# Photografting Polymerization of Polyacrylamide on PHBV Films (I)

**Yu Ke,[1] Yingjun Wang,[1,2] Li Ren,[1,2] Ling Lu,[1,3] Gang Wu,[1,2] Xiaofeng Chen,[1,2] Jingdi Chen[1]**

[1]*Biomaterial Research Institute, College of Material Science and Engineering, South China University of Technology, Guangzhou, China*
[2]*Key Laboratory of Specially Functional Material and Advanced Manufacturing Technology, South China University of Technology, Guangzhou, China*
[3]*College of Pharmaceutical Science, Southern Medical University, Guangzhou, China*

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**ABSTRACT:** Photografting polymerization of polyacrylamide (PAM) onto poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) films using benzophenone as photoinitiator was studied. The morphology and structure of the grafted PHBV film were characterized by Fourier transformed infrared spectroscopy (FTIR) with attenuated total reflectance (ATR) and scanning electron microscope (SEM) with energy dispersive X-ray spectrometer (EDX). The grafting percentage and grafting efficiency of the grafted PHBV film went up with the increase of acrylamide concentration and irradiation time. It was observed that photografting polymerization of PAM was not only limited to the film surface, but also *in situ* occurred inside the film to form the pore microstructure. Sheep bone marrow stromal cell studies showed that MSCs cells attachment efficiency on the grafted PHBV films increased and cells grew well. These results demonstrated the potentiality of PAM-photografting PHBV in medical applications. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104, 4088–4095, 2007

**Key words:** PHBV; acrylamide; graft copolymers; photopolymerization; biocompatibility

## INTRODUCTION

Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), commonly referred to PHBV, has received considerable attention in recent years as biodegradable biomaterials. PHBV is optical active polyester made by microorganisms via fermentation. Its various properties such as natural origin, biodegradability, biocompatibility, nontoxicity, piezoelectricity, and thermoplasticity make it suitable for a variety of applications in medicine.[1–6]

It may be advantageous for biomaterials to incorporate particular functionality, for example, the ability to induce cell adhesion, spread, migration, or proliferation. A common approach used to promote cell adhesion and proliferation on a foreign polymer surface is to provide a surface close to the natural cell environment. It is known that natural extra-cellular matrix can regulate the cell phenotype.[7,8] Therefore, extra-cellular matrix proteins are immobilized on polymer surface to mimic the natural cell environment on polymer surface. Because PHBV lacks functional groups to covalently anchor extra-cellular matrix protein, several approaches have been tried to perform functionalization of polymer surface. For example, Tesema et al. grafted polymethyl methacrylic acid (PMAA) onto ozone-activated PHBV surface, resulting in an active polymer surface that can immobilize collagen to mimic extra-cellular matrix.[9] Grondahl et al. grafted polyacrylic acid onto PHBV surface through $\gamma$ irradiation and have glucosamine linked to the functionalized surface.[10] Wang et al. have used oxygen and nitrogen plasma to treat PHBV film to get functionalized surface, which promoted cell growth and proliferation.[11]

Photografting polymerization is also a way to introduce functional groups on PHBV surface, which can further immobilize proteins or glucosamine to mimic extra-cellular matrix. Photografting polymerization is widely known to be useful because of its significant advantages: low cost of operation, mild reaction condition, selectivity to absorb UV light, and permanent alteration of substrate chemistry. Hydrophilic monomers, including 2-hydroxylethyl acrylate, acrylamide, and methacrylic acid, have been grafted onto PU and poly(L-lactic acid) through the combination of photooxidization in hydrogen peroxide and subsequent UV-induced grafting copolymerization.[12–14] Photografting polymerization of poly(ethylene glycol) monoacrylate

*Correspondence to:* Y. J. Wang (imwangyj@scut.edu.cn).
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upon premodified poly(tetrafluoroethylene) substrates and PHB was studied, with a view toward applying the grafted materials as biomaterials.[15–17]

Polyacrylamide (PAM) is a water-soluble polymer with reactive amide pendant groups along polymer's main chains, which make it desirable for functionalizing PHBV substrates. Lee and Leet grafted PAM onto PHBV films through chemical initiator at 70°C and tested the permselectivity of the grafted PHBV films.[18] The photografting polymerization of PAM onto PHBV has rarely been investigated.

