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Biocompatibility of poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) modified by silk fibroin

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1. Introduction

The materials used as tissue scaffolds play an important role for guiding the tissue to create a new functional structure when the damaged tissue is no longer regenerated naturally [1]. Whether or not the material is suitable for tissue scaffold depends on its physical and chemical properties, such as mechanical properties, degradability and, importantly, biocompatibility. Most of biomaterials used in tissue engineering are poly(lactic acid) (PLA) and poly(glycolic acid) (PGA) [2–4]. But their poor hydrophilicity [5, 6] and relatively high crystallinity [7, 8] restrict their application in some degree. For example, previous experiments of tissue engineered heart valves fabricated by PGA only successfully acted at the low-pressure pulmonary circulation [9]. Recently, polyhydroxyalkanoate (PHA), a thermoplastic elastomor class of biopolyesters synthesized by the bacteria as an intracellular carbon and energy storage compound [10–12], is widely applied as biodegradable plastics. Moreover, it is recognized as a candidate for tissue engineered heart valve and cartilage [13] because of its elastic and easily moldable properties [14]. Of PHAs family, the most common member as biomaterial studied *in vitro* and *in vivo* is poly(3-hydroxybutyric acid) (PHB) [15, 16], although PHB has relative poor mechanical properties [17]. Sodian et al. has already demonstrated that the trileaflet heart valve made from PHA matrices could open and close synchronously in a pulsatile bioreactor, while it was not possible to create a functional trileaflet from PGA [18]. The PHA-PGA composite scaffold with PGA as inner layer for good biocompatibility shows superior tensile strength and flexibility in tissue engineered pulmonary valve [18]. Nowadays, the copolymer of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx), a new member of PHAs family, has been developed for various potential medical applications [19, 20]. Chen et al. [21] proved that the mechanical properties of PHBHHx were superior to those of the previously utilized PHB. Meanwhile they have proved that PHBHHx has better biocompatibility than PLA for the fibroblast L929 cells [22]. These works demonstrated the potential of PHBHHx used as tissue engineered heart valve and lung, a tissue-engineering trend in recent years [23] because of its combined properties such as tensile strength, extensibility, biocompatibility, as well as the ability to be thermally molded without need for suturing leaflets [14]. However, the biocompatibility of PHAs is still not so good as, for example, PGA [18]. Therefore, it promotes us to try to improve the biocompatibility of PHBHHx for the requirement of tissue engineering.

There are many methods to improve the biocompatibility of materials [24–27]. One of the important ways is to modify the surface of material, that is, the surface of scaffold material is coated by the compound which is biocompatible [25]. In present work, we focused on this method.

Silk fibroin (SF), a natural protein regenerated from silkworm silk fiber, has attracted many attentions for its medical application in recent years [28–35]. Its protein chain is constructed by the crystalline motif (dominated by the

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hydrophobic amino acids) and amorphous motif (dominated by the hydrophilic amino acids) [36]. Previous experiments [28] *in vivo* showed that the regenerated SF had no thrombogenicity. And studies [29–33] *in vitro* also revealed that SF films could support the adhesion and growth of rodent fibroblasts and had no significant inflammatory properties. A number of researches [34, 35] have examined the tissue response to various silk protein based implants. These implants have been found to evoke a minimal foreign body reaction. Hence, SF has been not only as a potential biomaterial apt for the tissue regeneration and reparation but also as an ideal surface modificator [37–41]. Several researches have been reported about SF used to modify the synthetic polymers such as poly(D, L-lactic acid) (PDLLA) [37, 38], polyurethane (PU) [39, 40] and poly(ε -caprolactone) (PCL) [43]. These work revealed that SF modification could greatly enhance the cell adhesion and proliferation on the materials.

In present work, the silk fibroin is used to modify the surfaces of both dense and porous PHBHHx matrices. An optimal modifying condition for the adhesion and proliferation of human fetal lunge fibroblast, a kind of fibroblast that can be used for the tissue construction of heart valve and lung, on the matrices is attempted to be figured out. The results would be helpful to design some optimum materials for the application into the tissue engineered heart valve and lung.

2. Materials and methods

2.1. Materials

Poly(hydroxybutyrate-co-hydroxyhexanoate), PHBHHx, containing about 12% hydroxylhexanoate, was purchased from Shantou Lianyi Biotech Company (Guangdong, China). The purity of PHBHHx is 99% with molecular weight of 800,000 (M_W).

