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Biocompatibility and biodegradation of poly(hydroxybutyrate)/poly(ethylene glycol) blend films

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Using chloroform as co-solvent, a series of poly(3-hydroxybutyrate) (PHB) and polyethylene glycol (PEG) blend materials with different ratio ranging from 80 : 20 (wt %) to 20 : 80 (wt %) were prepared by solution blend. The blood-compatibility was evaluated by means of platelet clotting time test and exploring its morphological changes. The results showed that PEG played an important role in resisting platelet adhesion. With the increased addition of PEG, the clotting times became longer and the number of platelet adhesion decreased apparently. All platelets were in discrete state, no pseudopodium had been found and no collective phenomenon had been happened. The cell-compatibility was evaluated via Chinese Hamster Lung (CHL) fibroblast cultivation *in vitro*. The cells cultured on the matrix spread and proliferated well. With the increase of PEG content in the blend films, the number of live cells became more and more. These results indicated that PHB exhibited satisfying cell-compatibility and the addition of PEG also could improve the cell-compatibility of PHB. The biodegradation experiment indicated that the degradation of PHB/PEG was accelerated by enzyme *in vitro* and the blending of PEG was favorable to degradation.
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1. Introduction

Scaffolds fabricated from biodegradable polymers are used extensively in the field of tissue engineering. It is very important to choose the proper scaffold material as the template of inducing cell and tissue growth. So the development of biodegradable polymers is considered one of the major advances in biomedical material research. It is a fundamental requirement that the biodegradable polymers display adequate biocompatibility. Nowadays, there are a large variety of degradable polymers available for use in tissue engineering, which are generally based on blends and copolymers of poly(L-lactide) (PLA) [1, 2], poly(L-glycolide) (PGA) [3–5], etc. These materials have the advantage of possessing initially enhanced mechanical properties, but their relatively quick degradation profile diminishes these properties.

Poly(3-hydroxybutyrate) (PHB) was found in a variety of bacteria such as *Bacillus megaterium* located as granules in the cytoplasm of the bacterium [6]. Compared with other materials, PHB has found a special range of biomedical and friendly environment applications because of its combination of excellent mechanical properties [7], biocompatibility [8, 9] and sorption–diffusion properties [10] coupled with its biodegradation [11]. The main degradation product is 3-hydroxybutric acid, which is found in humans. To date, the available toxicology data are favorable indicating that the PHB is non-toxic. PHB has been used as biodegradable plastics [12] for a few years. Nowadays, PHB has attracted much attention for a variety of medical applications, which include controlled release system [13–17], surgical sutures [18], fracture repair [19, 20], bone and cartilage remolding [21–23], orthopedic uses [24] and as a pericardial substitute [25]. In recent years, PHB has been studied as biopolymer scaffolds in tissue engineering applications. Since the hydrophilicity of PHB is poor, it should be modified to suit the use in tissue engineering. The most effective and simply way is to blend PHB with hydrophilic polymer which is also easy to be blended.

Among the water soluble polymers, Poly(ethylene glycol) (PEG) is the most preferred candidate. PEG is a nontoxic polymer approved by the U.S. Food and Drug Administration (FDA) for internal consumption [26]. PEG is usually effective at excluding other polymers from its surroundings when in an aqueous environment. This property can be translated into protein rejection,

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TABLE I Composition and code of PHB/PEG blends

Blend composition PHB/PEG (w/w)	100/0	80/20	50/50	20/80	0/100
Code	PHB	PHB80	PHB50	PHB20	PEG

reduced platelet adhesion, bacterial repulsion, etc. Several techniques have been used to immobilize PEG onto a variety of polymer surfaces, including physical adsorption, graft copolymerization, covalent grafting, etc [27].

In this paper, we aimed to develop a PHB/PEG blend materials to be used as blood vessel. Since that is a blood-contact device and will be implanted back to body, the materials should have good biocompatibility. To satisfy the usage in tissue engineering, the effects of PEG content on the biocompatibility and biodegradability were evaluated.