This work reports the photografting polymerization of hydrophilic acrylamide onto the PHBV film to activate PHBV surface, with the aim to immobilize bioactive materials, such as oligopeptides, proteins, glycosaminoglycan, or growth factors. The effects of the monomer concentration and irradiation time on the grafting percentage (GP) and grafting efficiency (GE) were investigated. Moreover, sheep marrow stromal cells (MSCs) were cultured on the PHBV and grafted PHBV films, aiming to test the biocompatibility of the grafted films primarily.

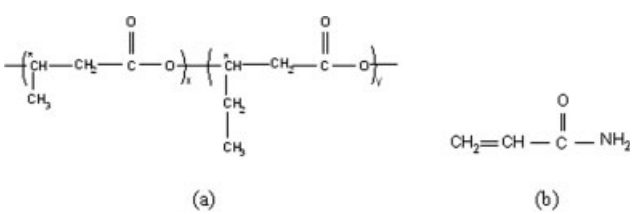
## EXPERIMENTAL

### Materials

PHBV (polyhydroxyvalerate 8% Grade: D 400P) powder was purchased from ICI®. Acetone and chloroform were purchased from Tianjin Chemical Reagent No. 1 Plant, China. Benzophenone (BP) and acrylamide (AAm) were obtained from Shanghai Runjie Chemical Reagent Company, China. BP was recrystallized from ethanol. All other reagents were used as received. The chemical structures of PHBV and AAm were shown in Scheme 1.

### Preparation of PHBV films

PHBV powder was dissolved in chloroform at 80°C at a concentration of 60 mg/mL, a known volume of which was cast on glass plate. After conditioned for 2 days at the room temperature, the film was further dried under vacuum at 60°C to remove the residual chloroform. The PHBV film with thickness of 0.15 mm was obtained and cut into 20 × 20 $mm^2$ pieces. The PHBV films were washed with acetone and then dried to constant weight ($W_0$) before photografting the polymerization.



**Scheme 1** The chemical structures of (a) PHBV and (b) acrylamide.

### Photografting polymerization

After soaked into 5 wt % BP solution with acetone as solvent for 24 h, PHBV films were dried at room temperature to remove acetone before irradiation. The films were then put into an aqueous acrylamide with different concentration contained in a small reactor with a quartz cover. Photografting polymerization was carried out with a high-pressure mercury lamp (Philips 400S) under nitrogen flows for different irradiation time. After reaction, the grafted PHBV films were taken out from the aqueous acrylamide in which homo-PAM was dissolved. The grafted PHBV films were then Soxhelt extracted with acetone for 48 h to get rid of homo-PAM, acrylamide, and BP attached that were easily dissolved in acetone. And the films were further rinsed with deionized water for 24 h, and deionized water was replaced three times in the meantime to get rid of residual PAM homopolymer and acrylamide that were also dissolved in water. The purifying treatment is known to basically remove physically attached homo-PAM from the grafted films. Finally, the grafted films were thoroughly dried under vacuum to a constant weight ($W_1$). The GP and GE were calculated according to the following formula:

$$\mathrm{GP} = (W_1 - W_0)/W_0$$

$$\mathrm{GE} = (W_1 - W_0)/W_2$$

where $W_2$ is the weight of acrylamide used for photografting polymerization. A student's $t$ test was performed in comparing means from two independent sample groups and a significance level of 0.05 was used in all the statistical tests performed. Data were expressed as the mean ± standard deviation of the mean (SD).

### Characterization of PHBV/PHBV-*g*-PAM films

Fourier transformed infrared spectroscopy (FTIR) spectra were recorded on a Bruker (Germany) Vector 33 Infrared Analysis equipped with an attenuated total reflectance (ATR) accessory providing analysis of the surface. Surface and cross section morphologies were observed with a Philips (Holland) XL 30 scanning electron microscope (SEM). An energy dispersive X-ray spectrometer (EDX) being attached to SEM was used to characterize the cross section component qualitatively. After swollen by water for at least 24 h, the PHBV and grafted PHBV films were

frozen at −50°C for 6 h and then freeze-thawed for 2 days. Films for cross section observation were snapped after plunged into liquid nitrogen for about 30 s. The cut samples were mounted on metal stubs and coated with gold through sputter (HITACHI E-1010). Films for surface observation were directly coated with gold without freeze-thawing treatment.

