Journal of Applied Polymer Science

Electrospinning of Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) Nanofibers with Feature Surface Microstructure

Dongzhi Yang, Jianfeng Zhang, Jie Xue, Jun Nie, Ziping Zhang
Key Laboratory of Chemical Resource Engineering, Key Laboratory of Carbon Fiber and Functional Polymers, Ministry of Education, Beijing University of Chemical Technology, Beijing, China
Correspondence to: D. Yang (E-mail: yangdz@mail.buct.edu.cn)

ABSTRACT: The development of surface microstructure with specific features in electrospun nanofibers has attracted more and more attention in recent years. In this article, a common biological polyester, poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) was electrospinning into nanofibers with "coral-like" surface microstructure by a conventional-electrospinning setup. The effect of the process parameters on the microstructure in electrospun nanofibers were investigated via a series of experiments. The formation mechanism of this feature structure and cytotoxicity assays of PHBV membrane were also discussed. The water contact angle of the electrospun PHBV membrane was higher than that of the PHBV cast film due to a very-rough fiber surface including porous beads when PHBV was electrospun from the concentration of 4 wt %. Because of special hole shape and size distribution, the physical structure of surface of PHBV electrospun fibers offered it special properties, such as specific-surface area, hydrophilic–hydrophobic properties, adhesion properties of cells and biological substances, etc. The demonstrated method of form coral structure would contribute to the areas such as filtration, sensor, tissue engineering scaffolds, and carriers of drugs or catalysis. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: electrospinning; microstructure; PHBV; 'coral-like' surface

Received 9 June 2011; accepted 9 March 2012; published online
DOI: 10.1002/app.37653

INTRODUCTION

Electrospinning is currently the only technique that allows the fabrication of continuous fibers with diameters down to a few nanometers.[1] Electrospinning generally afford fibers with smooth surface, while feature surface structures could be obtained in exceptional case. Nanofibers with feature surface microstructures such as bead,[2,3] porous,[4–7] and failure structures[8,9] are paid much attention because of their attractive and unprecedented properties, which cannot be achieved with macroscopic fibers. The feature surface structures would promise potential application in medical and industrial areas.

In previous researches, several techniques were developed for the surface microstructures. The bead-to-fiber transition was systematically investigated; the parameters such as polymer molecular weight, concentration, and solvent property were considered to be the most important influencing factors.[3] In general, beaded fibers have been considered as undesirable or defective products. However, a previous study reported that beaded fibers have increased hydrophobic property.[10] Especially, porous-beaded fibers have superhydrophobic properties. Superhydrophobic surfaces have important technical applications ranging from self-cleaning window glasses, paint, and fibers to low-friction surfaces. The superhydrophobicity can be adjusted by varying the electrospinning conditions and concentration of polymer solutions.[11] Nanofibers with porous structures could be produced by a rapid phase separation induced by the evaporation of the solvent and a subsequent rapid solidification,[5] and they found that electrospun fibers with porous structure will directly yield by choosing appropriate spinning parameters and solvent. Porous structures could be also obtained by electrospinning the fibers into liquid nitrogen before reaching the collector. The remaining solvent is frozen along with the polymer. In the freezing process, phase separation into solvent-rich and solvent-poor regions was induced. By controlling the method, the solvent was evaporated, a porous morphology can be easily obtained.[6] Megelski et al.[12] fabricated electrospun fibers with porous morphology using different high-volatility solvent, and they obtained high-surface area through the introduction of a micro- and nanostructured surface structure. Fail structures of nanofibers were significantly different from macroscopic fibers. Multiple necking and ripple structures were generated by inducing tensile stress to the fibers.[7,8]

Applied Polymer SCIENCE

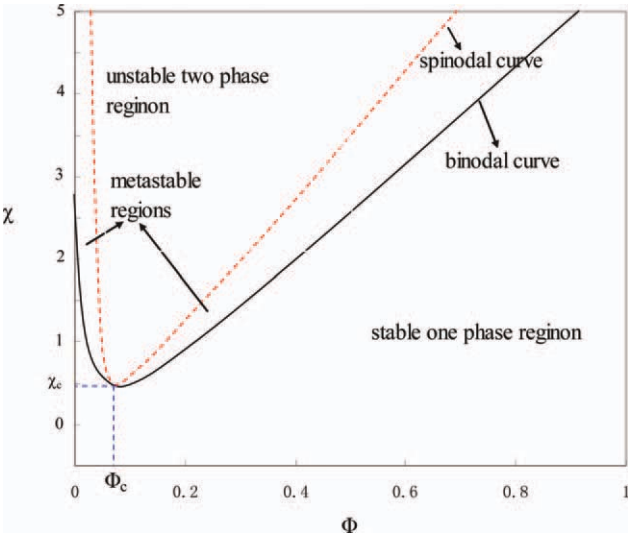

Figure 1. Phase diagram of polymer solution. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) is a complete biodegradable, good biocompatible, and thermoplastic polyester, which can be biosynthesized by various microorganisms, it has received much attention as a biomedical material. Such as the porous PHBV materials were used for tissue-engineering scaffold. Recently, some researchers were researched the effect of operation conditions on the morphology and structure of PHBV electrospinning fibers, or trying to use the electrospinning PHBV film to prepare microporous tissue-engineering scaffolds.[13–15]

In this article, a simple and versatile method was demonstrated to prepare porous "coral-like" surface structure on the PHBV-beaded nanofiber. And this feature microstructure could be controlled by the parameter including applied voltage, concentration, and temperature, etc. Formation mechanism of this coral structure was also interpreted preliminary by a phase diagram of polymer solution.

