# Evaluation of PHBHHx and PHBV/PLA Fibers Used as Medical Sutures (Postprint)

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## Abstract

Two types of fibers were prepared by using bio-based materials: a mono-filament made from poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) and a multi-filament made from poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and polylactic acid (PLA) b

## Full Text

## Abstract

Two types of fibers were prepared using bio-based materials for evaluation as medical sutures: a monofilament made from poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) and a multifilament made from a blend of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and polylactic acid (PLA). Both fibers were evaluated for mechanical properties, biocompatibility, and degradability. The PHBHHx fiber demonstrated remarkable biocompatibility via H.E. staining, producing minimal impact on surrounding tissues. Fiber degradation was observed by SEM after 36 weeks of implantation, with major degradation products detected after 96 weeks. Consistently, the PHBHHx fiber maintained more than half of its mechanical properties after 96 weeks. The other fiber was prepared by twisting PHBV/PLA blend strands into a bundle, showing high biocompatibility and relatively high degradability. The bunched structure loosened after 36 weeks of implantation. These low-cost, easily prepared fibers have great potential for medical applications, as they could avoid fibrous capsule formation, reduce scar size, and degrade into non-toxic and even beneficial products.

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## 1 Introduction

Ligation sutures are sterile fibers used for medical hemostasis and tissue fixation, playing a significant role in the early wound-healing stage. They are simple but essential articles in medical operations with a fast-growing market [?, ?]. Medical sutures can be divided into absorbable and non-absorbable categories depending on whether the body can naturally degrade and absorb them. Absorbable sutures, previously defined as sutures that lose most of their tensile strength within 60 days post-implantation [?], degrade in vivo to form non-toxic breakdown products, thus reducing infection risk by avoiding secondary operations.

An ideal absorbable medical suture should have the following characteristics [?, ?, ?]: a smooth surface to avoid scratching surrounding tissues; ease of knotting and handling; high biocompatibility (non-toxic with minimal tissue reactions and minimal influence on surrounding tissues); resistance against bacterial growth; high tensile strength to ensure wound closure; ease of sterilization; and appropriate absorption after serving its function (i.e., losing strength after the wound fully recovers). Therefore, specific absorbable sutures should be chosen according to the requirements of different wounds. In most cases, rapidly healing tissues require fast-degrading sutures, while long-lasting sutures are favorable for slowly healing tissues such as fascia, bone, and tendons [?, ?, ?, ?].

Based on their sources, absorbable sutures can be divided into natural and synthetic sutures. Catgut is the most commonly used natural absorbable suture due to its diverse sources and low cost, but it has many shortcomings. First, catgut tends to cause serious tissue reactions [?, ?, ?, ?]; second, its mechanical properties such as low flexibility cannot fulfill the requirements of different operations; and it loses all tensile strength within a few days [?]. Other natural fibers such as collagen fiber, chitin fiber, and chitosan fiber have also been developed, but they usually require specific processing technology. Furthermore, chitin and chitosan have complex degradation mechanisms and may cause severe immune reactions in some populations [?, ?].

Synthetic absorbable sutures are fibers produced from strands of synthetic polymers and are usually easier to process than natural fibers. As catgut can elicit intense tissue reactions, polyglycolide (PGA) suture with higher biocompatibility was developed in 1968 as the first synthetic absorbable suture [?, ?, ?, ?].

PGA suture also has better mechanical properties and handling characteristics than catgut, but it lacks flexibility and its rough surface may scratch surrounding tissues. It may also cause slight tissue reactions [?, ?]. Another synthetic absorbable suture, poly(lactide-co-glycolide) (PLGA), was developed in 1974 by random ring-opening polymerization of the cyclic dimers of glycolic acid (GA) and lactic acid (LA). Different degradation rates of PLGA can be obtained depending on the LA/GA ratio [?]. Two other types of synthetic absorbable sutures—polydioxanone (PDS) and polylactide (PLA)—were developed in 1981, which overcame one major disadvantage of previous synthetic sutures: handling problems. The smooth surface and soft texture allowed easy bending and knotting, making the handling process simple and safe. Also, with improved biocompatibility, PDS suture could be used in vascular and nervous tissues. However, the biocompatibility and mechanical properties of these sutures still need further improvement to meet the various requirements of different wounds. Therefore, other sutures made of biopolymers such as polyhydroxyalkanoates (PHAs) have drawn great attention in recent years as they can offer properties not available in existing synthetic absorbable polymers [?].