### Cell culture on the PHBV and grafted PHBV films

The PHBV and grafted PHBV films were sterilized by exposure to $H_2O_2$ vapor and washed three times with phosphate buffer solution (PBS), then placed on 96-well plates. Sheep MSCs (passages 3) were seeded evenly onto the PHBV and grafted PHBV films at about 1000 cells/well in DMEM supplemented with 20% fetal bovine serum. The cell-seeded films were maintained at 37°C under 5% $CO_2$ (Thermo Forma, USA). The cell attachment at 0.5 h was determined using a hemacytometer to count the number of cells. At the end of 7 days culture period, the cells on the films were fixed with 2.5% glutaraldehyde and dehydrated by sequential dipping in increasing concentrations of alcohol, and then dried at ambient temperature for SEM observation to visualize the manner of cell attachment.

## RESULTS AND DISCUSSION

### Photografting polymerization

UV spectrum is a requirement for photoinitiator to be activated and initiate the grafting polymerization. BP was used as an initiator in our photografting polymerization, where BP was transferred to an excited state as it absorbed in the UV spectrum, relaxed, and abstracted the tertiary hydrogen from the polymer main chains on the PHBV film surface by inelastic collision. Ketyl radical (BPH*) dimerized, while polymer radical (*P**) formed on the film surface.[19]

Because of high crystallinity of the PHBV films and heterogeneous photografting polymerization system, the formation of polymer radicals is not easily achieved, especially at room temperature. Therefore, photoinitiator preabsorbing treatment was carried out in this work. The polymer radicals formed on the film surface react with the vinyl functional group of acrylamide in solution and effectively initiate graft polymerization. In addition, BP preabsorbed on PHBV film may diffuse into monomer solution to initiate acylamide to form homo-PAM, which competes with photografting polymerization during the entire course of polymerization. BP preabsorbed in the PHBV films, being in the solid state, could enhance tertiary hydrogen abstraction from the PHBV

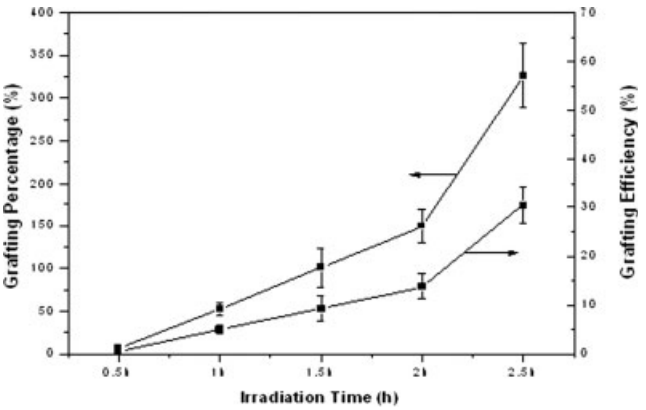



**Figure 2** Effect of the irradiation time on the grafting efficiency and grafting percentage (photoinitiator concentration 5 wt %; monomer concentration 10 wt %).

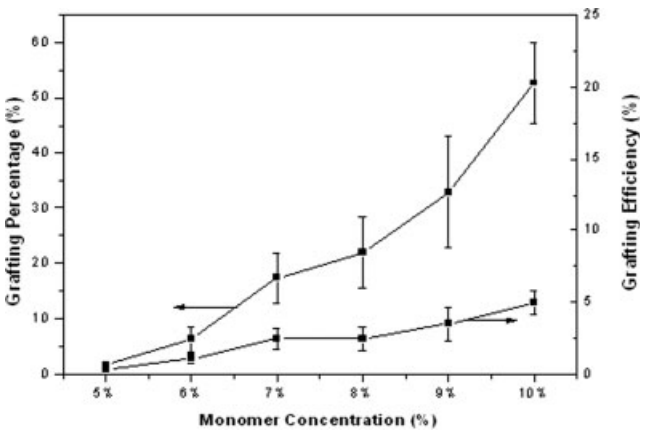



**Figure 1** Effect of the monomer concentration on the grafting efficiency and grafting percentage (photoinitiator concentration 5 wt %; reaction time 1 h).