EXPERIMENTAL

Electrospinning

PHBV, M_w = 1,000,000, was prepared into homogeneous solution with the concentration 4–16 wt % (6 wt % increment) by dissolving it in chloroform. During electrospinning, the solution was continuously pushed at by a syringe pump (WSZ-50FZ, ZHEJIANG University Medical Instrument); the feed rate was 0.12, 0.24, 0.48, and 0.50 mL/h. To investigate the effect of collect distance and applied voltage, the collect distance was varied from 15 to 30 cm with 5 cm increment, and the applied voltage ranged over 15–25 kV. The electrospinning schematic diagram of the setup was shown in Figure 1, the electrospinning device with a thermocouple, heating, and ventilation device, so which was temperature controllable (25, 30, and 35°C). The surface microstructure of electrospun nanofibers was characterized using a field-emission scanning electron microscope (FE-SEM, S-4700, Hitachi).

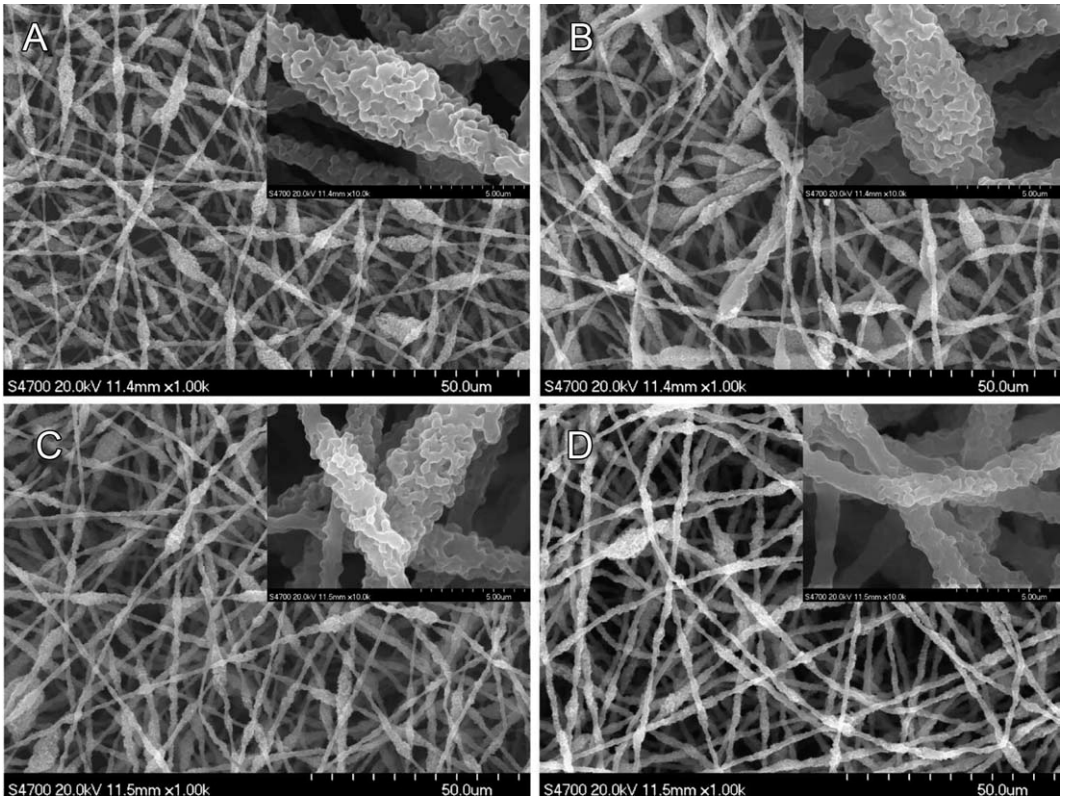

Figure 2. SEM images of PHBV nanofibers, which were spun at different applied voltage. (A) 15 kV, (B) 17 kV, (C) 20 kV, (D) 25 kV.