2. Materials and methods

2.1. Materials

The PHB, a white powder sample, was kindly provided by Tianjin TianLu Co. Ltd (Tianjin, China), $Mw = 4.3 \times 10^5$, $Mn = 2.9 \times 10^5$, $Mw/Mn = 1.49$ (obtained by G.P.C. in chloroform at 30 °C). It was purified by precipitation in *n*-hexane from chloroform solution, subsequently precipitated in methanol from chloroform solution. The sample of PEG was purchased from Tianjin TianTai Chemical Company (Tianjin, China, $Mn = 6 \times 10^3$), recrystallized from acetone and vacuum dried. Chloroform, *n*-hexane, methanol, ether was AR grade and used without further purification.

2.2. Preparation of PHB/PEG blend films

All PHB/PEG blend films were prepared by solution-cast technique using chloroform as the co-solvent. The PHB and PEG were dissolved separately in chloroform before blending. Since PHB is insoluble in chloroform at room temperature. An autoclave was used to stir it for 150 min at 70 °C. The polymer concentration of each solution was 30 g/l. After the PHB and PEG solutions were well homogenized, they were mixed and stirred for 12 h. The mixed solution was dipped on a poly(tetrafluoroethylene) dish, then left at room temperature for 48 h and subsequently dried at 60 °C for further 120 h under vacuum to eliminate the solvent completely. The blend composition was denoted as wt % and given in Table I.

2.3. Water contact angle measurement

The measurement of contact angle was performed at 25 °C in the range of 0.5–20 min by pendant drop method, employing a contact-angle measurement apparatus (type DSA-10, made in KURSS Company, Germany). The static contact angle was measured at contact time $t = 30$ s. Drops of liquid (1.5–2.0 mm diameter) were prepared with a microsyringe and were dropped onto the surface of polymer films. For each sample, the mean of five separate points was obtained based on the same contact time.

2.4. Evaluation of blood-compatibility

2.4.1. Blood coagulation time on polymer surface

The coagulation of blood on the polymer materials was investigated by the following method. This is known as the Lee–White method [28]. The test polymers were coated on the inside of glass test tubes (10 mm in i.d., 10 cm in length) by a solvent evaporation method. Five milliliters of rabbit whole blood without anticoagulant were introduced in the polymer-coated test tube, and the samples were shaken gently in a water bath at 37 °C. The time was measured until the blood fluidity disappeared. The experiments were carried out three times for each sample, and the mean value of each polymer was indicated. The hydrophilic control glasses were prepared by clearing glass microscope slides with detergent, and the hydrophobic control glasses were prepared by siliconizing the microscope slides.

The siliconizing process follows as below: pipetting dimethyl dichlorosilane (Sigma Chemicals) into the glass container, and ''roll'' it around to coat all surfaces. Tip off excess liquid and leave to dry in a safety cabinet to retain sterility. Wash in PBS three times, dry and sterilize by autoclaving.

2.4.2. Platelet adhesion experiments

Rabbit platelet-rich plasma was obtained from blood drawn by venipuncture into centrifuge tubes containing sodium citrate solution (9 : 1). Platelet-rich plasma was prepared by centrifuging at 800 rpm for 20 min at room temperature.

Platelet adhesion experiments were carried out at room temperature. Equilibrated with phosphate buffered saline (PBS; pH 7.4) for 1 h, a drop of platelet-rich plasma was placed on the surface of the PHB/PEG blend films prepared above. It was cultured in incubator at 37 °C for 1 h, then rinsed with phosphate buffered saline (PBS; pH 7.4) three times. The attached platelets were fixed with 2.5% glutaraldehyde buffer solution (pH 7.4) for 30 min at room temperature. The membranes were rinsed in distilled water and dehydrated by immersing them in increasing concentrations of ethanol (10, 30, 50, 60, 70, 80, 90, 95 and 100%) for about 15 min at each concentration. The platelets-attached surface were gold deposited in vacuum and examined by a scanning electron microscope SEM (Philips XL-30).

For each material, three separated experiments were carried out; and for each experiment three samples were conducted. Four different fields were randomly counted and values were expressed as the average number of adhered platelets per m^2 of surface.

2.5. Evaluation of cell-compatibility

2.5.1. Preparation of samples

The PHB/PEG blend films prepared previously were cut into 1 cm × 1 cm squares. These films were sterilized by gamma irradiation.