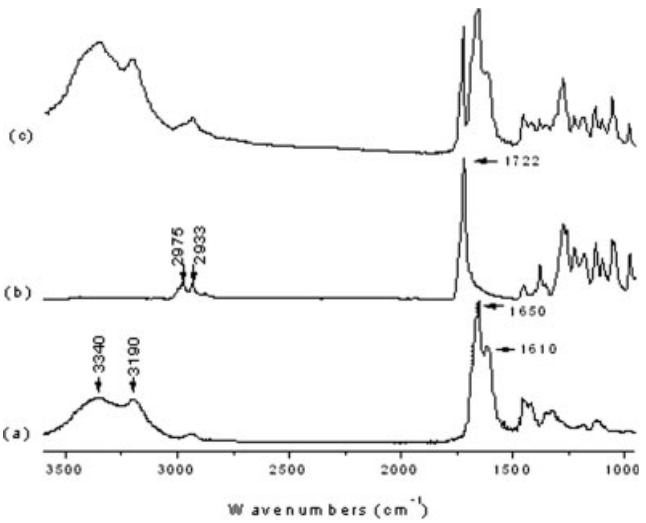



**Figure 3** ATR–FTIR spectra of (a) polyacrylamide, (b) the PHBV film, and (c) the grafted PHBV film with 53% GP.

**TABLE I**
**Effect of the Grafting Percentage on the Intensity Ratio**

| GP (%) | $R_1$[a] | $R_2$[a] | $R_3$[a] | $R_4$[a] | $R_5$[b] |
|---|---|---|---|---|---|
| 6 | 0.019 | 0.009 | 0.009 | 0.009 | 0 |
| 17 | 0.288 | 0.047 | 0.121 | 0.069 | 0 |
| 33 | 0.756 | 0.115 | 0.282 | 0.173 | 0.291 |
| 53 | 0.824 | 0.122 | 0.405 | 0.233 | 0.513 |
| 101 | 4.375 | 1.018 | 1.893 | 1.411 | 2.008 |
| 149 | 10.231 | 1.846 | 4.077 | 3.154 | 9.764 |
| 326 | 33.222 | 7.440 | 13.556 | 10.222 | 4.392 |

[a] Determined by peak height at half width of amide at 1650, 1610, 3340, and 3190 $cm^{-1}$ to that of ester at 1722 $cm^{-1}$ on the surface with irradiation, respectively.
[b] Determined by peak height at half width of amide at 1650 $cm^{-1}$ to that of ester at 1722 $cm^{-1}$ on the surface without irradiation.

films. And this treatment could also reduce homopolymerization because the diffusion of BP into aqueous acrylamide is slow and most of initiator still stays in PHBV film.

Figure 1 showed the development of the GP and GE as a function of monomer concentration. The results clearly showed that GP and GE went up gradually with the increase of acrylamide concentration because macromolecular radicals collide with monomer more effectively. It could also be found that GP and GE increased from 2% and 0.4% in the 5% acrylamide solution to 53% and 5.0% in the 10% acrylamide solution, respectively. The effect of monomer concentration on GP is greater than that on GE.

The effects of irradiation time on the GP and GE were shown in Figure 2. GP and GE increased gradually within 2 h. As irradiation time prolonged from 2 to 2.5 h, GP and GE increased from 149 and 14% to 326 and 31%, respectively. GP and GE increase abruptly after 2 h possibly because of autoacceleration in the free-radical polymerization. In comparison with the effect of monomer concentration, the effect of irradiation time on GP and GE is significantly more. Irradiation time is a more important and effective factor. And, as can be seen that it is possible to reduce homopolymer by using photoinitiator preabsorbing treatment at room temperature, which could be in favor of tertiary hydrogen abstraction from the PHBV films. It gains an advantage over grafting polymerization system with chemical initiator predissolving in monomer solution, which may yield more initiator radicals in the initial reaction stage, and thus lead to the fast increase of GP and a large amount of homopolymer as well. Subsequently, overconsumption of monomer in the initial stage or by homopolymerization may result in a slow increase of GP.

### ATR–FTIR analyses

The surface structures of the PAM, PHBV, and grafted PHBV film were characterized through ATR–

