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# The Properties of Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) and its Applications in Tissue Engineering

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**Abstract:** Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) is a member of the polyhydroxyalkanoate (PHA) family. It is the designation of molecules consisting of random co-polymers of 3-hydroxybutyrate and 3-hydroxyhexanoate. PHBHHx plays a significant role in the field of biomedical materials. It has good physical, chemical and mechanical properties, making it potentially useful for a wide range of biomaterials applications. In addition, it has also shown better biocompatibility with different cell types. This paper will introduce the physical, chemical and biological properties of PHBHHx, including biodegradation, hydrophilicity, surface properties and cytocompatibility. The development of PHBHHx in tissue-engineering applications will be discussed. PHBHHx used to repair bone, cartilage, tendons, nerves and vessels will be the focus of discussion.

**Keywords:** Biocompatibility, biodegradability, mechanical properties, osteoblast, poly(3-hydroxybutyrate-co-3-hydroxyhexanoate), tissue engineering.

## 1. INTRODUCTION

Polyhydroxyalkanoates (PHA) are a family of biopolymers consisting of polyesters of many different hydroxycarboxylic acid molecules. Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) is one of the few PHA molecules that can currently be produced on a scale large enough for use in both scientific research and medical device construction [1]. It is the designation of molecules consisting of random co-polymers of 3-hydroxybutyrate and 3-hydroxyhexanoate [2]. A co-polyester consisting of 3-hydroxybutyrate (3HB) and 3-hydroxyhexanoate (3HHx) can be synthesized by Aeromonas hydrophila strain 4AK4 with long-chain fatty acids as the carbon source [3, 4]. PHBHHx has good physical, chemical and mechanical properties [5-7], making it potentially useful for a wide range of biomaterials applications. In addition, it has also shown better biocompatibility with different cell types, such as smooth-muscle cells [8], fibroblasts [9], chondrocytes [10], osteoblasts [2] and bone marrow cells [11]. PHBHHX plays a significant role in the field of biomedical materials and has become a popular research field in recent years. In this paper, we will review this new material in biomedical tissue engineering research.

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## 2. PROPERTIES OF THE MATERIAL

### 2.1. Biodegradation

In tissue-engineering applications, it is desirable to match the rate of absorption of the scaffolds with biological tissue treatment periods. During treatment, the scaffolds must maintain their original mechanical properties and other various functions to play a supportive role in induced tissue growth. After treatment, the best scaffolds will degrade as soon as possible, absorbed by the body to reduce side-effects. Treatment cycles vary in different biological tissues in the human body. For example, skin tissue takes only 3 to 10 days, visceral tissue takes 1 to 2 months, while the major organs may require more than 6 months to degrade. In an *in vitro* degradation test [12] of the 3HHx content of 0%, 5%, 12% and 20% in four types of PHBHHX, the material containing 12% HHx PHBHHX showed the fastest degradation: After 50 days, the weight reduction was 7%, and sustained weight loss was observed. While the other three materials' weight reduction reached only about 3% after 50 days, and sustained weight loss was not observed. PHBHHx could be considered as being similar to polyhydroxybutyrate (PHB), which is a compact right-handed helix stabilized by carbonyl–methyl group interaction. It represents one of the few exceptions of a helix found in nature which does not depend on hydrogen bonding for its formation and stability [13]. Based on this structure, the high HHx content in PHBHHx increases the number of methyl groups compared with the increase of carbonyl groups in the polymer; at the same time, it decreases the oxygen-containing moieties on the polymer surface [8].

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Typically, the smaller blending modification of the polymer chain, so commonly used, was chemically modified to change the performance of the degradation of the material. In the study by Shangguan *et al.* [14], an ultraviolet (UV) radiation method was developed to achieve controlled degradation for bacterial biopolyester PHBHHx. In comparison, the PHBHHx films subjected to direct UV radiation became very brittle, although their degradation was faster than that of the PHBHHx powders subjected to direct UV radiation. After 15 weeks of degradation in simulated body fluid (SBF), films prepared from 8 and 16h UV-treated PHBHHx powders maintained 92% and 87% of their original weights, respectively, while the untreated PHBHHx films lost only 1% of their weight. In general, UV-treated PHBHHx powder had a broad Mw distribution that contributed to rapid degradation, due to dissolution of low-Mw polymer fragments, and strong mechanical properties due to high-Mw polymer chains.