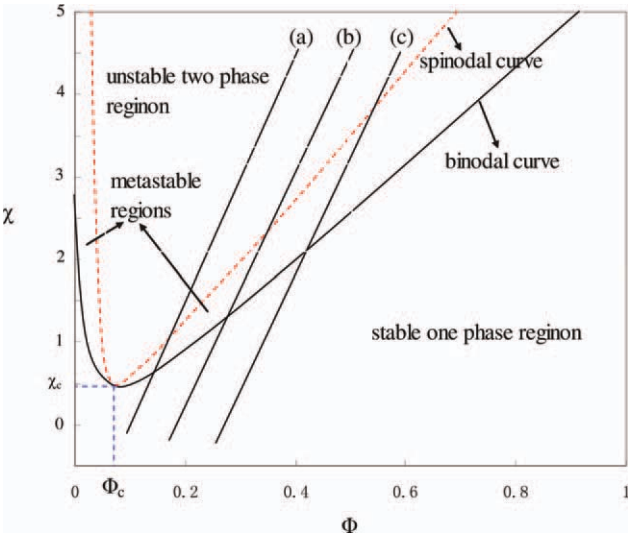

Figure 3. The fraction swept with different initial concentration. (a) 4 wt %, (b) 10 wt %, (c) 16 wt %. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The water contact angle measurements were performed on the cast films and electrospun membrane using a sessile-drop contact angle measuring device (JC2000C1, Powereach Digital Technology, Shanghai, China) with distilled water droplets at room temperature. The water contact angle value for each sample was obtained by averaging at five times.

Cell Test

Cytotoxicity Assays. The cytotoxicity of the electrospinning PHBV films was evaluated by methylthiazolydiphenyl-tetrazolium bromide (MTT) assay based on the ISO10993-5 standard test method. Mouse fibroblast (L929) was cultured in DMEM medium (10% fetal bovine serum, 1.0% penicillin–streptomycin, 1.2% glutamine) under 37°C in 5% CO_2. When the cells reached 70% confluence, they were trypsinized with 0.25% trypsin and 1 m*M* ethylenediamine tetraacetic acid. The viabilities of cells were determined by the MTT assay. For the MTT assay, the PHBV electrospun film were sterilized with highly compressed steam for 15 min and placed in 1 mL of DMEM medium, and then incubated at 37°C for 24 h. The extraction ratio was 6 cm^2/mL. L929 cells were seeded in wells of a 96-well plate at a density of 10^3 cells per well. After incubation for another 24 h, the culture medium was removed and replaced with the as-prepared extraction medium and incubated for another 24, 48, and 72 h, respectively, then 100 μL of MTT solution was added to each well. After 4 h incubation at 37°C, 200 μL of dimethyl sulfoxide was added. The dissolved solution was swirled homogeneously for about 10 min by the shaker. The OD value of the formazan solution was detected by an ELISA reader (Multiscan MK3, Labsystem, Finland) at 490 nm. For reference purposes, cells were seeded to medium containing 0.64% phenol (positive control) and a blank