PHAs are a family of intracellular polyesters synthesized by a variety of bacteria, which usually serve as carbon source and energy storage materials [?, ?]. The general structure of PHAs is shown in Fig. 1 [FIGURE:1]. PHA materials are biodegradable and highly biocompatible, and the first-generation product polyhydroxybutyrate (PHB) has already been used as sutures [?, ?]. In comparison, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) has higher flexibility, thermal stability, and processability, making it promising as a medical suture. When PHB and PHBV were implanted intramuscularly in animals, the tissue reaction was similar to the reaction to silk and less severe than the reaction to catgut [?, ?]. Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) is considered the third-generation PHA, consisting mainly of 3-HB (3-hydroxybutyrate) and partially of 3-HHx (3-hydroxyhexanoate). Large-scale production of PHBHHx has been accomplished after years of trials [?]. This polymer shows higher break strength and flexibility than PHB and PHBV [?], greatly higher biocompatibility and hemocompatibility [?, ?], and high affinity to a variety of cells [?, ?, ?], which makes it even more advantageous for medical suture applications.

However, despite their biodegradability, high biocompatibility, and unique properties, PHBV and PHBHHx could be further modified for better applications as medical sutures. Previous studies on PHBV and PHBHHx films indicated that the compositions of the monomers greatly affect the mechanical properties of the polymer [?, ?, ?]. For example, the elastic modulus and toughness were enhanced and the crystallization rate was reduced as the 3-HHx content in PHBHHx increased. Therefore, the compositions should be appropriately designed for the preparation of sutures. In this study, the compositions for preparation of PHBHHx and PHBV fibers (see Sect. 2) could balance the mechanical properties and crystallization rate [?, ?]. Twisted multi-filaments with ultra-thin strands usually have better properties and higher handleability. With

PLA (72 wt% of the total fiber) added into PHBV, the resulting PHBV/PLA blend strands have better handleability [?, ?].

In this research, we used PHBHHx and PHBV/PLA fibers as medical sutures and implanted them in rats for as long as 96 weeks. Their qualities were evaluated during the process, including mechanical properties, biocompatibility, degradation, and tensile strength maintenance. These results suggest these two biocompatible materials could be used as medical sutures for further studies.

## 2 Materials and Methods

### 2.1 Preparation of Fibers and Measurement of Mechanical Properties

The materials used to spin strands in this study were PHBHHx (12 mol% 3-HHx, 88 mol% 3-HB) and PHBV/PLA (28 wt% PHBV with 1.2 mol% 3-HV/98.8 mol% 3-HB, blended with 72 wt% PLA). The PHBHHx fiber was prepared according to patents [?, ?] and Ref. [?], and the PHBV/PLA strands were prepared by Ningbo Tian' an Institute of Materials [?].

The diameters of the strands were measured using scanning electron microscope (SEM), and other mechanical properties were measured with an electronic pull test machine (GOTECH, AI 7000S). The drawing speed was 50 mm/s and the interval of fixtures was 15 cm.

### 2.2 Implantation

Three-month-old male Sprague–Dawley (SD) rats (180–200 g) were used and randomly divided into 16 groups with 3 rats each, with 8 groups for PHBHHx implantation and 8 groups for PHBV/PLA implantation. The rats were injected with pentobarbital into the celiac region as anesthetic. The hair on the back near the tail was shaved with blades to expose the skin, and then the skin was incised longitudinally from the middle. A syringe needle inserted with sutures was used to pierce the tissues, and the needle was pulled out quickly to leave the fibers in the tissues. Medical catgut was used as control. The PHA sutures (left side) and catgut (right side) were implanted symmetrically in the subcutaneous tissues and muscle tissues (Fig. 2 [FIGURE:2]).