Chinese Hamster Lung cells (CHL), which have been used in many research fields such as chromosome mutation [29] and DNA fingerprint analysis [30] etc, were used in this study and were cultured in Dulbecco's

modified Eagles medium (DEME/F-12), supplemented with gentamycin (50 μ g/ml), 10% fetal calf serum (FCS) and 5.6% sodium bicarbonate adjusted to pH 7.0–7.4.

2.5.2. Cell culture

The square films of 1 cm × 1 cm and 300 μm in thickness were placed in the bottom of each well of a 24-well tissue-culture plate. Single-cell suspensions were added to the polymers at a density of 1.5×10^4 cells/ml; and the polymer membranes were incubated in 1 ml medium for 24 h at 37 °C/5% CO_2.

2.5.3. Determination of cell adhesion and growth

For the measurement of cell adhesion, cells were washed twice with PBS to remove the non-adhered cells and the attached cells were fixed with 2.5% glutaraldehyde buffer solution (pH 7.4) for 30 min at room temperature. Furthermore, the membranes were rinsed in distilled water and dehydrated by immersing them in increasing concentrations of ethanol (10, 30, 50, 60, 70, 80, 90, 95 and 100%) for about 20 min at each concentration. For each experimental value, three independent experiments were conducted.

The cell morphology on polymer surface was observed by scanning electron microscope SEM (Philips XL-30), and the cell number, at eight random fields, was counted visually.

2.6. Biodegradation test

The enzymatic degradation of the PHB/PEG blend films was carried out at 37 °C in 0.1 M phosphate buffered saline (PBS; pH 7.2–7.4) containing lysozyme (Sigma, 0.2% solution in PBS). The dried films were cut into squares and incubated in the reaction solution with shaking, and the samples were removed at various time points, washed in distilled water and allowed to dry in air to constant weight. For each polymer sample, three films were used and the degradation rate was determined by the ratio of the weight loss to the initial weight of samples as shown below.

$$S = \frac{W_0 - W_t}{W_0} \times 100\% \qquad (1)$$

where S is the degradation rate, W_t and W_0 are the weight of samples after dried and the initial weight respectively.

These degraded samples were coated with gold and observed under SEM.

3. Results and discussion

3.1. Hydrophilicity of PHB/PEG blend films

The surface energy of the solid can be estimated by contact angle measurement, together with a theory of intermolecular forces. In this experiment, the drop contour analysis is used for determining the surface tension and the contact angle. The water contact angle and surface tension of the PHB/PEG films are shown in Fig. 1. With the increase of PEG content in blend films, the water contact angle decreases, but the surface tension increased, as seen in Fig. 1. For pure PHB, the water contact angle value is 64.6°, while with the PEG content increased to 80% the water contact angle value lowered to 34.8°. Dissimilarly, the surface tension value of pure PHB is 26.52 mN/m^2. With the PEG incorporation up to 80% the surface tension value of blend film is up to 41.32 mN/m^2. These results indicate that with the increase of PEG content the PHB/PEG film became more and more hydrophilic. Such a result is probably accounted that the specific interaction involves the carbonyl group of PHB and the –OH in PEG [31, 32].

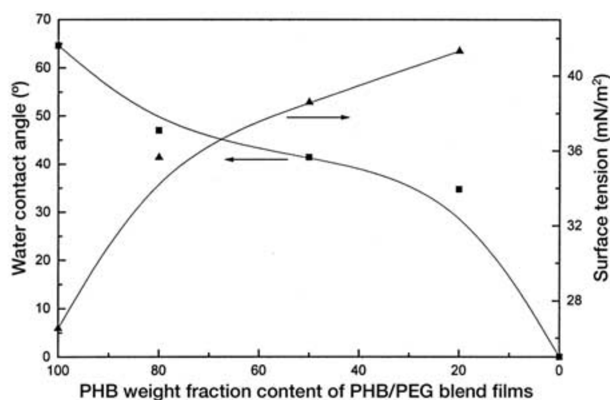


Figure 1 Water contact angle and surface tension of the PHB/PEG blend films.