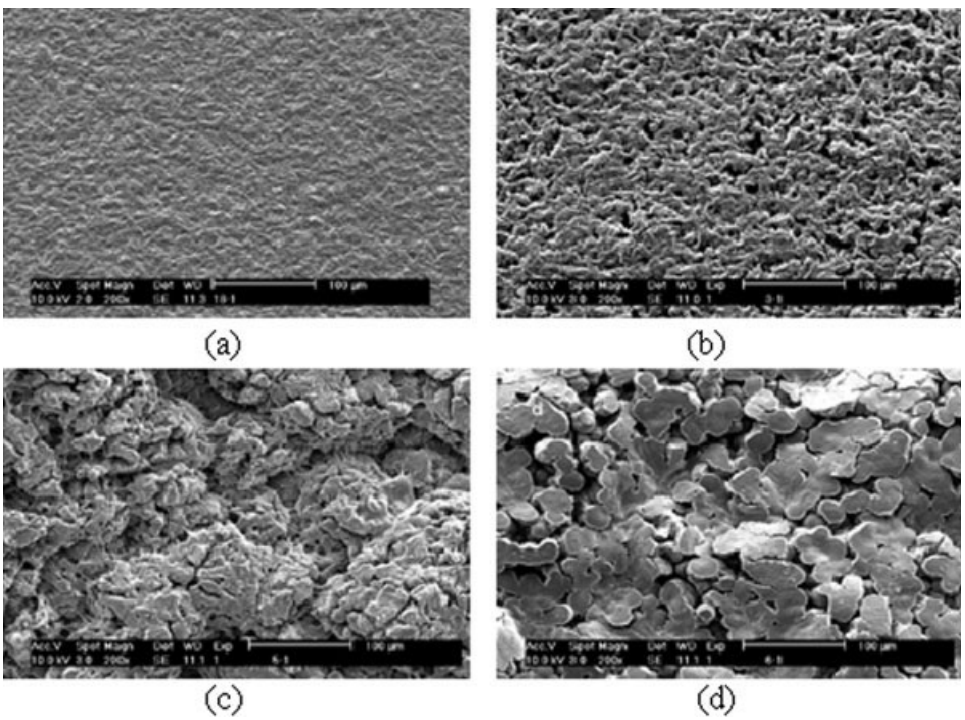



**Figure 4** SEM micrographs of the PHBV film and grafted PHBV films with different GP: (a) the PHBV film, (b) the grafted PHBV film with 6% GP, (c) the grafted PHBV film with 33% GP, and (d) the grafted PHBV film with 53% GP.