Physical and chemical methods could control PHBHHx degradation to match the periods needed to build the tissues and organs for treatment.

PHB, the component of PHBHHx, is a normal ingredient of blood. Oligomeric-3HB is the main degradation product present in the cell membranes of eukaryotes. Investigation of the degradation products of PHAs, especially the oligomers, which are not harmful to the cells and surrounding tissues, is important. In a study by Yang and co-workers [15], the *in vitro* effects of oligo(3-hydroxybutyrate) (OHB), oligo(3-hydroxybutyrate-co-4-hydroxybutyrate) (O3HB4HB) and oligo(3-hydroxybutyrate-co-3-hydroxyhexanoate) (OHBHHx) on growth and differentiation of the murine beta cell line NIT-1 were investigated. Among the three oligo-hydroxyalkanoates (oligo-HAs), cells treated with OHBHHx displayed higher viability, as measured by the CCK-8 assay. The results demonstrated that the degradation products of PHAs, especially OHBHHx from PHBHHx, were not harmful to beta cells.

### 2.2. Hydrophilicity

The hydrophilicity of the material was examined by measurement of the contact angle to water. Considerable research has shown that the surface properties of a biomaterial, especially its hydrophilicity, influence cell adhesion and proliferation [16-18]. Wang *et al.* [9] reported that, with increasing HHx content in PHBHHx, contact angles increased from 68° to 85°, indicating a decreasing hydrophilicity on the polymer surface, while changing HHx content from 5% to 20% did not lead to dramatic changes in surface hydrophilicity. All PHBHHx and PHB were more hydrophobic than polylactic acid (PLA). Sun *et al.* [19] measured the water contact angle, and surface-free energy analysis showed that the silk fibroin is adsorbed into the surface of the PHBHHX. The water contact angle of the material surface is reduced from 90° to 51°, and the surface-free energy increased from 37.9mJ/$m^2$ to 57.4mJ/$m^2$. Hydrophilicity improved with the addition of polycaprolactone (PCL) [20]. PHBHHx/PCL testing by accelerated hydrolytic studies has indicated that a 30/70 blend exhibited similar degradation behavior to PCL in terms of changes in crystallinity, molecular weight, morphology and mass loss.

### 2.3. Mechanical Properties

Scaffolds for tissue engineering, in addition to the requirements of good biocompatibility and biodegradability, must also induce cell adsorption and promote tissue growth, contribute good mechanical properties and match adjacent tissue. Scaffolds can be used to meet the requirements of the defect in terms of mechanical support and morphology before new tissue matures. Chen's study [21] researched the mechanical properties of solution-cast P(3HB-co-3") films. The tensile strength of the films decreased from 43 to 20 MPa as the 3HH fraction was increased from 0 to 17mol %. In contrast, the elongation to break increased from 6 to 850%. This result indicates that the P(3HB-co-3") films become soft and flexible with an increase in the 3HH fraction.

However, its brittleness and degradation characteristics can be improved upon by blending with other biomaterials. Enhanced yield strength, yield strain and Young's modulus of solvent-blended PHBHHx/PCL occurred at a 30/70 blend when compared with PHBHHx and PCL [20].

### 2.4. Surface Properties

Surface properties including hydrophilicity, surface appearance and functional groups were characterized by water contact angle measurement, scanning electron microscopy (SEM) and x-ray photoelectron spectroscopy (XPS). Most polymer materials show poor cell adhesion properties due to their low surface energy, chemical inertness and smooth surface. For this reason, it is crucial for polymeric films intended for biomedical applications to be surface-modified by additional treatments to raise surface bioactivity, thus enhancing hydrophilic as well as adhesive properties.