### 2.3 Histological Analysis

After 1, 3, 6, 12, 24, 36, 48, and 96 weeks of implantation, 3 rats were randomly chosen as a group and injected with overdose pentobarbital. The chest was cut open to puncture the left atrium, and 40 ml of formalin (10% formaldehyde, PBS buffer) was injected into the right ventricle. After formalin circulated throughout the body and reached the left atrium, the hair on the back near the implantation site was shaved and the strands along with the surrounding tissues were removed. Then 2/3 of the samples were fixed in 10% neutral buffered formalin for 24 h and embedded in paraffin for hematoxylin-eosin (H.E.) staining, while the other samples were used for other experiments such as molecular weight measurement, retained tensile strength measurement, and 3-HB assay. Each sample was cut at 3–5 positions and the resulting 5-µm thick sections were used for H.E. staining.

The paraffin embedding and H.E. staining were performed at the Center of Biomedical Analysis, Tsinghua University.

### 2.4 Molecular Weight Measurement by Gel Permeation Chromatography (GPC)

A SHIMADZU Prominence GPC system (LC-20AT Solvent Delivery Unit, RID-10A Differential Refractive Index Detector, Column Oven CTO-20A, and Autosampler SIL-20A) was used to measure the molecular weight of the samples. The PHBHHx strands after 36 weeks of implantation were lyophilized and dissolved in chloroform, and after filtration through a 0.22-µm filter, the molecular weight was measured using GPC. Non-implanted PHBHHx strands were used as control.

### 2.5 Surface Observation by SEM

Scanning electron microscope (FEI Quanta 200, Center of Biomedical Analysis, Tsinghua University) was used to observe the surface of the PHA fibers. All samples were sputter-coated before observation.

### 2.6 3-HB Monomer Assay

The muscle tissues where PHBHHx fiber was implanted were analyzed to detect the concentration of 3-HB. Three pieces of muscle tissue were used: one piece in the back that was in direct contact with the fiber, one piece in the back near the implantation site, and one piece in the leg. Two parallel samples were analyzed for each group.

The concentration of 3-HB was determined using a $\beta$-hydroxybutyrate (Ketone Body) Colorimetric Assay Kit (Cayman Chemical Company, America). The samples were prepared as follows: First, 400 mg of muscle tissue was obtained from each sample and minced into small pieces. Each tissue was immersed in 200 µl of assay buffer, and then the minced tissues were homogenized with an OSE-Y10 electric tissue grinder (Tiangen, China) in 1.5-ml centrifuge tubes. Another 800 µl of assay buffer was added, following the protocol of the kit.

## 3 Results

### 3.1 Preparation and Mechanical Properties of PHBHHx and PHBV/PLA Fibers

Figures 3 and 4 show the structures of the PHBHHx fiber (a monofilament) and the PHBV/PLA fiber (consisting of 20 twisted strands), respectively. The diameters and other mechanical properties of the strands are listed in Table 1

. The very thin PHBV/PLA strand was relatively easier to crystallize, so it was stretched with the help of an automatic spinning machine. However, it was difficult to draw PHBHHx strand in a common way due to its high viscosity and low crystallization rate. Therefore, a semi-automatic machine was used for the preparation of PHBHHx fibers [?]. Even better mechanical properties of PHBHHx fibers could be expected if an automatic spinning method were developed. Both PHBHHx and PHBV/PLA fibers had regular structures with smooth surfaces and uniform diameters (Figs. 3a and 3b), and they were easy to stain according to our previous studies (data not shown), which allowed them to be used as medical sutures.

### 3.2 Implantation and Biocompatibility

Both the PHBHHx and PHBV/PLA fibers were used as medical sutures for operations in muscular and subcutaneous tissues in rats, as described in Sect. 2. Catgut was used as control. The operations proceeded smoothly with neither toxic symptoms nor tumors at the implantation sites. Muscular and subcutaneous slices (5 µm) were removed at different time points and stained for observation (Figs. 4 and 5).