3.2. Blood-compatibility

Blood-compatibility was evaluated by means of platelet clotting time test and exploring its morphological changes. It had been shown that the clotting times of all PHB/PEG blend films were longer than that of reference glass. With the PEG content increasing, the clotting time became longer; and as to PHB50 and PHB20 the clotting time was even longer than that of silicon (seen in Fig. 2).

Fig. 3 shows the SEM pictures of platelets attached to the surfaces of the films after 1 h incubation at 37 °C. The number of platelets adhered on the film surface decreases with increasing PEG content. As shown in Fig. 3, the shape of platelet changed obviously on glass, thrombus were formed locally. While all platelets on PHB and PHB/PEG films were in discrete state, no pseudopodium

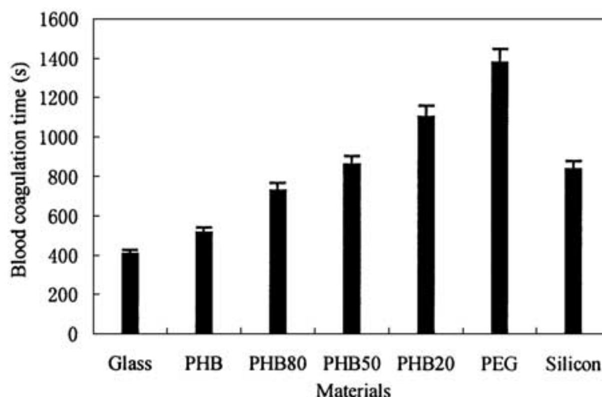


Figure 2 Test of clotting time ($n = 3$).