Regenerated SF solution was prepared according to the literature [42]. Raw silk fiber from *Bombyx mori* silkworm was degummed twice with 0.5 wt% $NaHCO_3$ aqueous solution at 100°C for 1 hour, and then washed with water. The degummed silk fiber was dissolved in a 9.3 mol/L LiBr solution at room temperature. After dialysis against deionized water for three days to remove LiBr, the solution was centrifuged to remove other impurities and diluted with deionized water. Two SF aqueous solutions with the concentration of 0.75% and 3.0% (w/v) were prepared.

2.2. Matrix fabrication

2.2.1. Preparation of PHBHHx films and PHBHHx scaffolds

About 0.3 g PHBHHx was dissolved in 5 mL chloroform. The solution was cast in a 60 mm diameter glass plate at room temperature for 12 h. A PHBHHx dense film with thickness about 0.3 mm was formed in the dish, and then dried again under vacuum to remove completely solvent remaining in the film. The obtained film was stored in desiccator for further study.

Serious porous scaffolds were fabricated by the particle-leaching technique using sodium chloride (NaCl) particles as porogen. It was demonstrated in our previous work [41, 43, 44] that the porosity of scaffold was almost independent on the porogen size, but dependent on the amount of used porogens instead. And the porosity of scaffold could be made maximum, about 85%, when the weight ratio of porogens to polymers was 9:1 [45]. Therefore, 2.7 g sieved NaCl particles with different diameters (ranging from 30 to 150 μm) were added into 5 mL 5% (w/v) PHBHHx dichloromethane solutions. The solutions were stirred vigorously to disperse the particles evenly, and cast in a circular glass dish with diameter of 60 mm. The solvents were allowed to evaporate for 24 h in a fume hood, achieving the PHBHHx/NaCl composite scaffolds. After 10 h vacuum dried to eliminate the rudimental dichloromethane, the scaffolds were immersed into the deionized water for 3 days to leach out the NaCl, and the deionized water was freshen every 6 h. After dried in vacuum, the porous scaffolds is with thickness of 0.7 mm were formed with 85% porosity and the porous size of scaffold equivalent to that of the porogen particle.

2.2.2. Preparation of SF modified PHBHHx matrices

The PHBHHx film was immersed into a mixture solution of acetone/water (3:1, v/v) to be swelled for 1 h, and then immersed into SF solution (0.75 or 3.0%) for 24 h to form the SF-coated scaffold. The SF-coated scaffold was frozen in liquid nitrogen for 5 min, followed by lyophilization overnight under a vacuum of 0.1 torr at −50°C. The freeze-dried scaffold was immersed into the methanol for 1 h to fix further the SF coating [41, 46], and then followed by rinsing three times with distilled water and phosphate buffer saline (PBS, pH = 7.4) solution, and vacuum dried.

2.3. Characterization of PHBHHx matrices

2.3.1. ATR-FTIR

Attenuated total reflection Fourier transformed infrared spectra (ATR-FTIR) were performed on a Nexus 470 spectrometer (Nicolet), equipped with a multiple reflection horizontal ATR attachment. The spectra were recorded with resolution of 4 cm^{-1} and 128 scans for the SF-modified scaffolds before (Fig. 1B) and after (Fig. 1C) methanol treatment referred to the pure PHBHHx 3D scaffold as control (Fig. 1A).

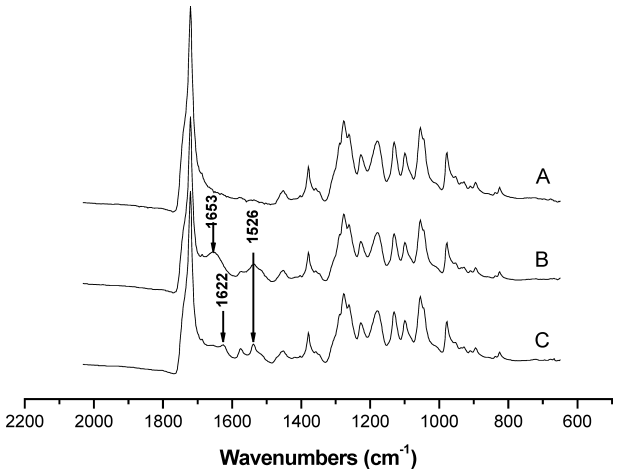

Fig. 1 ATR-FTIR spectra of the surfaces of (A) PHBHHx 3D scaffold; (B) the SF-modified PHBHHx 3D scaffold without methanol treatment; (C) the same scaffold as (B) but with methanol treatment.