After 1 week of implantation, apparent tissue reactions were observed when catgut was implanted in muscular or subcutaneous tissues, with numerous inflammatory cells (mostly lymphocytes and monocytes, and a few macrophages and giant cells) penetrating the implantation area (Figs. 4c and 5c). The inflammatory cells (dark stained spots indicated by arrows) gathered around the implantation sites. The PHBHHx and PHBV/PLA fibers caused much less severe inflammatory reactions, indicating greatly improved biocompatibility (Fig. 4a). Catgut had already degraded by week 1, with inflammatory cells invading inside (Fig. 4c), while no significant degradation of PHBHHx or PHBV/PLA was observed. The PHBV/PLA fiber showed higher cell affinity than the PHBHHx fiber (Fig. 4a, b), which was consistent with a previous study [?].

Three weeks after implantation, the catgut was entirely digested into pieces and the implantation area was large (indicated by arrows in Figs. 4f and 5f), indicating severe inflammation. The PHBHHx and PHBV/PLA groups maintained their tissue reaction shapes with slight changes (Figs. 4d, e, 5d, e). Similar phenomena were observed at 6 weeks after implantation, when the catgut fiber was completely degraded and the interspace was filled by collagen and fibroblasts to form a spindle-shaped fibrous capsule (Fig. 4i). In contrast, the PHBHHx fiber merely degraded, and collagen and fibroblasts gathered outside the implantation site. The inflammatory area in the PHBV/PLA group expanded slightly, suggesting that the fiber started to degrade. Additionally, the twisted strands began to loosen (Fig. 4h).

From week 12 to week 48, the spindle-shaped fibrous capsules entirely replaced the catgut, similar to the results at week 6 (Fig. 4l, o, r), and the PHBV/PLA fiber further loosened and totally lost its bundle structure (Fig. 4k, n, q). However, the diameter of each remaining strand remained almost unchanged, indicating that the degradation rate was relatively low, which is similar to PHBHHx implanted in muscular and subcutaneous tissues (Figs. 4j, m, p, 5j, k, n).

### 3.3 Biodegradation of the PHBHHx Fiber

Degradation of the PHBHHx fiber did occur, although not apparent from the tissue slices. After 36 weeks of implantation into muscle tissues, slight erosion appeared on the surface of the PHBHHx fiber (Fig. 6b [FIGURE:6]). There were stripped defects on the edge and the degradation was heterogeneous. As a result, the diameter of the fiber decreased from the original size (Fig. 6a, b).

To further investigate the degradation process, the molecular weight of the PHBHHx fiber was measured using GPC. After 36 weeks of implantation, the number-average molecular weight (nAMW) was reduced slightly, while the weight-average molecular weight (wAMW) remained the same (Table 2 ). The reason might be that the low-molecular-weight components in PHBHHx degraded faster than the high-molecular-weight counterparts, so nAMW was reduced faster than wAMW.

The concentration of 3-HB was detected after PHBHHx was implanted in muscles for 96 weeks. Samples were taken from tissues at different distances from the PHBHHx fiber (Fig. 7 [FIGURE:7]). The adjacent muscle samples contained higher 3-HB content than the distant ones, indicating that the degradation process resulted in a concentration gradient of the released 3-HB.

### 3.4 Tensile Strength Maintenance in Muscle Tissues

In suture selection, the properties of the suture must match the requirements of the tissue [?]. The mechanical properties of the PHBHHx suture, including retained tensile strength, maximum stretch ratio, and elastic modulus, were measured after 0, 24, 48, or 96 weeks of implantation (Fig. 8 [FIGURE:8]). The retained tensile strength and maximum stretch ratio were stable within the first 24 weeks, then dropped after 48 weeks. After 96 weeks, the PHBHHx fiber still maintained more than half of its tensile strength, indicating possible application for chronic wounds.

## 4 Discussion

One major problem of existing medical sutures is unsatisfactory biocompatibility leading to tissue reactions. PHA-based sutures, such as PHBHHx and PHBV used in this study, have superior biocompatibility. Their degradation products including 3-HB, 3-HV, and 3-HHx are completely non-toxic and even beneficial for the growth of vascular smooth muscle cells [?]. In this study, the PHBHHx and PHBV/PLA fibers were evaluated for application as medical sutures and both demonstrated high biocompatibility.