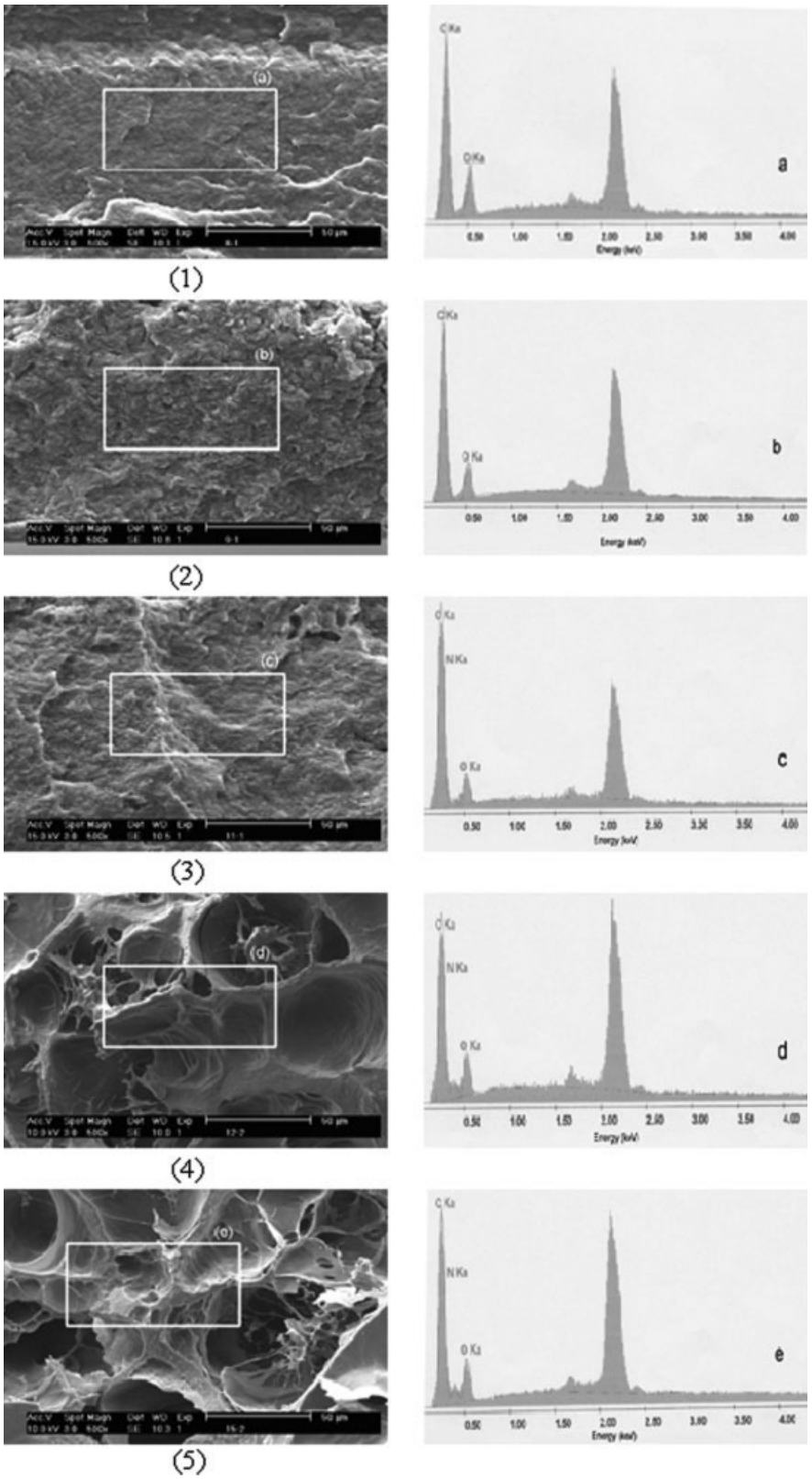



**Figure 5** Cross section SEM micrographs and EDX spectra in rectangle areas of the PHBV and grafted PHBV films: (1) the PHBV film, (2) the grafted PHBV with 6% GP, (3) the grafted PHBV with 33% GP, (4) the grafted PHBV with 53% GP, and (5) the grafted PHBV with 326% GP.

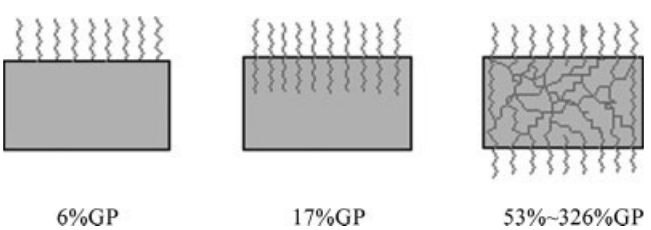
FTIR as shown in Figure 3. When compared with the PHBV film, the PAM-grafted PHBV film with 53% GP possessed three characteristic absorbance peaks of PHBV at 2975, 2933, and 1722 $cm^{-1}$, which belonged to asymmetric and symmetric stretching vibration of $CH_3$ and stretching vibration of C=O in ester, respectively. In addition, the grafted PHBV film exhibited four new peaks at 3340, 3190, 1650, and 1610 $cm^{-1}$. The first two peaks are assigned to asymmetric and symmetric stretching vibration of N—H, respectively. The last two are attributed to the strong stretching vibration of C=O and the medium bending vibration of N—H, respectively. These four peaks are characteristic absorbance peaks of acrylamide group.[20] Because the PAM homopolymer had been removed basically, the four characteristic peaks of acrylamide shown in Figure 3(c) could be attributed to the grafting PAM. ATR–FTIR results confirmed that PAM was successfully grafted on the PHBV film.

To evaluate the function of GP on the amount of PAM grafted on the film surface, intensity ratio $R$ was introduced in this work. The absorption peak because of ester C=O at 1722 $cm^{-1}$ was used as a reference peak. The intensity ratios for the grafted PHBV films with different GP were determined by using the peak height at half width of acrylamide at 1650, 1610, 3340, and 3190 $cm^{-1}$ against reference peak, respectively. As can be seen from Table I that all the four intensity ratios increased, indicating that the amount of PAM grafted on the surface with irradiation increased with the increase of GP. It could also be seen that $R_1$ changed most significantly among these four intensity ratios. To understand the chemical structure of surface without irradiation, the intensity ratio at 1650 $cm^{-1}$, $R_5$, was used. The amide absorbance peak began to appear on the surface without irradiation when GP was 33%, suggesting that PAM-grafted chains penetrated through the film and accumulated on the surface without irradiation. It is presumed that the initial grafting polymerization occurs on the film surface with irradiation. Once there are PAM chains photografted on the surface with irradiation, they will facilitate the diffusion of acrylamide in the PHBV films because of the approximate solubility parameter of PAM and acrylamide when compared with that of PHBV. During photografting polymerization, grafted PAM may induce more acrylamide to be transported into deeper region from outer surface and then more grafted PAM was formed locally. Even though the exact distribution of the grafted chain inside the grafted PHBV films cannot be assessed from these results, it is clear that the intensity ratio of the surface with irradiation is always higher than that of the surface without irradiation. From Table I, it could be seen that $R_5$ increased basically as GP went up.