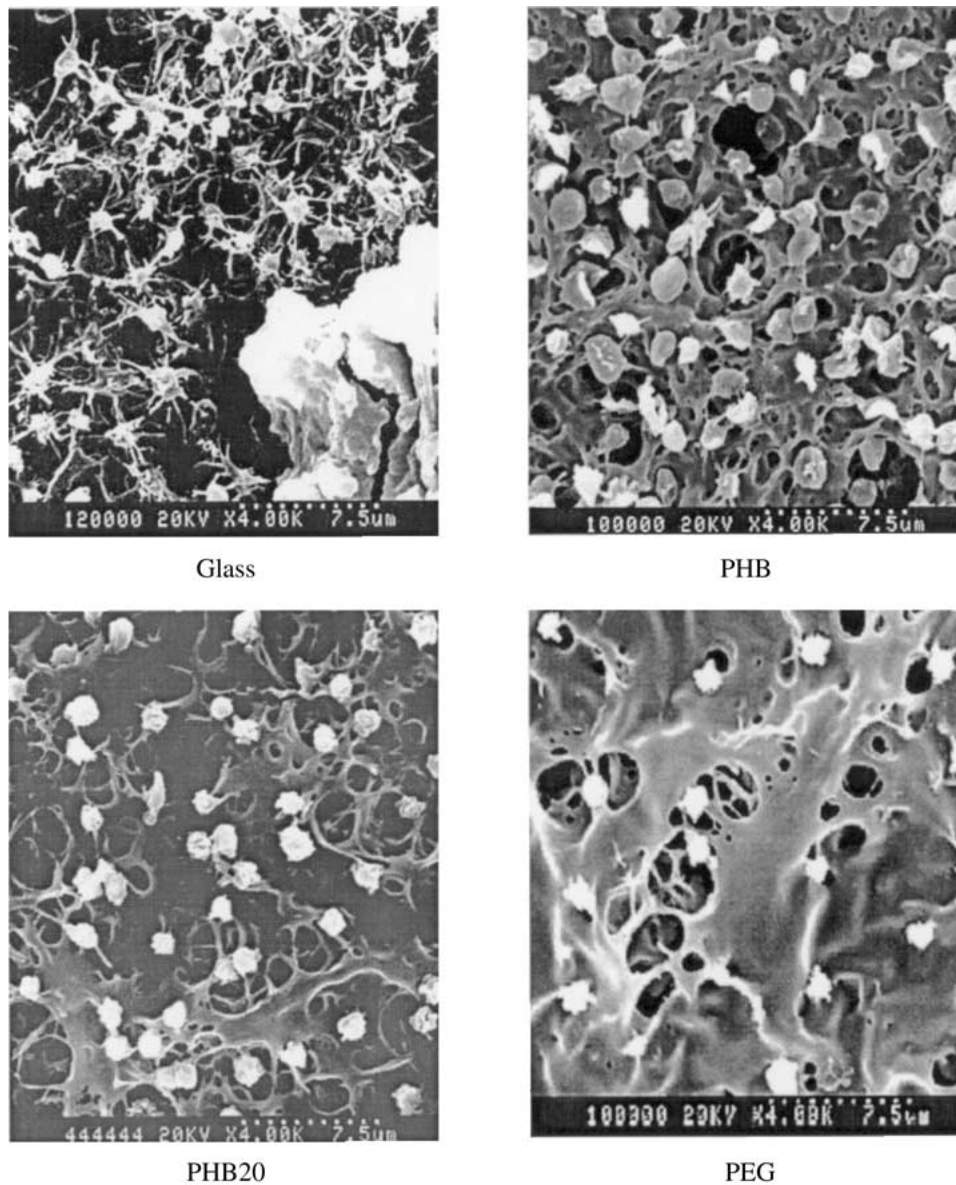


Figure 3 The morphology of platelets cultured on the surface of the materials for 1 h at 37 °C.

had been found and no collective phenomenon had happened. Seen from Table II, with the addition of PEG increased, the clotting time became longer and the number of platelets adhered decreased apparently. The film surfaces containing PEG were effective for the prevention of platelet adhesion. Possible explanations include its hydrophilicity, high surface mobility and its minimum surface tension with water, as reported previously [33]. In conclusion, the addition of PEG played an important role in improving the blood-compatibility.

3.3. Cell-compatibility

Cell cytotoxicity testing is one of the important factors that affected the use of polymers in tissue engineering [34]. In this study, CHL fibroblast was used to evaluate PHB-based membranes cell-compatibility via cell culture *in vitro*. As shown in Table III, live cell adhered on the surface of membrane increases as the PEG content increases in the blend films. In general, cells grow on a layer of protein that interacts with cellular receptors and the hydrophilic surface is favorable to adhesion and growth of cell [35]. As the PEG content increases, the

TABLE II The number of platelets adhered on surface of films after incubation for 1 h at 37 °C and its morphology ($n = 3$)

Materials	Number of platelets ($10^8/m^2$)[a]	Morphology
galss	—	Platelets aggregate thrombus formed locally
PHB	834 ± 67	Discrete state, some platelets with pseudopodium
PHB80	576 ± 52	Discrete state, few platelets with pseudopodium
PHB50	219 ± 24	Discrete state, no pseudopodium
PHB20	180 ± 16	Discrete state, no pseudopodium
PEG	Barely	—

[a]The number of platelets adhered on surface of materials was calculated by geometrical average.

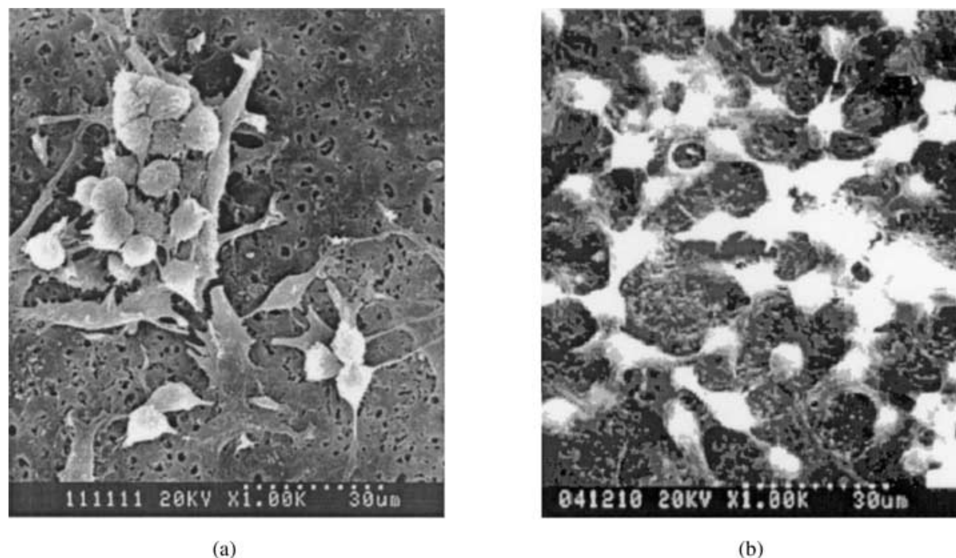


Figure 4 SEM of CHL fibroblasts cultured on PHB-based films *in vitro* for 24 h at 37 °C. (A, CHL cell cultured on PHB; B, CHL cell cultured on PHB50).