Surface properties of PHBHHx consisting of different HHx contents were investigated. It was found that increasing HHx content in PHBHHx changed the properties of polymer surfaces, including polymer crystallinity [21], surface roughness and surface hydrophilicity. These changes were interdependent. For example, the discrepancy in the surface morphology of PHB and PHBHHx may be a major reason for their different contact angles [22]. With the increased HHx content in PHBHHx, the polymer surface properties changed accordingly. P(HB-co-20%-HHx) had the smoothest surface, while the PHB surface was most hydrophilic among the evaluated PHB and all the PHBHHx. With increasing HHx content, PHBHHx films became less porous on the surface. When HHx content increased to 20%, a dramatic change in the surface topology of PHBHHx was observed [9].

### 2.5. Modification of Properties

Several tissues and organs in the human body have different anatomical and physiological features, so it is difficult to meet their requirements by relying on a scaffold made of a single material. To make PHBHHx able to adapt to more requirements and have better biocompatibility, scientists use a variety of methods to modify the material. Different characteristics of the materials are mixed to yield scaffolds with new levels of performance. Techniques used to modify material properties include surface hydrolysis [23], grafting technique [24], ultraviolet treatment [14] and plasma treatment [25, 26]. Additives might have a positive impact on the deg-

radation of the material, or the final degradation products will be toxic to the tissue.

To enhance the thermal physical properties of PHBHHx, Luo and co-workers blended PHBHHx with poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P3HB4HB) [27]. All PHBHHx/P3HB4HB blends showed improved physical properties compared with those of PHBHHx, including higher thermal stability, flexibility and mechanical strength. The PHBHHx/P3HB4HB blend in a 4:2 weight ratio showed the roughest surface and also had the highest chondrocyte viability among all blends.

Shen *et al.* [28] fabricated the PHBHHx film by a solution-casting method that was subsequently modified by NaOH treatment to improve surface hydrophilic properties. The results showed that the hydrophilicity of PHBHHx film was obviously improved by the NaOH treatment, due to the topographic changes promoted by NaOH-etching and the introduction of polar groups, including hydroxyl and carboxyl, on the topmost surface layers. However, the modified film exhibited an aging effect: the hydrophilicity decreased with time elapsed during storage. It was found that the aging rate was strongly dependent on both the crystallinity of the film and the storage environment.

Surface modification can also change the properties' modification of PHBHHx. The water contact angle was decreased dramatically compared with that of NaOH- or lipase-treated PHBHHx films and untreated PHBHHx films, and the number of attached neural stem cells (NSC) significantly increased. NSCs survived well on treated PHBHHx films and differentiated into neurons and glial cells.

### 2.6. Cytocompatibility

PHBHHx has excellent cell compatibility with a variety of primary cells and cell lines such as cartilage cells [29], bone-marrow-derived mesenchymal stem cells [30], adipose-derived stem cells [31], fibroblasts [32] and osteoblasts [9].

The behaviors of cells differed on PHBHHx. The highly porous electrospun PHBHHx membranes are better substrates for the culturing of human mesenchymal stem cells (hMSCs), since they can maintain cell viability higher than that of the compression-molded PHBHHx membranes [30].

Also, different cells had different responses to the materials. For fibroblasts, the smoothest P(HB-co-20%-HHx) was preferred, while for osteoblasts, the P(HB-co-12%-HHx) surface with appropriate roughness was the most favorable. This roughness may also promote osteoblast differentiation, indicated by round cells on the P(HB-co-12%-HHx) surface, which indicated activated osteoblasts [9].

Human embryonic stem cells (hESCs), spontaneously differentiated hESCs (SDhESCs) and mesenchymal stem cells (hMSCs) co-culture with PHBHHx and collagen. Following exposure to the appropriate induction medium [33], those three groups were shown to promote retention of osteogenic, chondrogenic and adipogenic differentiation by the expression of RUNX2, SOX9 and PPARγ genes, respectively. This suggests that PHBHHx/collagen scaffolds have the potential for use as biocompatible scaffolds in future tissue engineering.

