanent disability.[93, 109] Therefore, the fabrication of effective biomaterial scaffolds is a more successful treatment to promote nerve tissue regeneration in nerve injuries.[118] Some studies have shown that PHA can promote nerve repair and is widely used in nerve tissue engineering. Köse et al.[91] combined random or arranged electrospun nanofibre PHB membranes with human mesenchymal stem cells to form axon scaffolds for bone and spinal cord, and they found that the PHB membrane has high biocompatibility with human mesenchymal stem cells and provides good biomaterials for bone or nerve tissue engineering. Because Schwann cells physiologically promote the growth of regenerated axons, the interaction between Schwann cells and scaffolds is particularly important in neural tissue engineering. The data presented by Masaeli et al.[94] manufactured PHB/PHBV/collagen fibres can effectively promote the proliferation of Schwann cells and facilitate the regeneration of the myelin membrane. Therefore, PHB/PHBV electrostatic spinning nanofibres can be used in nerve tissue engineering.[93, 94] The PHB scaffold prepared by Khorasani et al.[92] can promote the attachment and differentiation of mouse embryonic cells into nerve cells. The results show that PHB can be used as a material for neural tissue engineering. Kuo et al.[95] found that poly(ε-caprolactone)-PHB scaffolds transplanted with neuron growth factor can induce pluripotent stem cells to differentiate into neurons, and this scaffold is promising in neural tissue engineering. Novikova et al.[119] found that PHBs support the survival and regeneration of neurons after spinal cord injury.

In addition to PHB, other PHA materials can also effectively promote the repair of nerve injuries. Bian et al.[96] found that the porous nerve conduit prepared by PHBHHx not only has good mechanical properties but also can promote nerve regeneration and rapid functional recovery of damaged nerves. In addition, it has been found that PLA, PHB, the copolymer of 3HB and 4HB, and PHBHHx can promote the growth and differentiation of neural stem cells. PHBHHx has the strongest potential to promote the differentiation of neural stem cells into neurons. Therefore, these materials are conducive to the repair of the central nervous system.[97, 120]

Applications of Polyhydroxyalkanoate Carriers in Drug Delivery in the Musculoskeletal System

Drug delivery technology, as an important part of healthcare, aims to deliver pharmacologically active drugs to specific sites of action using appropriate drug carriers at the most appropriate

drug delivery rate and dose, while minimising the impact on the body and the occurrence of adverse reactions. Silicone, as an organic compound, is widely used to encapsulate hydrophobic drugs, but recent studies have shown that silicone has the potential to cause cancer.[121, 122] In recent decades, researchers have shown great interest in developing biodegradable polymeric materials from natural sources as drug delivery materials and have achieved positive results. Due to their good biocompatibility and biodegradability, PHAs have attracted great attention in drug delivery systems.[123, 124] The reason why PHAs can serve as good drug carriers is not only because of their unique physicochemical properties but also because they can be processed into films, scaffolds, microspheres, and nanoparticles as needed, which facilitates drug encapsulation. Some studies have shown that drug delivery systems such as hydrogels, microspheres, microcapsules, polymer vesicles, microns and nanoparticles are widely used to deliver a variety of therapeutic drugs, such as proteins, narcotics, antibiotics, anti-inflammatory drugs, anticancer drugs, hormones, etc. PHA can be used as a drug delivery carrier to participate in these processes.[124-129]

Considering the properties of tetracycline, Sendil et al.[130] prepared PHBV microspheres and microcapsules that can be successfully used to load tetracycline, an antibiotic for periodontitis. The author investigated their encapsulation efficiency, loading, release kinetics, and morphological properties. At the same time, it was found that the antibiotics were fully released before degradation of PHBV was observed.[130] Kassab et al.[131] performed experiments using PHB microspheres, with a drug loading of up to 407.6 mg of rifampicin/g PHB. The release rate of the drug is very high. Xiong et al.[132] compared the controlled intracellular drug release behaviour encapsulated in PHB, PHBHHx, and PLA nanoparticles. The results showed that PHB and PHBHHx nanoparticles could load over 75% of rhodamine B isothiocyanate, while PHB and PHBHHx nanoparticles continued to release drugs for at least 20 days, while PLA nanoparticles only for 15 days release. This study is the first to demonstrate that PHB and PHBHHx can effectively control intracellular drug release.[132] Because the PHA delivery system can deliver and maintain sufficient antibiotic concentrations at the infection site, PHB can be widely used for local administration to treat osteomyelitis. However, since systemic administration may be ineffective due to damage to the vascular system, in diseases such as osteomyelitis it is best to ensure local administration through the implantation of pharmaceutical preparations. A study found that PHBV robs containing 7%, 14% and 22% (mol) 14-hydroxyvalvonic acid exhibit late antibiotic release behaviour lasting approximately 2 months in physiological phosphate buffers under *in vitro* conditions, whereas appropriate antibiotic treatment produces a minimum effective concentration of at least 6 weeks.[133] It was found that, in addition to PHBV, PHB and P(3HB-4HB) can also be processed into implantable rods for local administration of antibiotics for the treatment of osteomyelitis.[133-135] Scheithauer et al.[136] prepared PHBV microspheres loaded with phytoestrogens; daidzein did not cause any shear or temperature stress on the drugs. The encapsulated daidzein was initially released at a low level (6.1% for 7 hours) and then continuously for 3 days. Therefore, PHBV microspheres with daidzein delivery functions can also be used in the treatment of osteoporosis and bone tissue engineering.[136] Peng et al.[137] explored a novel PHB nanoparticle loaded with hydrophilic recombinant human bone morphogenetic protein 2 and amphiphilic phosphatide (BPC-PHB NP). The osteogenic differentiation gene markers of BPC-PHB NPs samples were significantly upregulated, suggesting that BPC-PHB NPs could serve as a fast-acting and long-acting bone morphogenetic protein 2 delivery system for osteogenic differentiation.[137] Chen et al.[138] designed a novel long-acting bone morphogenetic protein 7 release system based on poly(4-hydroxybutyric acid) nanoparticles to achieve osteogenic differentiation of human adipose mesenchymal stem cells. The results indicate that bone morphogenetic protein 7-soybean lecithin-poly(3-hydroxybutyrate-co-4-hydroxybutyrate) nanoparticles can be used as a fast and long-acting bone morphogenetic protein 7 delivery system for osteogenic differentiation.[138] PHA drug delivery systems have great potential due to their non-immunogenic, sustained and controlled drug release, targeted delivery, and high drug loading capabilities.[139] Nevertheless, certain properties of PHAs may hinder their use in drug delivery systems. Some of these include high hydrophobicity, low thermal stability, and slow degradation rates.[140]