TABLE III The number of live cells adhered on surface of materials after incubation for 24 h at 37 °C ($n = 3$)

Materials	Number of live cells ($10^8/m^2$)[a]
PHB	13.10 ± 1.36
PHB80	15.26 ± 1.47
PHB50	20.32 ± 1.94

[a]The number of live cells adhered on surface of materials was calculated by geometrical average.

blend films became more hydrophilic and easy to adsorption proteins [36].

Fig. 4 shows the morphology of CHL cultured in PHB-based films at 37 °C for 24 h. As seen in Fig. 4, the newly developed biodegradable PHB50 film exhibited favorable cell-compatibility. Cells cultured on the matrix spread and maintained their phenotype. The cellular interaction between the cells and the polymer was inseparable. This indicates that PHB has good cell-compatibility, and modification by PEG can improve its cell-compatibility [37].

3.4. Biodegradability of PHB/PEG films

It has been reported that PHB can be degraded by many enzymes, for example, depolymerase from *Alcaligenes faecalis* T1 [38], *Pseudomonas lemoignei* [39] etc. *In vivo*, enzyme such as lysozyme may also accelerate the degradation rate of PHB. In this study, degradation rate of PHB/PEG blend films was tested by measuring the weight loss as they were degraded by PBS/lysozyme buffer solution (pH: 7.2–7.4) at 37 °C and the results were plotted in Fig. 5. Pure PHB is degraded with a relatively low biodegradation rate in the scale of the experiment. After degradation for 25 days, the weight loss ratio is less than 5% of original weight, which is much faster than that of non-ion water as reported previously [40, 41]. Blending with the PEG, the degradation rate of blend films improves greatly. The reason is perhaps due to the hydrophilicity of PEG, which make the surface PEG molecules dissolved in water. Under a scanning microscope, it can be seen that the

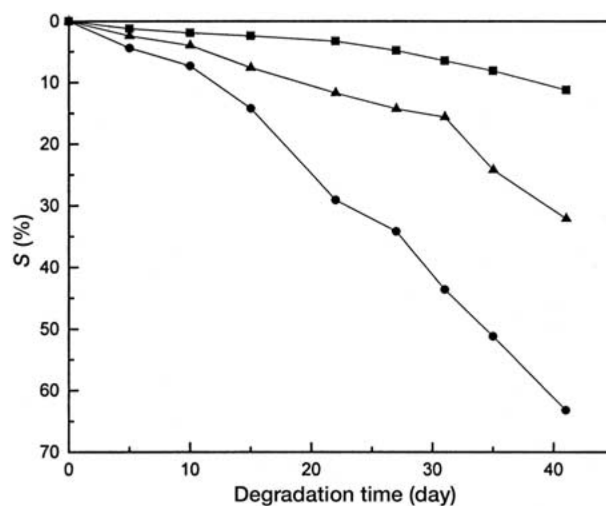
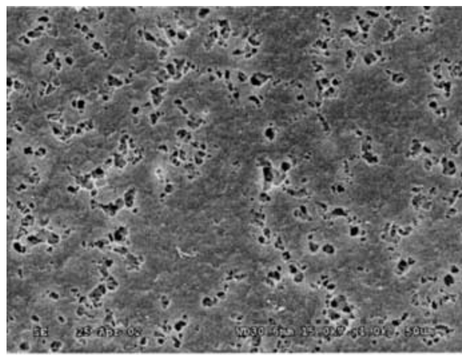


Figure 5 The biodegradation test of PHB/PEG blend films in PBS/ lysozyme buffer solution at 37 °C (■), PHB; (▲), PHB80; (●), PHB50.

surface of PHB/PEG blend films turns porous and phase separated, which is very favorable for enzyme attacking the PHB chain, due to the dissolved surface PEG molecules (seen in Fig. 6(b)). With the PEG content increasing in blend films, this tendency becomes more significant. For PHB50, after degradation for 41 days, the weight loss is about 63%, in which most of the weight loss is due to the dissolving of PEG. However, with the PEG dissolving, more specific areas that can be attacked by enzyme are produced, which in turn accelerates the biodegradation course.