2.3.2. Image observation

To observe the morphology of the prepared PHBHHx matrices and evaluate the effect of SF coating on the dense and porous PHBHHx matrices, the surfaces of both PHBHHx 2D film and porous 3D scaffold were observed by scanning electron microscopy (SEM, Philips XL30) at an accelerating voltage of 20 kV.

2.3.3. Coated SF content and stability in the modified PHBHHx matrices

The coated SF content in the modified PHBHHx scaffold is evaluated with the following expression:

$$SF\% = (W_m - W_{PHBHHx})/W_m \times 100\% \quad (1)$$

where W_{PHBHHx} represents the weight of the PHBHHx scaffold without SF coated, whereas W_m represents the weight of the SF-modified scaffold after methanol treatment.

Stability of coated SF in the modified PHBHHx matrices is estimated in the physiological-like conditions. The SF-modified scaffolds after methanol treatment were immersed into PBS solution and incubated at 37°C for periods of time. At different time courses of incubation, the scaffolds were taken out from PBS solution, washed by distilled water, vacuum-dried and weighed.

The SF-retained-ratio (R_t) is defined as following expression to evaluate the coated SF stability in the PHBHHx scaffold:

$$R_t\% = (W_t - W_{PHBHHx})/(W_0 - W_{PHBHHx}) \times 100\% \quad (2)$$

where W_{PHBHHx} represents the weight of PHBHHx scaffold before SF coated, and W_0, W_t represent the weights of the SF-modified scaffolds before and after immersion into PBS solution for a given time course, respectively. This definition implicates that the initial SF-retained-ratio R_0 before incubation is 100%, while R_t is that measured at various immersion time courses.

2.3.4. Hydrophilicity analysis

Water contact angles of PHBHHx and SF-coated PHBHHx dense 2D films were determined by the measurement for the deionized water on the films using a sessile drop method with an image analysis system (OCA 15 plus, data physics) at 25°C. The data of one site on the film surface was collected after 2 minutes of the water dropped on the surface. Five measurements on different surface sites were averaged as the final result.

2.4. Cell culture on the matrices

2.4.1. Preparation of cells

The human fetal lung fibroblasts (HFL-1) were purchased from American Type Culture Collection (ATCC). After cells in the culture flask grew confluent, they were digested with 1 mL 0.5 w/t% trypsinase for 2 minutes to create a single cell suspension, and then 3 mL of culture medium was added to stop the digestion. Cell numbers were determined by counting *via* a haemacytometer, and then diluted to the concentration of 7.5×10^5 cells/mL.

2.4.2. Cell culture on the matrices

Both SF-modified PHBHHx matrices and control ones (unmodified matrices) were cut into circular samples with 15 mm in diameter. The samples were sterilized with 75% (v/v) ethanol overnight and then by ultraviolet light for 2 h. The sterilized matrices were pre-incubated in PBS (pH = 7.4) solution to replace ethanol remaining in the samples and then were transferred to a sterile 24-well cell culture plate (Costar™). 100 μL cell suspension prepared previously was dripped into each matrix. After the cells were cultured in a humidified incubator (5% CO_2, 37°C) for 1 h, additional 1 mL culture medium was added into each well. The medium was replenished every 2 days.

2.4.3. MTT assay

Cell numbers of viable fibroblasts in the PHBHHx matrices were quantitatively assessed with 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide (MTT, Sigma) at various cultural time courses up to 28 days.

MTT assay is a rapid colorimetric method based on the mitochondrial conversion of tetrazolium to MTT formazan

to determine the viable cell numbers. The cell-contained PHBHHx matrices were rinsed with serum free medium to remove the unattached obituary cells and transferred to another cell culture plate. The cells in the matrices were incubated at 37°C for 4 h for the formation of MTT formazan after 100 μL MTT stock solution (0.5 mg/mL in RPMII 1640) was added into each sample. The MTT formazan was then dissolved into 300 μL/well dimethyl sulfoxide (DMSO) solution, and then transferred to a 96-well plate for MTT assay. Optical density (OD) of the MTT formazan in DMSO solution was measured with an automatic microplate reader (ELX 800, Bio-Tek) at wavelength of 570 nm referred to the value at 630 nm. Both DMSO and the PHBHHx dense 2D film without cells cultured were assayed as the background. Four parallel samples were measured in each time, and the mean value was used as the final result.