Application of Polyhydroxyalkanoate Degradation Products and Derivatives in the Musculoskeletal System

PHA is a biodegradable biopolymer, and as one of the degradation products of PHA, 3HB is a component of the blood and is widely used in the musculoskeletal system.[30, 141-143] Zhao et al.[144] found that 3HB can promote osteoblast growth *in vitro* and anti-osteoporosis *in vivo*. Therefore, 3HB monomers containing PHA can be used as an effective bone implant material.[144] Skibiński et al.[68] found that novel composite materials based on β-tricalcium phosphate and poly (3-hydroxybutyric acid) bacteria-derived biopolymer can promote the adhesion and proliferation of mesenchymal stem cells and consider this material as a potential candidate for bone tissue regeneration. Czechowska et al.[145] found that the β-tricalcium phosphate-based polyporous scaffold modified with silver and coated with the biopolymer poly (3-hydroxybutyrate)-poly (3-hydroxybutyric acid) bacteria-derived biopolymer can promote the regeneration of bone tissue, and this scaffold is considered more promising bone substitute. A study has found that 3HB can also significantly inhibit the loss of muscle weight, muscle fibre size, and muscle fibre diameter, so it can effectively treat muscle atrophy.[146]

4HB and 3HHx are also degradation products of PHA, and when they coexist with 3HB, they can also be used in the musculoskeletal system. Wang et al.[147] found that the electrospun poly (3-hydrobutyric acid co-4-hydrobutyric acid)/octacalcium phosphate nanofibre membrane had good mechanical properties and bone induction ability. Ang et al.[148] found that the mixture of P(3HB-co-3HHx) and fibroin protein could promote the proliferation and osteogenic differentiation of human umbilical cord mesenchymal stem cells. Studies have shown that poly(3-hydroxybutyrate-co-4-hydroxybutyrate-co-3-

hydroxyhexanoate) (P(3HB-4HB-3HHx)) can significantly promote cell proliferation, so P(3HB-4HB-3HHx) can be used in the musculoskeletal system.[34]

Conclusions and Future Perspectives

The musculoskeletal system, as the heart of human movement, has certain peculiarities in its regeneration and recovery: repairing bone tissue requires a certain level of hardness, while tendons and ligaments pay more attention to toughness. Many treatment methods have their advantages and disadvantages, and biomaterials have provided new ideas for the treatment of musculoskeletal disorders in recent years. The selection of biomaterials has also attracted considerable attention from researchers. In addition to its special mechanical properties, a suitable biomaterial must also have good biocompatibility, a suitable degradation time and certain antibacterial properties in order to avoid local infections. As a biomaterial in regenerative medicine, PHA has great potential for use in the musculoskeletal system. With the addition of many manufacturing technologies and other materials, the various properties of PHA have been significantly improved. Furthermore, PHA scaffolds have shown great potential for drug delivery.

The production process and material properties of PHA are constantly improving, but there are still many challenges.[149] Unlike other biopolymers (such as marine-derived collagen, which has a wide range of sources and simple extraction methods), one of the most significant disadvantages of PHA is its high production cost, and its high price is the result of the need for large quantities of high-purity substrates as well as labour-intensive production and downstream processing.[150] At the same time, there may be some cases during PHA processing where organic solvents are not completely removed, which may lead to PHA being cytotoxic and not conducive to musculoskeletal tissue repair.[151, 152] We can control the physical and chemical properties of PHA through certain technical means, but it is undeniable that there are reports that PHA can cause acute and chronic inflammation, and at the same time, the particle size and distribution of PHA are still very uncontrollable.[65, 147] According to the current trend of PHA application, it has inestimable potential in the musculoskeletal system, but the great challenges it faces are also a fact that cannot be ignored. On the one hand, the large-scale high-quality production of PHA requires researchers to devote more energy to research, on the other hand, the specific problems encountered in the specific application of PHA, for example, How the hydrophobicity is applied in human tissues, the degradation rate under specific conditions, whether there is a suitable drug release rate, how to make the elasticity and hardness meet the tissue requirements, and maintain the integrity of the scaffold structure need further research and exploration.

Author contributions

Conceptualization: DXW, JWD; data collection and literature reviewing: CHM, XYQ and JZ; manuscript draft: CHM; manuscript revision: CHM, DXW, YWD. All authors read and approved the final version of the manuscript.

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Conflicts of interest statement

The authors declare that they have no competing interests.

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