4. Conclusion

The blood-compatibility of PHB/PEG blend films was evaluated by the test of clotting time and platelet adhesion. The results show that with the increase of PEG content the clotting time becomes longer and the number of platelet adhered on the surface of films decreases remarkably. At the same time, the decreasing of contact angle after PEG incorporation results in an increased fibroblast. All these results indicate that the


(a)

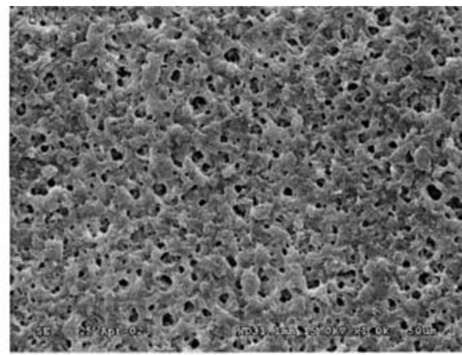

(b)

Figure 6 SEM of the surface of PHB/PEG blend films after degradation in PBS/lysozyme buffer solution at 37 °C for 15 days, (a), PHB; (b), PHB80.

biocompatibility of PHB has been improved with the addition of PEG. The degradation experiment indicated that the degradation of PHB/PEG blend films was accelerated by enzyme *in vitro* and the porous configuration was favorable to its degradation.