## 3. PHBHHX APPLICATIONS IN TISSUE ENGINEERING

### 3.1. Bone Tissue Engineering

In general, bone tissue engineering scaffolds must have the features described above to be considered useful. They must be biocompatible, support cell growth, guide and organize the cells, allow for tissue ingrowth and, ultimately, degrade to non-toxic products [34]. Currently, investigators have conducted considerable research on bone tissue engineering scaffolds, and many experimentally validated scaffolds have greatly promoted the development of bone tissue engineering, but the ideal scaffold for bone tissue engineering requirements has not been identified. In recent years, much has been achieved with the use of PHBHHX in the bone tissue engineering field.

Recently, 3D porous scaffolds loaded with specific living cells have been investigated for natural tissue regeneration. Xi *et al.* [35] blended PHBHHx-hydroxyapatite (HAP) composite scaffolds prepared by phase separation and subsequent sublimation of the solvent for bone tissue engineering. The HAP platelets, ranging from 10 to 100mm in size, were randomly distributed in the PHBHHx matrix. Good adhesion between PHBHHx matrix and HAP platelets was observed. Compared with those of the PHBHHx scaffolds, both the compressive modulus and the maximum stress of the composite scaffold were significantly higher. Biocompatibility and osteoconductivity were assessed by examination of the morphology, proliferation and differentiation of MC3T3-E1 osteoprogenitor cells seeded on the scaffolds. The PHBHHx-HAP composite scaffolds showed better mechanical properties, biocompatibility and osteoconductivity than the PHBHHx scaffolds. However, studies by Wang and co-workers [36] yielded opposite results, perhaps due to different preparation methods for scaffold materials with different properties, among other factors.

For bone tissue engineering, electrospinning techniques have been used in recent years to create fibrous scaffolds [37]. Microgrooved topography (10 μm) on PHBHHx surfaces was proved to promote initial osteogenesis of MSCs [38]. As reported, the aligned PHBHHx electrospun fibers impaired adipogenic but enhanced osteogenic differentiation in the absence of soluble inductive factors [39]. The MAPK-dependent PPAR signaling pathway was responsible for the influence of fiber alignment on MSCs.

### 3.2. Cartilage Tissue Engineering

Adult articular cartilage contains no blood vessels, neural network or lymphatic drainage and has a limited intrinsic capacity to heal once damaged by injury or disease [40]. To repair damaged cartilage, many researchers are turning toward tissue engineering approaches involving the fabrication of cartilage constructs by culturing cells on porous and resorbable scaffolds [41, 42].

PHB/PHBHHx is a suitable material for cartilage tissue engineering [31]. The mRNA level of type II collagen of chondrocytes seeded on all scaffolds consisting of PHBHHx was obviously higher than that of the PHB-only scaffold throughout the culture period, suggesting the positive effect of PHBHHx on extracellular matrix production [29]. Human

adipose-derived stem cells (hASCs) were grown on a three-dimensional PHB/PHBHHx scaffold *in vitro* with or without chondrogenic media for 14 days [31]. The differentiated cells grown on the (PHB/PHBHHx) scaffold were then implanted into the subcutaneous layer of nude mice. After 24 weeks of implantation, the differentiated cells/(PHB/PHBHHx) implants formed cartilage-like tissue and stained positive for collagen type II, safranin O and toluidine blue. In addition, typical cartilage lacuna was observed, and there were no remnants of PHB/PHBHHx [31].

Three-dimensional scaffolds made of PHBHHx have been used to repair a rabbit articular cartilage defect model [40]. After 16 weeks of *in vivo* implantation, the defects in both the engineered cartilage constructs and the bare scaffolds were found to be filled with white cartilaginous tissue, with the engineered constructs showing histologically good subchondral bone connection and better surrounding cartilage infusion. More accumulation of extracellular matrix (ECM), including type II collagen and sGAG, was achieved in the engineered cartilage constructs. The repaired tissues possessed an average compressive modulus of 1.58MPa.