According to our results, the PHBHHx fiber is a promising material for cosmetic surgical sutures. Its high elasticity could accommodate wound deformation, while its high biocompatibility could avoid tissue reactions and reduce irritation to surrounding areas. Additionally, the PHBHHx fiber maintained more than half of its tensile strength after long-term implantation, and its high elasticity helps maintain the original shape and fix the wound. Therefore, this type of suture is particularly suitable for treatment of chronic wounds such as bone tissue repair or Achilles tendon repair. Applications in other tissues are also possible.

Reportedly, PHBHHx showed high hemocompatibility [?] and has great potential for use in vascular closure. Furthermore, PHBHHx could accelerate the growth and differentiation of nerve stem cells [?], and PHBHHx-woven conduits were used to repair nerve defects [?]. These findings indicate that PHBHHx can be used as a nerve repair material.

Although PHA-based sutures have many advantages including high biocompatibility and elasticity [?, ?, ?], they need further improvement, especially in handleability. Due to the low crystallization rates of PHBV and PHBHHx, it was difficult to draw very long, thin fibers with uniform texture, which limited their applications. PLA is relatively more prone to crystallization and degradation than PHBV [?], and films and electro-spinning scaffolds made of PHBV/PLA and PHBHHx/PLA have been explored [?]. To overcome the drawbacks of PHA-based sutures, PLA was blended into PHBV to accelerate crystallization and enhance handleability. As a result, very thin strands with a diameter of about 14 μm were produced, and by bundling 20 strands together, the fiber maintained stable mechanical properties while keeping a small overall diameter. Using this method, these materials can also be made into other medical items such as patches, artificial vascular grafts, or artificial nerve conduits. Thin fibers of the blends could be directly woven into different shapes, avoiding any re-heating that would greatly damage the mechanical properties of the blends [?]. While maintaining high biocompatibility, the addition of PLA also greatly improved the handleability of the suture, resulting in a smoother surface and easier knotting that would make operations simpler and less risky.

Certainly, there are some limitations for these fibers to be used as medical sutures. Both PHBHHx and PHBV/PLA fibers are hydrophobic, which reduces cell affinity. It is generally considered that higher hydrophilicity provides a suture with higher cell affinity [?]. The hydrophilicity of these fibers can be improved by many methods, such as treatment with NaOH [?] and silk fibroin modification on the surface [?]. Additionally, the hydrophobicity of PHBHHx or PHBV/PLA fibers could be utilized to avoid intensive adhesion of some tissues to other tissues. Therefore, these fibers could be used to suture hernia and can stay in vivo for a long time as a tissue adhesion prevention material [?]. Other functional units (e.g., PGA which accelerates degradation) can be incorporated into the material to further improve mechanical properties.

## 5 Conclusion

In this work, PHBV/PLA and PHBHHx strands were evaluated in terms of appearance, mechanical properties, biocompatibility, and biodegradation. The PHBHHx strand showed high tensile strength, elasticity, and biocompatibility. The PHBV/PLA strands showed great biocompatibility as PHBV and high processability as PLA. Thus, both PHBHHx and PHBV/PLA fibers are appropriate for use as medical sutures.