**SEM analyses**

Figure 4 presented the surface morphologies of PHBV films before and after photografting polymerization as viewed by SEM. Figure 4(a) clearly showed that the surface of the PHBV film was uniform and wavelike with small pores, probably resulted from the evaporation of solvent. Figure 4(b–d) exhibited the surface topographies of the grafted PHBV films with the GP of 6, 33, and 53%, respectively. The surface of the grafted PHBV films became very rough because of the formation of PAM layer. Voids could be clearly seen on the surfaces of the grafted PHBV films. These voids may be resulted from shrinkage of PAM chains during the desiccating process. When compared with that of the PHBV film, the surface of the grafted PHBV films changed notably, which reconfirmed that PAM was successfully grafted onto the surface of PHBV films.

Figure 5 showed the cross section morphologies of the PHBV and grafted PHBV films through freeze-thawing method, as well as the EDX analyses in rectangle areas of the corresponding films. The cross section of the PHBV film as viewed in Figure 5(a) was compact and smooth. Figure 5(b,c) exhibited the cross section of the grafted PHBV films with 6 and 33% GP, respectively. They were uneven with small pores that may result from the sublimation of water being absorbed in the grafted PHBV films. During freeze-thawing treatment, the space occupied by water remained and formed the pores inside the films. With the GP increased further, the cross section of the grafted PHBV films changed notably as shown in Figure 5(d,e). The grafted PHBV films with 53% GP showed large columned channels, most of which were closed-pore structure, and irregular interconnected open-pore between these channels. Inside the columned channels, a small cluster extends its tiny thread sticking to the wall of the channels. The grafted PHBV films with 326% GP possessed better interconnectivity. The formation of columned channels is probably due to the fact that the grafted PAM encloses large amount of water and penetrates into the films, which will loose the structure of PHBV film and form the channel inside the film. After freeze-thawing process, the films main-




**Scheme 2** Schematic structures of the grafted PHBV film with different grafting percentages.

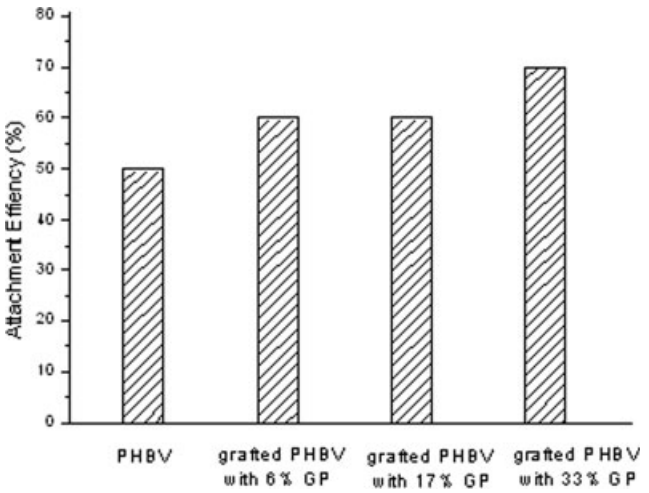



**Figure 6** The attachment efficiency of MSCs cells on the PHBV and grafted PHBV films.

tain their original shape and leave columned pores inside the films.

The element analysis of cross section of the PHBV and grafted PHBV films was determined by EDX. The PHBV film mainly consists of C and O, which was confirmed by EDX in Figure 5(1). There was no presence of *N* element in the middle section for the grafted PHBV with 6% GP as shown in Figure 5(2). It may conclude that the PAM-grafted chains mainly located on the surface. Nitrogen was observed in the grafted PHBV films with 33, 53, and 326% GP as shown in Figure 5(3–5), indicating that the grafted PAM chains penetrate inside the PHBV films and form physical semi-interpenetrating network through photografting polymerization. It is presumed that acrylamide is subjected to polymerization on the surface of the preformed PHBV film and to pervasion through PHBV film at the same time.

According to the above-mentioned SEM observation with EDX analysis and ATR results, schematic structures of the grafted PHBV films with various GPs were put forward, as shown in Scheme 2. When GP was 6%, PAM chains distributed evenly on the side with irradiation; when it was 17%, PAM chains appeared not only on the side with irradiation but also inside the PHBV films, however, did not arrive at the side without irradiation yet; when it was higher than 53%, PAM chains appeared on both sides of the PHBV film. And the grafted PHBV films formed the physical semi-interpenetrating network, with columned pore structure inside the films after freeze-thawing treatment.