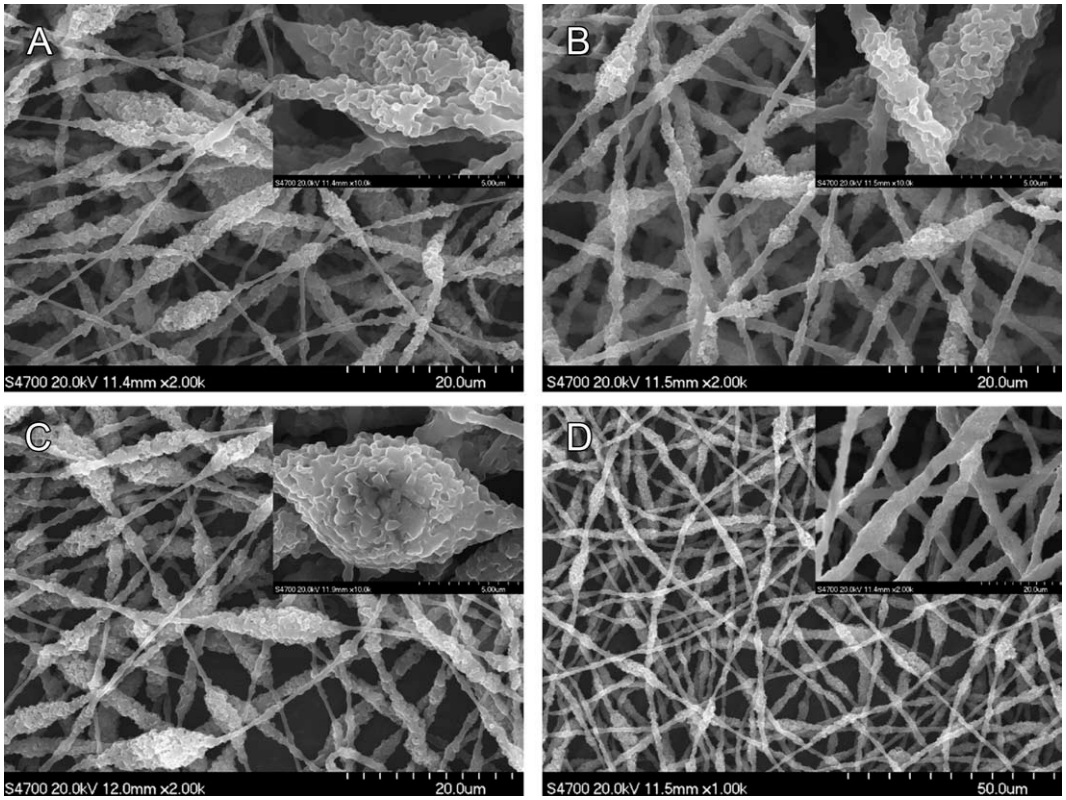

Figure 4. SEM images of PHBV nanofibers which were spun with different collect distance. (A) 15 cm, (B) 20 cm, (C) 25 cm, (D) 30 cm. To observe the precise structure of the electrospinning mats, the SEM images of local areas of the mats with higher magnification was inserted in the SEM micrographs

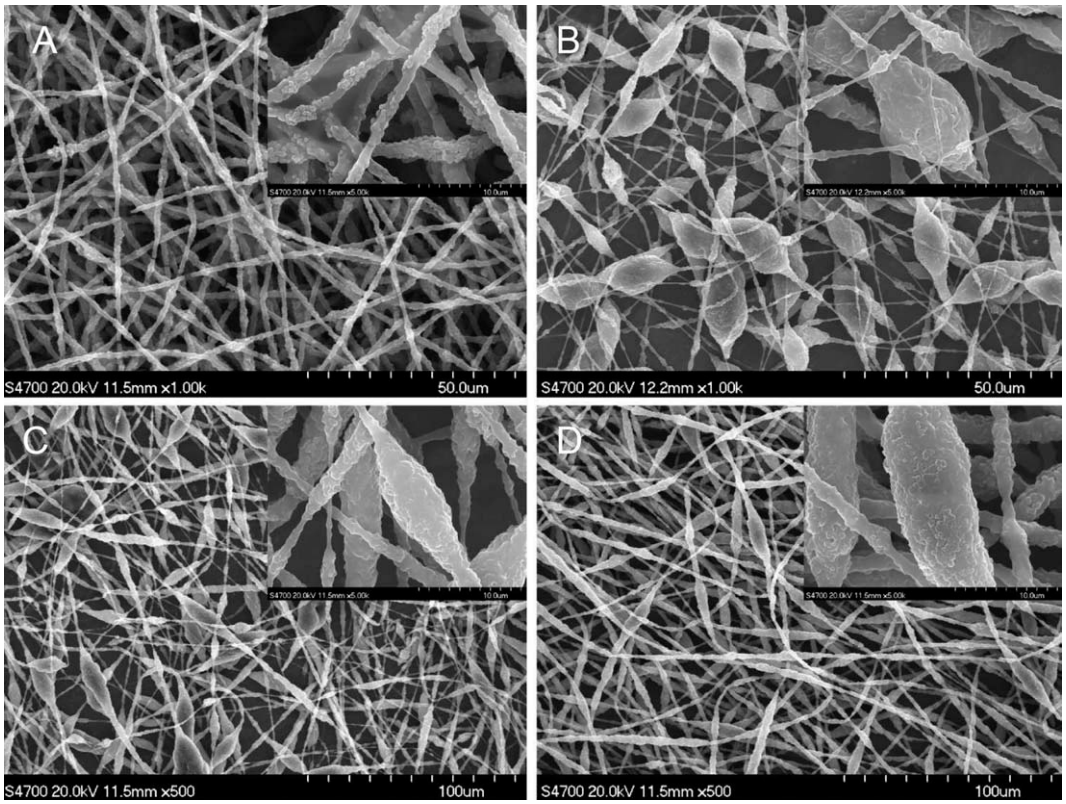

Figure 5. SEM images of PHBV nanofibers, which were spun with different feed rate. (A) 0.12 mL/h, (B) 0.24 mL/h, (C) 0.48 mL/h, (D) 0.50 mL/h.

culture medium (DMEM, negative control) under the same seeding conditions, the each sample was measurement six times.

Morphology Observation. The prepared samples (area 1 cm^2, and thickness 0.1 mm) soaked in the 250 mL phosphate buffer solution (PBS pH = 7.4) for 24 h before sterilization with highly compressed steam for 15 min. Then, the samples were transferred to the 24-well plate. One milliliter of L929 suspension with 1.5×10^4 cells/mL was seeded on the samples. After 48 h of culture, collected cellular was rinsed twice with PBS to remove nonadherent cells. And subsequently fixed by 75% alcohol solution, Hochest staining, the samples were observed by the fluorescence microscope.

RESULTS AND DISCUSSION

The microstructure of the electrospinning nanofibers was governed by the factors of the competition between the rates of phase separation and solvent evaporation, and the process parameters such as applied voltage, feed rate, collect distance, temperature, and concentration.