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## References

1. Pillai CKS, Sharma CP. Review paper: absorbable polymeric surgical sutures: chemistry, production, properties, biodegradability, and performance. J Biomater Appl. 2010;25(4):291–366.
2. Singer AJ, Thode HC Jr, Hollander JE. National trends in ED lacerations between 1992 and 2002. Am J Emerg Med. 2006;24(2):183–8.
3. Bennett RG. Selection of wound closure materials. J Am Acad Dermatol. 1988;18(4):619–37.
4. Hon LQ, Ganeshan A, Thomas SM, Warakaulle D, Jagdish J, Uberoi R. Vascular closure devices: a comparative overview. Curr Probl Diagn Radiol. 2009;38(1):33–43.
5. Moy R, Waldman B, Hein D. A review of sutures and suturing techniques. J Dermatol Surg Oncol. 1992;18(9):785–95.
6. Singhal JP, Singh H, Ray AR. Absorbable suture materials: preparation and properties. Polym Rev. 1988;28(3–4):475–502.
7. Edlich R, Szarmach RR, Livingston J, Rodeheaver GT, Thacker JG. An innovative surgical suture and needle evaluation and selection program. J Long-term Eff Med Implant. 2002;12(4):211–230.
8. Grisham JE, Zukin DD. Suture selection for the pediatrician. Pediatr Emerg Care. 1990;6(4):301–4.
9. Pavan A, Bosio M, Longo T. A comparative study of poly(glycolic acid) and catgut as suture materials. Histomorphology and mechanical properties. J Biomed Mater Res. 1979;13(3):477–487.
10. Tachibana M, Yaita A, Taniura H, Fukasawa K, Nagasue N, Nakamura T. The use of chitin as a new absorbable suture material—an experimental study. Jpn J Surg. 1988;18(5):533–9.

11. Shishatskaya E, Volova T, Puzyr A, Mogilnaya O, Efremov S. Tissue response to the implantation of biodegradable polyhydroxyalkanoate sutures. J Mater Sci: Mater Med. 2004;15(6):619–628.

12. Vasenius J, Vainionpää S, Vihtonen K, Mäkelä A, Rokkanen P, Mero M, et al. Comparison of in vitro hydrolysis, subcutaneous and intramedullary implantation to evaluate the strength retention of absorbable osteosynthesis implants. Biomaterials. 1990;11(7):501–504.

13. Burton OT, Zaccone P. The potential role of chitin in allergic reactions. Trends Immunol. 2007;28(10):419–22.

14. Reese TA, Liang HE, Tager AM, Luster AD, Van Rooijen N, Voehringer D, et al. Chitin induces accumulation in tissue of innate immune cells associated with allergy. Nature. 2007;447(7140):92–6.

15. Hochberg J, Meyer KM, Marion MD. Suture choice and other methods of skin closure. Adolesc Med Clin. 2009;89(3):627–41.

16. Taylor B, Bayat A. Basic plastic surgery techniques and principles: choosing the right suture material. Student BMJ. 2003;11:139–141.

17. Takizawa T, Akizuki S, Horiuchi H, Yasukawa Y. Foreign body synovitis caused by a broken poly-L-lactic acid screw. Arthroscopy. J Arthrosc Relat Surg. 1998;14(3):329–30.

18. Matsusue Y, Yamamuro T, Oka M, Shikinami Y, Hyon SH, Ikada Y. In vitro and in vivo studies on bioabsorbable ultra-high-strength poly(L-lactide) rods. J Biomed Mater Res. 1992;26(12):1553–67.

19. Miller RA, Brady JM, Cutright DE. Degradation rates of oral resorbable implants (polylactates and polyglycolates): rate modification with changes in PLA/PGA copolymer ratios. J Biomed Mater Res. 1977;11(5):711–9.

20. Williams SF. Applications of PHAs in medicine and pharmacy. Series of Biopolymers. Weinheim: Wiley-VCH Verlag; 2002.

21. Jacquel N, Lo CW, Wei YH, Wu HS, Wang SS. Isolation and purification of bacterial poly(3-hydroxyalkanoates). Biochem Eng J. 2008;39(1):15–27.

22. Rocha RC, da Silva LF, Taciro MK, Pradella JG. Production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) P(3HB-co-3HV) with a broad range of 3HV content at high yields by Burkholderia sacchari IPT 189. World J Microbiol Biotechnol. 2008;24(3):427–31.

23. Volova T, Shishatskaya E, Sevastianov V, Efremov S, Mogilnaya O. Results of biomedical investigations of PHB and PHB/PHV fibers. Biochem Eng J. 2003;16(2):125–33.

24. Chen G, Zhang G, Park S, Lee S. Industrial scale production of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate). Appl Microbiol Biotechnol.

2001;57(1-2):50-5.