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References

1. S. F. EL-AMIN, M. ATTAWIA, H. H. LU, A. K. SHAH, R. CHANG and N. J. HICKOK, *J. Ortho. Res.* **20** (2002) 20.
2. X. B. YANG, H. I. ROACH, N. M. P. CLARKE, S. M. HOWDLE, R. QURIK, K. M. SHAKESHEFF and R. O. C. OREFFO, *Bone* **29** (2001) 523.
3. V. SALDANHA and D. A. GRANDE, *Biomaterials* **21** (2000) 2427.
4. G. ZUND, Q. YE, S. P. HOSRSTRUP, A. SCHOEKERLEIN, A. C. SCHMID and J. GRUNENFELDER, *Eur. J. Cardio-Thoracic Surg.* **15** (1999) 519.
5. G. ZUND, C. K. BREUER, T. SHINOKA, P. X. MA, R. LANGER, J. E. MAYER and J. P. VACANTI, *ibid.* **11** (1997) 493.
6. E. A. DAWES and P. J. SENIOR (eds), ''Advances in Microbid Physiology'', vol 10 (London, 1973).
7. YAMANE, K. TERAO, S. HIKI and Y. KIMURA, *Polymer* **42** (2001) 3241.
8. X. S. YANG, K. ZHAO and G. Q. CHEN, *Biomaterials* **23** (2002) 1391.
9. N. C. FORSTER, H. POMMERENKE, G. HULDA, D. BEHREND and V. BERNEWSKI, *ibid.* **22** (2001) 2425.
10. MIGUEL, M. J. FERNANDEZ-BERRIDI and J. J. IRUIN, *J. Appl. Polym. Sci.* **64** (1997) 1849.
11. P. J. HOCKING and R. H. MARCHESSAULT, *Macromolecules* **36** (1996) 2472.
12. S. Y. LEE, *TIBTECH* **14** (1996) 431.
13. M. ASANO, M. YOSHUDA, H. OMICHI, T. MASHIMO, K. OHABE, H. YUASA, H. YAMANAKA, S. MORIMOTO and H. SAKAKIBARA, *Biomaterials* **14** (1993) 797.
14. H. ABE and Y. DOI, *Macromol. Rep.* **29** (1992) 229.
15. W. P. COLIN and A. SAGHIR, *Adv. Drug Delivery Rev.* **18** (1996) 133.
16. SAGHIR, W. P. COLIN, J. N. LIDIA, *Polymer* **23** (1992) 117.
17. B. W. WAGENAAR and B. W. MULLER, *Biomaterials* **15** (1994) 49.
18. J. N. BASPIST and J. B. ZIEGLER, Method of Making Absorbable Surgical Sutures from Poly(Hydroxyacid), *Eur. Pat. Appl.* E.P No. 46334, 1982.
19. N. GALEGO, C. NOZSA, R. SANCHEZ, J. FUNG and V. ANALIA, *Polymer Testing* **19** (2000) 485.
20. LOOTZ, D. BEHREND, S. KRAMER, T. FREIER, A. HAUBOLD, G. BENKIEBER and K. D. SCHMITZ, *Biomaterials* **22** (2001) 2447.
21. N. R. BOEREE, J. DOVE and J. J. COOPER, *ibid.* **14** (1993) 793.
22. Y. KINOSHITA, M. KIRIGAKUBO, M. KOBAYASHI, T. TABATA, K. SHIMURA and Y. IKADA, *ibid.* **14** (1993) 729.
23. D. E. T. TANNER and W. BONFIELD, *ibid.* **12** (1991) 841.
24. P. J. HOCKING and R. H. MARCHESSAULT, in ''Chemistry and Technology of Biodegradable Polymers'', edited by G. J. L. Griffin (Chapman and Hall, London, 1994) chap. 4, p. 48.
25. J. P. VACANTI, J. E. MAYER and R. LANGER, *Ann. Thorac. Surg.* **60** (1995) 513.
26. J. M. HARRIS (ed.), ''Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications'' (Plenum Press, New York, 1992).
27. S. W. SHALABY, Y. IKADA, R. LANGER and J. WILLIANS (eds.), ''Polymer of Biological and Biomedical Significance'' (Ameriacn Chemical Society, Washington, DC, 1994) pp. 135–146.
28. K. ISHIHARA, H. OSHIDA, Y. ENDO, T. UEDA, A. WATANABE and N. NAKABAYASHI, *J. Biomed. Mater. Res.* **26** (1992) 1543.
29. T. MATSUSITA, H. NAKAMICHI, S. ITOH and H. SUMITOMO, *Environ. Eng. Res.* **34** (1997) 29.
30. T. KIKUNO, M. HONMA, S. OGURA, H. MIZUSAWA, M. HAYASHI and T. SOFUNI, *Mutation Res.* **338** (1995) 87.
31. M. AVELLA and E. MARTUCELL, *Polymer* **29** (1988) 1731.
32. M. AVELLA, E. MARTUCELL and P. GRECO, *ibid.* **32** (1991) 1647.
33. J. H. LEE, Y. M. JU and D. M. KIM, *Biomaterials* **21** (2000) 683.
34. S. SABITA and D. G. STEPHEN, *ibid.* **11** (1990) 133.
35. S. M. KUO, S. W. TSAI and L. H. HUANG, *Art. Cell Blood Subs. Immo. Biotech.* **25** (1997) 551.
36. T. CHANDY and P. SHARMA, *Biomater. Artif. Cells. Artif. Organs.* **18** (1990) 1.
37. N. GURAV, S. DOWNES, *J. Mater. Sci.: Mater. Med.* **5** (1994) 784.
38. T. TANIO, T. FUKUI, Y. SHIRAKURA, T. SAITO, K. TOMITA, T. KAIHO and S. MASEMUNE, *Eur. J. Biochem.* **124** (1982) 71.
39. M. SCANDOLA, M. L. FOCARETE, M. GAZZANO, A. MATUSZOWIC, W. SIKORSKA, G. ADAMUS, J. BARANOWSKA, S. SWIERCZEK, M. GNATOWISKI, P. KURCOK, M. KOWALCZUK and Z. JEDLINSKI, *Macromolecules* **30** (1997) 7743.
40. J. C. KNOWLES and G. W. HASTINGS, *Biomaterials* **12** (1991) 210.
41. S. J. HOLLAND, A. M. JOLLY, M. YASIN and B. J. TIGHE, *ibid.* **8** (1987) 289.

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