PHBV Electrospinning

Phase Separation. In the processes of membrane preparation, the formation of nano- and microstructures by phase-separation technique is very well-known and widely discussed. There was a series of phase-separation technique such as (a) immersion precipitation, (b) air-casting of the polymer solution, (c) thermally induced phase separation, and (d) vapor-induced phase separation.[12] An understanding of these techniques may also help to explain the pore formation in electrospun fibers. Of course, the electrospinning process is more complex since the fibers are carrying charge, which is not present in the formation of other porous structures. If a polymer solution (polymer and solvent) is cast on a support, evaporation takes place under convective conditions.

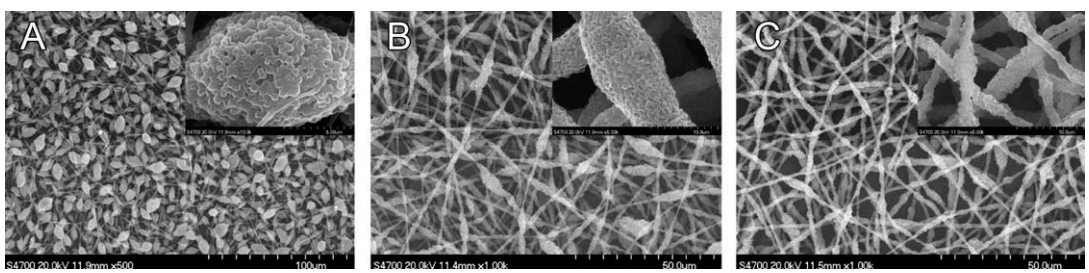

Figure 6. SEM images of PHBV nanofibers which were spun in series concentration. (A) 4 wt %, (B) 10 wt %, (C) 16 wt %.

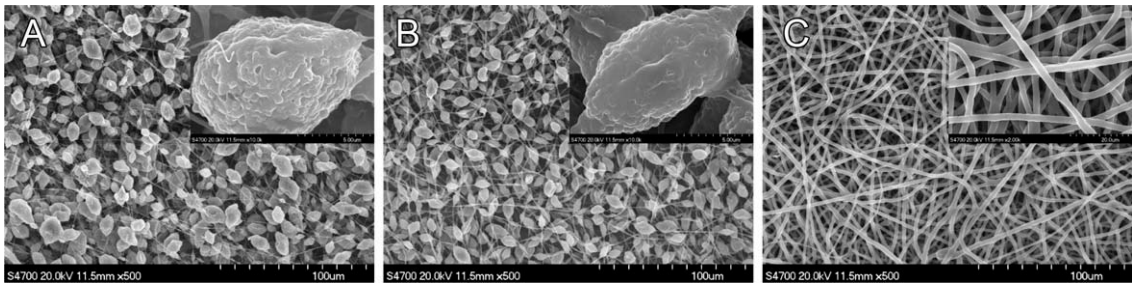

Figure 7. SEM images of PHBV nanofibers, which were spun in series temperature. (A) 25°C, (B) 30°C, (C) 35°C.

During solvent evaporation, the solution becomes thermodynamically unstable and phase separation occurs into a polymer-rich and a polymer-lean phase. The concentrated phase solidifies shortly after phase separation and forms the matrix, whereas the polymer lean phase forms the pores. A phase diagram coupled with thermodynamic and kinetic aspects provides information about structure formation in membranes. All these general aspects may be transferable to the formation of porous polymer fibers.[16]

During the electrospinning process, even if the temperature was kept constant, evaporative cooling occurs as the fiber traverses the distance between the syringe and the collective device. So that the solvent loss and the simultaneous temperature decrease would lead to thermodynamically unstable state of electrospinning solution.

To elucidate the formation of the feature microstructure, a phase diagram of polymer and solution was produced (Figure 1). This phase diagram was characterized by three distinct regions, stable one phase region, metastable region, and unstable two-phase region. The bimodal curve separated the phase diagrams into a single-phase region and a two-phase region. Depending on the thermal quenching or slow cooling into unstable or metastable regions, liquid–liquid-phase separation was known to occur by spinodal decomposition (SD) or by nucleation and growth (NG).[7] And in polymer solutions, the phase diagram was strongly asymmetric with low-critical composition (φ_c) and critical interaction parameter (χ_c) close to 0.5. Empirically, the temperature dependence of the Flory interaction parameter (χ) is often written as the sum of two terms:

$$\chi(T) \cong A + B/T \quad (1)$$

where A is referred to as the "entropic part" of χ, and B is called the "enthalpic part." In the PHBV solutions, $B > 0$, the χ decreased as the temperature was raised. During the electrospinning process, the composition (φ) raised rapidly with the evaporation of the solvent, and the temperature (T) declined synchronously. Then, the NG occurred when the solution passed through the binodal curve of the phase diagram from stable one phase region to metastable regions. In the process of NG, the phase separation was initiated by the nucleation event in supercooled state, which contributed to the bead structure. With the further change of φ and T, phase separation would continue with the process of SD. The formation of "coral-like" microstructure could be due to the process of SD.