25. Doi Y, Kitamura S, Abe H. Microbial synthesis and characterization of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate). Macromolecules. 1995;28(14):4822-8.

26. Wu Q, Wang Y, Chen GQ. Medical application of microbial biopolyesters polyhydroxyalkanoates. Artif Cell Blood Substit Biotechnol. 2009;37(1):1-12.

27. Chen GQ, Wu Q. The application of polyhydroxyalkanoates as tissue engineering materials. Biomaterials. 2005;26(33):6565-78.

28. Wang YW, Wu Q, Chen GQ. Attachment, proliferation and differentiation of osteoblasts on random biopolyester poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) scaffolds. Biomaterials. 2004;25(4):669-75.

29. Wang YW, Wu Q, Chen GQ. Reduced mouse fibroblast cell growth by increased hydrophilicity of microbial polyhydroxyalkanoates via hyaluronan coating. Biomaterials. 2003;24(25):4621-9.

30. Yang X, Zhao K, Chen GQ. Effect of surface treatment on the biocompatibility of microbial polyhydroxyalkanoates. Biomaterials. 2002;23(5):1391-7.

31. McChalicher CW, Srienc F. Investigating the structure-property relationship of bacterial PHA block copolymers. J Biotechnol. 2007;132(3):296-302.

32. Chen GQ. Biofunctionalization of polymers and their applications. Biofunctionalization of Polymers and their Applications. Berlin: Springer; 2011. p. 29-45.

33. Xu J, Guo BH, Yang R, Wu Q, Chen GQ, Zhang ZM. In situ FTIR study on melting and crystallization of polyhydroxyalkanoates. Polymer. 2002;43(25):6893-9.

34. Cai H, Qiu Z. Effect of comonomer content on the crystallization kinetics and morphology of biodegradable poly(3-hydroxybutyrate-co-3-hydroxyhexanoate). Phys Chem Chem Phys. 2009;11(41):8851-8857.

35. Ferreira B, Zavaglia C, Duek E. Films of PLLA/PHBV: thermal, morphological, and mechanical characterization. J Appl Polym Sci. 2002;86(11):2898-906.

36. Chen P, Gu Q, Li J, Zhou J, Wang ZB, Gou QT, Yan Q (2011) China Patent No. CN102181960A: a kind of PHBV fiber and method of production, 14 Sep 2011.

37. Wu Q, Ren MD, Ding CK, Chen GQ, Cheng BW (2011) China Patent No. CN102108563A: method of producing PHA fibers. Chinese, 29 Jun

2011.

38. Cheng BW, Ding CK, Wu Q, Reng MD, Chen GQ (2011) China Patent No. CN102108562A: a kind of method of producing PHA fibers, 29 Jun 2011.

39. Ding CK, Cheng BW, Wu Q. Mechanical properties and ordered structure of bacterial poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) fibers stretched after isothermal crystallization near Tg. Adv Mat Res. 2011;284:1774–7.

40. Dong Y, Li P, Chen CB, Wang ZH, Ma P, Chen GQ. The improvement of fibroblast growth on hydrophobic biopolyesters by coating with polyhydroxyalkanoate granule binding protein PhaP fused with cell adhesion motif RGD. Biomaterials. 2010;31(34):8921–30.

41. Patel KA, Thomas W. Sutures, ligatures and staples. Surg (Oxf). 2008;26(2):48–53.

42. Qu XH, Wu Q, Zhang KY, Chen G. In vivo studies of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) based polymers: biodegradation and tissue reactions. Biomaterials. 2006;27(19):3540–8.

43. Qu XH, Wu Q, Chen GQ. In vitro study on hemocompatibility and cytocompatibility of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate). J Biomater Sci Polym Ed. 2006;17(10):1107–21.

44. Xu XY, Li XT, Peng SW, Xiao JF, Liu C, Fang G, et al. The behaviour of neural stem cells on polyhydroxyalkanoate nanofiber scaffolds. Biomaterials. 2010;31(14):3967–75.

45. Bian YZ, Wang Y, Aibaidoula G, Chen GQ, Wu Q. Evaluation of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) conduits for peripheral nerve regeneration. Biomaterials. 2009;30(2):217–25.