2.4.4. Cell morphologies

After cells in the PHBHHx matrices were cultured for different days, the cell-contained PHBHHx matrices were washed with PBS solution three times, and then the cells were fixed with 5% glutaraldehyde in PBS solution for 24 h at 4°C. The matrices were dehydrated sequentially in 50%, 70%, 90%, 100% ethanol, each for 15 minutes and then freeze-dried, sputter-coated with gold, and examined by a scanning electron microscope.

2.5. Statistical analysis

Experiments were run at least triple in parallel tests. Data were expressed as means ± standard deviation (SD) for $n \geq 3$. Statistical evaluation was performed by one-way analysis of variance for multiple comparisons using the Bonferroni procedure. The mean values were considered to be significantly different if the probability of difference fell below 5% (i.e. $P < 0.05$) or below 1% (i.e. $P < 0.01$).

3. Results and discussions

3.1. Scaffold characterizations

3.1.1. ATR-FTIR analysis

ATR-FTIR analysis is performed to examine the presence of SF on the surface of SF-modified PHBHHx porous scaffold as well as the behavior of SF conformation. The bands of amide I and amide II in IR spectrum are very useful for the conformational analysis of SF protein. In general, the amide I mode of SF associating with the α-form conformation occurs at 1650-1660 cm^{-1}, whereas the random coil conformation at 1640-1650 cm^{-1} and the β-form conformation at 1620 and 1640 cm^{-1}; the amide II of SF occurs at 1520–1530 cm^{-1} [47].

Fig. 1 represents the ATR-FTIR spectra of PHBHHx porous scaffold (A), SF-modified scaffold without (B) and with methanol treatment (C), respectively. It can be seen that there are some new peaks, 1653, 1622 and 1526 cm^{-1} appear in both spectra of Fig. 1B and 1C when compared with Fig. 1A, which demonstrates the presence of SF on the surfaces of SF-modified PHBHHx scaffolds as have been proved in a 2D film by Cai et al [37]. Notably, the peak at 1653 cm^{-1} (Fig. 1B) indicates that the SF conformation is α-form before the modified scaffold is treated by methanol. However, a SF conformational transition from water-soluble α-form to water-insoluble β-form happens after the scaffold is treated by methanol, which is evidenced by the SF Amide I peak shifting from 1653 cm^{-1} (Fig. 1B) to 1622 cm^{-1} (Fig. 1C), resulting in the immobilization of the SF on the scaffolds [41]. Therefore, to prevent the dissolution of the coated SF from the scaffolds in our experimental conditions, all modified scaffolds used in further experiments are treated by methanol if there is no special illustration.

3.1.2. Coated SF stability on the PHBHHx porous scaffold

The contents of coated SF on the PHBHHx scaffolds with pore size between 50–75 μm are evaluated about 5.7 to 9.2 ± 0.1 wt% in the scaffolds modified with SF solutions of 0.75% to 3.0% based on the evaluation of expression (1). Fig. 2 shows the SF-retained-ratio (R_t) of porous SF-modified scaffold treated under physiological-like conditions for 4 and 7 days. It can be observed that R_t is higher than 90% after 7 days immersed into the PBS solution for the SF-modified PHBHHx scaffolds. The slight R_t decreases possibly arise

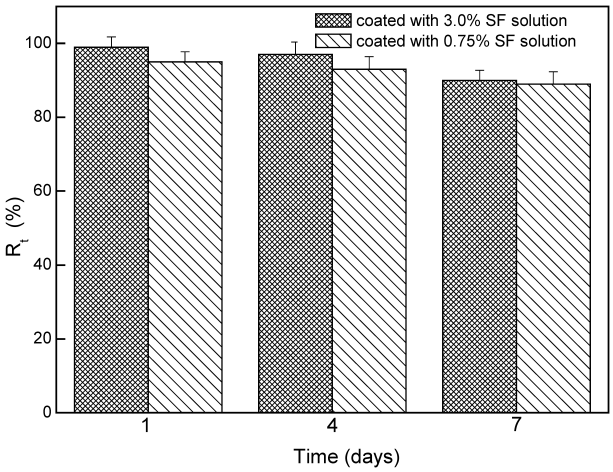

Fig. 2 SF-retained-ratio (R_t%) of SF-modified PHBHHx 3D scaffolds with pore size between 50–75 μm at various immersion time courses in the PBS (pH = 7) solution.