Applied Voltage. The SEM images in Figure 2 demonstrated that the PHBV nanofibers were spun in different applied voltage (1525 kV) with the same of all other parameter (feed rate 0.12 mL/h, concentration 10 wt %, collect distance 20 cm, temperature 25°C). The distribution of fiber diameter became narrow with the increasing applied voltage. The bead structure almost disappeared when the voltage was 25 kV. However, the surface microstructure would be not affected by varying the voltage.[12] The "coral-like" microstructure on the surface of PHBV nanofibers maintained, shown in the inset of Figure 3. The formation process of this microstructure could be corresponding to the process of (a) in the phase diagram (Figure 3).

Collect Distance. To assess the effect of collect distance on the surface microstructure, the distance between nozzle and collect was narrowed from 30 to 15 cm in 5 cm step, keeping all other parameter constant (feed rate 0.12 mL/h, concentration 10 wt %, applied voltage 20 kV, temperature 25°C). As shown in Figure 4, the "coral-like" surface microstructure was not strongly influenced by collect distance. But the fiber surface was smooth with the increasing of the collect distance. Our interpretation is that there is not enough time to evaporate solvent completely

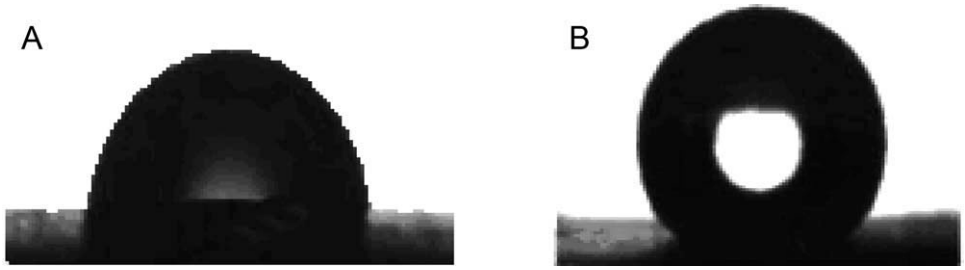

Figure 8. Water contact angle on PHBV cast film (A) and PHBV electrospun film (B).