46. Guo WH, Frey MT, Burnham NA, Wang YL. Substrate rigidity regulates the formation and maintenance of tissues. Biophys J. 2006;90(6):2213–20.

47. Luo L, Wei X, Chen GQ. Physical properties and biocompatibility of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) blended with poly(3-hydroxybutyrate-co-4-hydroxybutyrate). J Biomater Sci Polym Ed. 2009;20(11):1537–53.

48. Nanda MR, Misra M, Mohanty AK. The effects of process engineering on the performance of PLA and PHBV blends. Macromol Mater Eng. 2011;296(8):719–28.

49. Gogolewski S, Jovanovic M, Perren S, Dillon J, Hughes M. Tissue response and in vivo degradation of selected polyhydroxyacids: polylactides (PLA), poly(3-hydroxybutyrate) (PHB), and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHB/VA). J Biomed Mater Res. 1993;27(9):1135–48.

50. Cheng ML, Chen PY, Lan CH, Sun YM. Structure, mechanical properties and degradation behaviors of the electrospun fibrous blends of PHBHHx/PDLLA. Polymer. 2011;52(6):1391-401.

51. Rasal RM, Hirt DE. Toughness decrease of PLA-PHBHHx blend films upon surface-confined photopolymerization. J Biomed Mater Res. 2009;88(4):1079-86.

52. Kubová O, Švorčík V, Heitz J, Moritz S, Romanin C, Matějka P, et al. Characterization and cytocompatibility of carbon layers prepared by photo-induced chemical vapor deposition. Thin Solid Films. 2007;515(17):6765-72.

53. Shen F, Zhang E, Wei Z. In vitro blood compatibility of poly(hydroxybutyrate-co-hydroxyhexanoate) and the influence of surface modification by alkali treatment. Mater Sci Eng. 2010;30(3):369-75.

54. Sun M, Zhou P, Pan LF, Liu S, Yang HX. Enhanced cell affinity of the silk fibroin-modified PHBHHx material. J Mater Sci: Mater Med. 2009;20(8):1743-51.

55. Dai ZW, Zou XH, Chen GQ. Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) as an injectable implant system for prevention of post-surgical tissue adhesion. Biomaterials. 2009;30(17):3289-3296.

## Figures

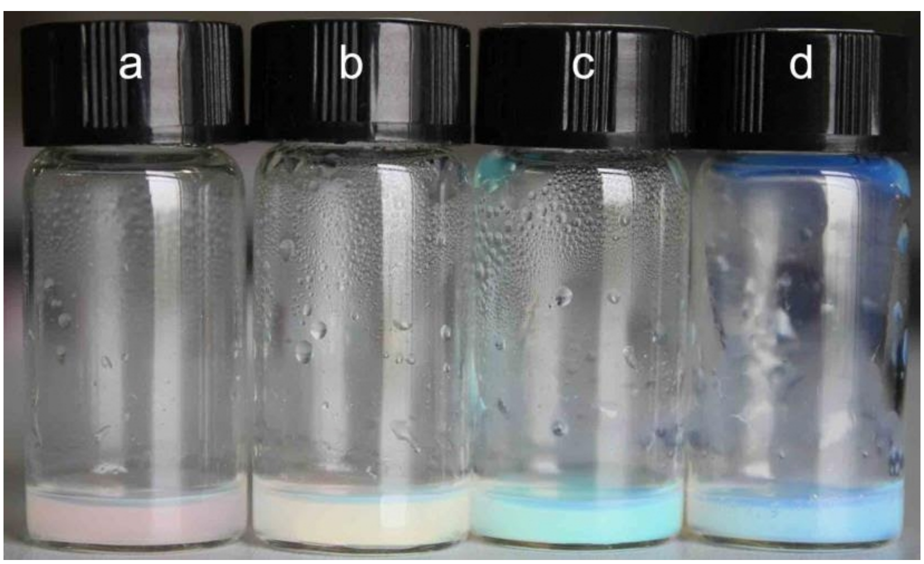


Figure 1: Figure 3