### 3.3. Tendon Tissue Engineering

Tendon tissue is characterized by poor repair following injury or disease, with the majority of tendon ruptures occurring at the midpoint of the Achilles tendon, which is relatively acellular [43]. Tenocytes are the major cell group present in tendons, constituting around 95% of the cellular mass [44]. Tenocytes can adhere to and spread across PHBHHx films over 24-hours/day time periods [45].

For tendon injuries and defects, there are, at present, no synthetic/biosynthesized implants available that can restore full function or match the mechanical properties of native tendon [46]. Therefore, PHBHHx was investigated for its utility as a scaffold in a rat Achilles tendon repair model [47]. An accelerated return of function was observed when compared with that in control, with no prolonged immunological or inflammatory response in the rat model observed over 40 days. It was also shown that cells not only migrated into the PHBHHx–collagen core, but also that implanted tenocytes became organized along the fiber surface within the PHBHHx fiber–collagen core *in vivo*. This result demonstrated that PHBHHx in conjunction with collagen and tenocytes can be used as a scaffold material for the treatment of damaged tendon tissue *in vivo*.

### 3.4. Vascular Tissue Engineering

Cardiovascular disease is a major cause of high mortality rates [48], usually considered as a surgical treatment for patch-filled, reorganization or defective angiogenesis [49]. Ideal cardiovascular patch material should be resistant to degradation and infection, non-toxic and non-immunogenic, have good plasticity and good scalability and be durable over the long term [50, 51]. PHBHHx can be an ideal material for use in cardiovascular tissue engineering.

The study by Qu and co-workers [8] study revealed that PHBHHX has functions that induced vascular-related cells. Results demonstrated that RaSMCs adhered better to PHBHHx containing 12% HHx (12%HHx), although they proliferated better on 20% HHx-containing PHBHHx films (20%HHx). This was explained by the difference in cell-cycle progression observed by flow cytometry, since it was found that only 20%HHx-containing polymer could maintain normal cell-cycle evolution as TCPs did after 3-day incubation.

Qu's team further investigated the effects of PHBHHX on vascular-related cells by surface modification [52]. The improved results were demonstrated by better growth of human umbilical vein endothelial cells (HUVECs) and rabbit aorta smooth-muscle cells (SMCs) on the surface of ammonia plasma-treated PHBHHx coated with fibronectin (PFn-PHBHHx), compared with that on fibronectin-coated (Fn-PHBHHx) or uncoated PHBHHx. The most prominent effect of PFn-PHBHHx was its stimulation of HUVEC proliferation. HUVECs on PFn-PHBHHx formed a confluent monolayer after 3 days of incubation, while SMCs were unable to form a sub-confluent layer.

To overcome the deficiencies of heart valve fillings, an acellular porcine aortic valve was prepared as a scaffold by coating a composite valve PHBHHx patch [53]. Mechanical testing in *in vitro* experiments showed that, through PHBHHx coating, the tensile strength of the composite valve was increased. In an *in vivo* study, hybrid valve conduits were implanted into pulmonary positions in sheep without cardiopulmonary bypass. The valves were explanted and examined histologically and biochemically 16 weeks after surgery. These results showed that, *in vivo,* PHBHHx coating can reduce calcification and promote the repopulation of hybrid valves with the recipient's cells, resembling native valve tissue.

Uniaxial microtubular structures made of PHBHHx were successfully prepared by directional freezing and phase-separation techniques [54]. The structure of the scaffolds included tubular size and architecture. The scaffold's mechanical properties could be adjusted by changing the PHBHHx concentration, PHBHHx solvent and phase-separation temperature. These special structures guided the rabbit aorta smooth-muscle cells (RaSMCs) to grow along the tubular structure. Scanning electron microscopy and H&E staining demonstrated that RaSMCs were guided to grow along the microtubular structures of scaffolds prepared from the 2.5% or 3.0% PHBHHx/benzene solution.