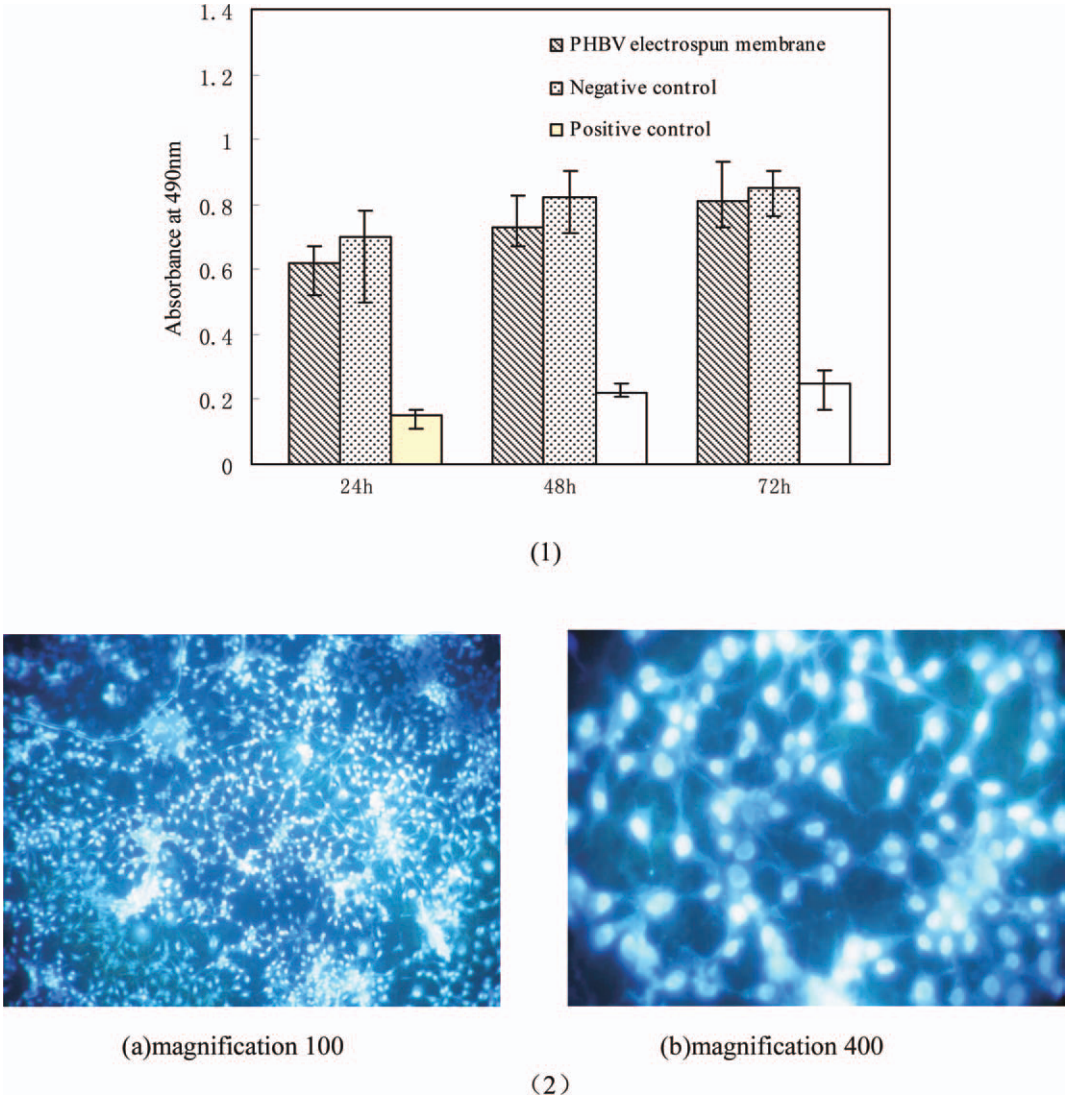

Figure 9. Cytotoxicity test of PHBV electrospinning membrane. (1) MTT test of PHBV membrane with positive and negative controls, $^*P > 0.05$ predicated statistically no significant differences when compared to the negative control of indirect cytotoxicity. The data represented mean and standard deviations of six samples. (2) Fluorescence microscope images of L929 cell seeded on the PHBV membrane. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

from the electrospinning bead-fiber in the shorter receive distance during the electrospinning process. In addition, the decreasing-collected distance resulted that electric-field strength increases, which led to the further increasing of moving rate of the drop, these factors also increased the solvent does not evaporate completely. So that, the shorter-collected distance, the more bead-fibers.

Feed Rate. The feed rate could be controlled by the syringe pump to determine the effect on the fiber microstructure; the feed rate was set at 0.12, 0.24, 0.48, and 0.50 mL/h (concentration 16 wt %, applied voltage 20 kV, collect distance 20 cm, temperature 25°C). Different with the series experiments of applied voltage and collect distance. The "coral-like" microstructure was not significant as before (Figure 5), which may because the higher concentration. And the microstructure formation could be simulated by process (c) in the phase diagram (Figure 3). As the process demonstrated, the SD occurred in a more concentrated solution; the motion of polymer chains would be more difficult. However, the effect of feed rate on the bead structure had been system discussed by Zuo.[17] Bead structure would be eliminated in a proper feed rate.

Concentration. The concentration of PHBV solution was varied from 4 to 16 wt %. All the other parameters were kept constant (feed rate 0.12 mL/h, applied voltage 20 kV, collect distance 20 cm, temperature 25°C). The electrospun nanofibers were shown in Figure 6. It could be found that the "coral-like" surface microstructure was razed, and the bead structure was disappeared with the increasing of concentration. The trends could be explained by phase diagram (Figure 4). As the discussion in

the part of phase separation, with the elevation of the solute fraction and χ, phase separation occurred in binodal curve contributed to bead structure, and the phase separation occurred in the spinodal curve contributed to the structure of coral-like. For the higher initial concentration of electrospinning solution, the phase separation was difficult to occur, so the coral-like structure did not appear. And the mobility was inversely proportional to the solution concentration, so that, the structure of bead structure also would be difficult to form.

Temperature. Temperature was an important factor, which would influence the phase separation process. As eq. (1) demonstrated, χ was inversely proportional to T, which meant that χ increased as temperature was decreased. Phase separation occurs by lowering the temperature of the solution. The solution then passes through the binodal curve of the phase diagram to enter the metastable region. Amorphous or semicrystalline polymers are known to produce microporous structures by liquid/liquid phase separation.

The PHBV nanofibers shown in Figure 7 were obtained under different temperature (25, 30, 35°C) by keeping all other parameters constant (feed rate 0.50 mL/h, concentration 16 wt %, collect distance 20 cm, applied voltage 20 kV). It was found that the surface of electrospun nanofibers was smooth with the increasing of temperature. And the bead structure was disappeared when temperature was at 35°C. When the solution was spun in lower temperature (25 and 30°C), the loss of solvent was finite. The process of NG would contribute to a bead structure, and the "coral-like" surface microstructure was also appeared, shown in the inset of Figure 8. The NG was followed by the process of SD, which contributed to the structure of coral-like. As the spun temperature was elevated, the saturated-vapor pressure of solvent greatly reduced. Therefore, the solvent is almost completely vaporized in a very-short distance and time, there is not enough time to form NG and SD. So that, polymer matrix was tensed to the smooth fibers.

Contact Angle

Figure 8 showed the contact angle of the PHBV cast film and PHBV electrospun film with same concentration of 4% was 88° and 130°, respectively. PHBV electrospun film had a much higher contact angle due to its surface roughness. On the surface of electrospun film, the bead-fiber structure provided to the macro-scale roughness, at the same time, the porous structure on the beads additionally provided to the micro-scale roughness. The study of Yoon et al. clearly showed that it is possible to obtain a superhydrophobic surface by the CF4 plasma treatment of porous and beaded fibers.[16] Superhydrophobic surfaces have important technical applications ranging from self-cleaning window glasses, paint, and fibers to low-friction surfaces.