## Cell studies

Cell attachment experiments with sheep bone MSC were utilized to illustrate the improvement of cell affinity after photografting polymerization. Figure 6

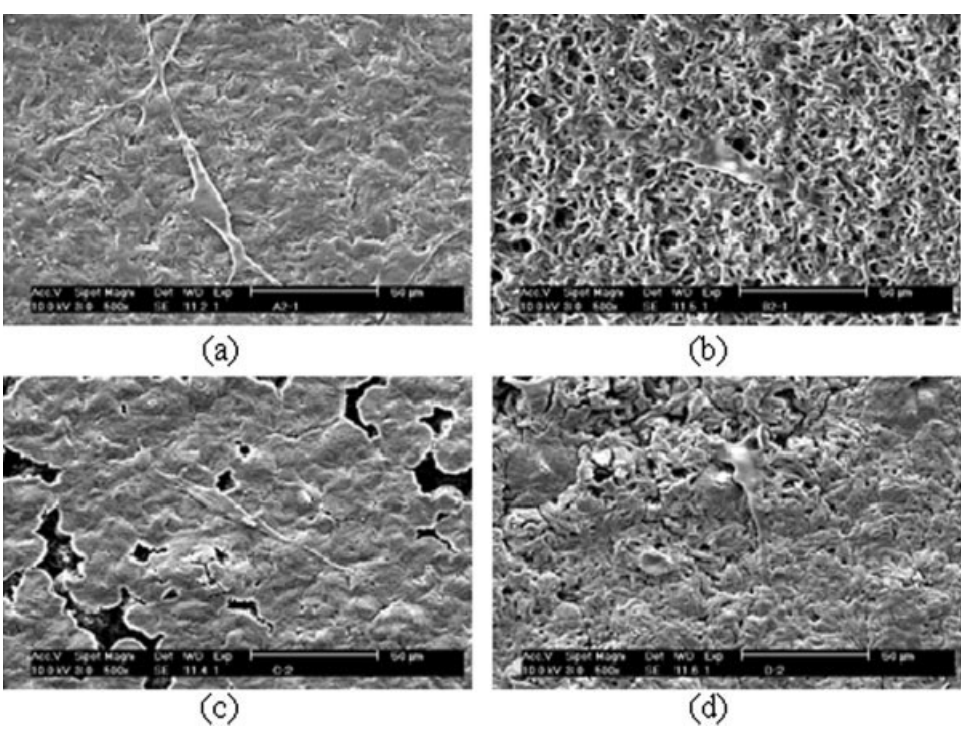



**Figure 7** Scanning electron micrographs of MSCs cells on: (a) the PHBV film, (b) the grafted PHBV film with 6% GP, (c) the grafted PHBV film with 17% GP, and (d) the grafted PHBV film with 33% GP. After 7 days of cell seeding with an initial cell seeding density of 1000 cells/cm$^2$.

showed the attachment efficiency of MSCs cells on the PHBV and grafted PHBV films after 0.5 h. It indicated that the cell attachment efficiency went up with the increase of the GP. SEM was used to visualize cells on the surface of the PHBV and grafted PHBV films. At the end of 7 days incubation, attached cells were observed on the films. Figure 7(a) showed a MSCs cell with filopodia extensions on the PHBV film. Figure 7(b–d) showed that the MSCs cells spread well on the grafted PHBV films. It is concluded that the PHBV and grafted PHBV films have a good biocompatibility with MSCs. A more detailed cell studies is still under way.

## CONCLUSIONS

PAM was successfully grafted onto PHBV films at room temperature by photografting polymerization despite PHBV's high degree of crystallinity and nonactive chemical structure. The GP and GE increased with the increase of acrylamide concentration and irradiation time. The chemical and morphological structures of the grafted PHBV films changed according to their GPs. The grafted PAM chains could be limited to the irradiated surface, or pervade inside of upper section of films. In addition, it could penetrate through the films and accumulate on the surface without irradiation. The various distributions of PAM-grafted chains will provide different reactive sites for further fictionalization. Preliminary cell studies indicated that PAM-grafted PHBV films possessed enhanced MSCs cell affinity and good cell compatibility.

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