Currently, PHBHHx and other polymeric materials in cardiovascular tissue engineering applications are still facing challenges, mainly application compatibility issues [55]. An electro-spinning technique [56] and a 3D weaving technique [57] have been developed to prepare polymers with suitable mechanical properties. We have reason to believe that the adoption of a new PHBHHx production preparation process, as well as an appropriately modified PHBHHx surface, will further improve its bio-mechanical properties, resulting in wide use in cardiovascular tissue engineering.

### 3.5. Nerve Tissue Engineering

In recent years, the clinical peripheral nerve injury repair technique has continued to rely on the autologous transplant method, but this method has many disadvantages, such as the need for the tissue engineering of a single nerve [58]. With the development of tissue engineering, the more synthetic neural tube is used for the repair of peripheral nerve injury.

**Table 1. Research into tissue engineering applications of Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) since 2005.**

| Year | Title |
|---|---|
| 2013 | Evaluation of PHBHHx and PHBV/PLA fibers used as medical sutures |
| 2013 | The application of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) scaffolds for tendon repair in the rat model |
| 2013 | Biocompatibility studies and characterization of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)/polycaprolactone blends |
| 2013 | Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)/collagen hybrid scaffolds for tissue engineering applications |
| 2013 | The implantable and biodegradable PHBHHx 3D scaffolds loaded with protein-phospholipid complex for sustained delivery of proteins |
| 2012 | Biocompatibility of surface modified PHBHHx with rat embryonic neural stem cells |
| 2012 | Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) supports adhesion and migration of mesenchymal stem cells and tenocytes |
| 2012 | Novel poly(hydroxyalkanoates)-based composites containing Bioglass® and calcium sulfate for bone tissue engineering |
| 2012 | The differential effects of aligned electrospunPHBHHx fibers on adipogenic and osteogenic potential of MSCs through the regulation of PPARγ signaling |
| 2012 | Phase morphology, physical properties, and biodegradation behavior of novel PLA/PHBHHx blends |
| 2012 | The differential effects of aligned electrospunPHBHHx fibers on adipogenic and osteogenic potential of MSCs through the regulation of PPARγ signaling |
| 2011 | Chondrogenic differentiation of human bone marrow mesenchymal stem cells on polyhydroxyalkanoate (PHA) scaffolds coated with PHA granule binding protein PhaP fused with RGD peptide |
| 2010 | The improvement of fibroblast growth on hydrophobic biopolyesters by coating with polyhydroxyalkanoate granule binding protein PhaP fused with cell adhesion motif RGD |
| 2010 | The use of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) scaffolds for tarsal repair in eyelid reconstruction in the rat |
| 2009 | Influence of poly(3-hydroxybutyrate-co-4-hydroxybutyrate-co-3-hydroxyhexanoate) on growth and osteogenic differentiation of human bone marrow-derived mesenchymal stem cells |
| 2009 | Biocompatibility of surface modified PHBHHx with rat embryonic neural stem cells marrow mesenchymal stem cells |
| 2009 | Enhanced cell affinity of the silk fibroin-modified PHBHHx material |
| 2008 | Preparation and evaluation of porous poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) hydroxyapatite composite scaffolds |
| 2008 | Evaluation of three-dimensional scaffolds prepared from poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) for growth of allogeneic chondrocytes for cartilage repair in rabbits |
| 2008 | Interactions between a poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyhexanoate) terpolyester and human keratinocytes |
| 2008 | Intravascular biocompatibility of decellularizedxenogenic vascular scaffolds/PHBHHx hybrid material for cardiovascular tissue engineering |
| 2008 | The expression of cross-linked elastin by rabbit blood vessel smooth muscle cells cultured in polyhydroxyalkanoate scaffolds |
| 2008 | Differentiation of smooth muscle progenitor cells in peripheral blood and its application in tissue engineered blood vessels |
| 2007 | Study on decellularized porcine aortic valve/poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) hybrid heart valve in sheep model |
| 2006 | Effect of 3-hydroxyhexanoate content in poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) on *in vitro* growth and differentiation of smooth muscle cells |
| 2006 | The effect of D,L-beta-hydroxybutyric acid on cell death and proliferation in L929 cells |
| 2005 | Effects of crystallization of polyhydroxyalkanoate blend on surface physicochemical properties and interactions with rabbit articular cartilage chondrocytes |
| 2005 | The application of polyhydroxyalkanoates as tissue engineering materials |
| 2005 | Effects of surface modification of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) on physicochemical properties and on interactions with MC3T3-E1 cells |