Cytotoxicity Assays

The ability to support the attachment and proliferation of cultured cells is a prerequisite of a functional scaffold. To evaluate cellular cytotoxicity, the MTT assays were carried. Figure 9(1) shows that the absorbance illustrating the viability of L929 cells that were cultured with the extraction medium. No statistically significant differences ($P > 0.05$) were observed in the cell activity of L929 culture for 24, 48, and 72 h in the presence of the PHBV membrane extracts in comparison with negative control, and the average absorbance values were lower than that of the negative control, but the cell relative proliferation rate still reached above 90% of that of the negative control at 72 h in Figure 9(1). This indicates that the PHBV nanofibers were less toxic to L929 cells.

The interaction between membrane and tissue cells was observed by fluorescence microscope at two different magnification [100× and 400×; Figure 9(2)], which showed the L929 cells distribution on the surface of PHBV membrane after 48 h culture. For the low magnification images (100×), a good number of cells were attached on the surface of PHBV electrospun fibers scaffold. The high magnification image (400×) showed that the attached cells exhibited the spindle shape, typical of active fibroblastic cells, and normal cell nucleus morphology.

CONCLUSIONS

This study described investigation on the formation of "coral-like" porous beaded fiber surface microstructure of electrospinning PHBV. The feature surface microstructure of "coral-like" was contributed to the two step phase separation (NG and SD). The parameters of applied voltage, feed rate, and collect distance had a small effect on the surface structure but a strong effect on the bead structure. However, the parameters of solution concentration and spun temperature played a decisive role in the formation of the surface porous microstructure. Cell culture results showed that fibrous mats were good in promoting the cell attachment and proliferation. PHBV electrospun matrix would be used as potential as filtration, sensor, tissue-engineering scaffolds, and carriers of drugs or catalysis.

ACKNOWLEDGMENTS

The authors would like to thank the National Natural Science Foundation of China (50803004), Beijing Natural Science Foundation (2112033), Study on the molecular design and properties of new photocurable antifouling coatings), and the Fundamental Research Funds for the Central Universities (ZZ1115) for its financial support.

REFERENCES

1. Greiner, A.; Wendorff, J. H. *Angew. Chem. Int. Ed.* **2007,** *46*, 5670.
2. Larsen, G.; Spreta, R.; Ortiz, R. V. *Adv. Mater.* **2004,** *16*, 166.
3. Eda, G.; Shivkumar, S. J. *Appl. Polym. Sci.* **2007,** *106*, 475.
4. McCann, J. T.; Li, D.; Xia, Y. N. *J. Mater. Chem.* **2005,** *15*, 735.
5. Bognitzki, M.; Czado, W.; Frese, T. *Adv. Mater.* **2001,** *13*, 70.
6. McCann, J. T.; Marquez, M.; Xia, Y. N. *J. Am. Chem. Soc.* **2006,** *128*, 1436.

7. Dayal, P.; Liu, J.; Kumar, S.; Kyu, T. *Macromolecules* **2007,** *40*, 7689.
8. Zussman, E.; Rittel, D.; Yarin, A. L. *Appl. Phys. Lett.* **2003,** *82*, 3958.
9. Mohammad, N.; Loannis, C.; Kahn, H.; Wen, Y.; Dzenis, Y. *Appl. Phys. Lett.* **2007,** *91*, 151.
10. Woodward, I.; Schofield, W. C. E.; Rouconles, V.; Badyal, J.P. S. *Langmuir* **2003,** *19*, 3432.
11. Yoon, Y. I.; Sikmoon, H.; Iyoo, W. S.; Lee, T. S.; Park, W. H. *J. Colloid Interface Sci.* **2008,** *320*, 91.
12. Megelski, S.; Stephens, J. S.; Chase, D. B.; Rabolt, J. F. *Macromolecules* **2002,** *35*, 8456.
13. Choi, J. S.; Lee, S. W.; Jeong, L.; Bae, S. H.; Min, B. C.; Youk, J. H.; Park, W. H. *Int. J. Biol. Macromol.* **2004,** *34*, 249.
14. Suwantong, O.; Waleetorncheepsawat, S.; Sanchavanakit, N.; Pavasant, P.; Cheepsunthorn, P.; Bunaprasert, T.; Supaphol, P. *Int. J. Biol. Macromol.* **2007,** *40*, 217.
15. Kim, Y.-J.; Bae, H.-I.; Kwon, O. K.; Choi, M.-S. *Int. J. Biol. Macromol.* **2009,** *45*, 65.
16. Yoon, Y. I.; Moon, H. S.; Lyoo, W. S.; Lee, T. S.; Park, W. H. *J. Colloid Interface Sci.* **2008,** *320*, 91.
17. Zuo, W. W.; Zhu, M. F.; Yang, W.; Yu, H.; Chen, Y. M.; Zhang, Y. *Polym. Eng. Sci.* **2005,** *45*, 704.