An ideal neural tube should be able to play a protective role in injured nerves and can also promote axonal growth [59]. As a new generation of PHA polymers, PHBHHx usage has been considered a good application in nerve tissue engineering, due to its excellent mechanical and biological properties.

PHBVHHx scaffolds with pore sizes of 30-60 microns increased the differentiation of hBMSCs into nerve cells, whereas they decreased cell proliferation. Results indicated that PHBVHHx scaffolds could be used in nerve tissue engineering for the treatment of nerve injury [60].

Three PHAs including PHBHHx verified that the materials supported neural stem cell (NSC) growth and differentiation, on both 2D films and 3D matrices [61]. Among three PHA nanofiber matrices, PHBHHx showed the strongest potential to promote NSC differentiation into neurons, which is beneficial for CNS repair. Compared with 2D films, 3D nanofiber matrices appeared to be more suitable for NSC attachment, synaptic outgrowth and synaptogenesis.

Both uniform and non-uniform wall porosity were prepared by the particle leaching method [6]. The conduits were used to bridge the 10-mm defects in the sciatic nerve of Sprague–Dawley (SD) rats. Mechanical tests showed that the PHBHHx nerve conduits had suitable mechanical properties, including maximal loads of 3.1 N and 1.3 N for the conduits with non-uniform wall porosity and with uniform wall porosity, respectively, and maximal stresses of 2.3 MPa and 0.94 MPa for the conduits with non-uniform wall porosity and with uniform wall porosity, respectively. After 1 month of implantation, rapid functional recovery of the disrupted nerves was indicated. After 3 months of implantation in the rats, the conduits with uniform wall porosity and those with non-uniform wall porosity lost 24% and 20% of their original average molecular weights, respectively.

### 3.6. Other

PHBHHx was studied for application as a tarsal substitute [62]. PHBHHx scaffolds were implanted into tarsal defects of Sprague-Dawley rats. PHBHHx scaffolds provided satisfactory repair, even though the implanted PHBHHx scaffolds showed inflammation at 2 weeks. Fibrous encapsulation and scaffold degradation were observed on the PHBHHx implants. Combined with its strong, elastic mechanical properties, the tissue-compatible and biodegradable PHBHHx was proven to be a suitable candidate for tarsal repair.

Murine islet beta cells incubated on PHBHHx have been reported to enhance insulin production [63]. More importantly, insulin gene expression as well as extracellular secretion was un-regulated after growth on PHBHHx for 72h. The effects of oligo(3-hydroxyalkanoates), which are the degradation products of PHBHHx, were not harmful to the beta cells. Therefore, PHBHHx warrants further study for application as a pancreatic tissue engineering material [15]. Research into the tissue engineering applications of PHBHHx has been summarized in Table **1**.

## 4. PROSPECTS

PHBHHx is a new member of the PHA family, and even though it has made great progress in the biomedical field, there are still many problems that have not yet been fully resolved, such as: how to improve PHBHHx degradation rates, mechanical characteristics according to different tissue regeneration needs, how to carry biological activity signal molecules, and how to control the geometry suitable for the content of different organs. PHBHHx promises to extend the range of biomaterial suitability for tissue engineering.

## CONFLICT OF INTEREST

There are no any financial or other relationships with other people or organizations that might lead to a conflict of interest.

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