from the degradation of some PHBHHx scaffolds whose residual weigh are about 90% when immersed in PHB/lipase solution for 7 days [48]. The achieved high R_t value indicates that SF conformational transition from α- to β-form induced by the methanol treatment stabilizes the SF component in the scaffolds. This is hence an advantage of SF coating, compared with the widely used collagen coatings that often fall off from the surface of polymeric materials when exposed to the culture medium for a period of time [49]. Our previous study [44] showed that there is no big difference in R_t between 2D- and 3D-SF modified scaffolds and between the scaffolds with different pore size. Therefore, the SF-modified scaffold with pore size of 50–75 um is used as a representative.

3.1.3. Scaffold hydrophilicity

Water contact angle can be used as an indicator of the hydrophobicity or hydrophilicity for a material. Generally, a more hydrophilic film has a smaller water contact angle, therefore, favoring the cell attachment. It can be seen in Fig. 3 that both water contact angles of PHBHHx films modified by 3% (53°) and 0.75% (60°) SF solutions are smaller than that of control (PHBHHx, 72°). This implies that the introduction of SF enhances efficiently the hydrophilicity of PHBHHx surface. And obviously, the more SF content, the better hydrophilicity there would be for a 2D film.

3.1.4. Scaffold morphologies

Fig. 4 shows the surfaces of 2D PHBHHx dense films without and with SF modification observed by SEM. Compared with control (Fig. 4A), the SF modification does not considerably change the surface morphology of PHBHHx film when a lower SF concentrated solution (0.75%) is used (Fig. 4B).

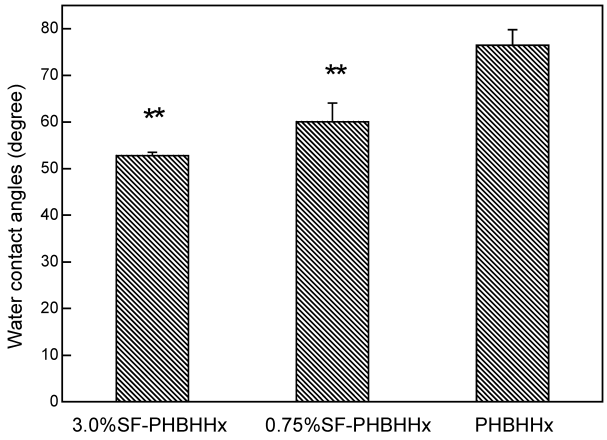

Fig. 3 Water contact angles of two SF-modified PHBHHx 2D films and control (PHBHHx).
(**: $P < 0.01$ compared to control).

But PHBHHx film modified by a higher SF concentrated solution (3.0%) has a relatively rough surface and there are some crinkles appearing (Fig. 4C).

To observe the morphologies of SF-modified PHBHHx porous 3D scaffolds, a porous scaffold with 85% porosity fabricated by 50–75 μm NaCl particles is exampled in Fig. 5. It is found that the PHBHHx scaffold modified by a lower SF concentrated solution (0.75%) has no evident sponge structure formed by SF itself on the freeze-dried scaffold surface (Fig. 5B). SF works only as coating and is difficult to form sponges with SF itself, nothing more than leading to a somewhat decrease in numbers of pores and interconnections between pores formed by PHBHHx in the SF-modified scaffold (Fig. 5B) compared with control (Fig. 5A). The morphology of the SF-modified PHBHHx porous scaffold is kept basically as original one when the used SF solution is low concentrated. In contrast, the SF form the spongy structures, with the elongated-pores and layers, enveloped in the PHBHHx porous scaffold after this scaffold is modified by a higher SF concentrated solution (3.0%) (Fig. 5C). This considerably affects the morphology of PHBHHx porous scaffold, therefore, possibly restricting the cell growth.

3.2. Cell culture on the matrices

3.2.1. Cell growth on 2D PHBHHx dense films

The optical density (OD) values of the cell cultured for 1 day on two types of 2D PHBHHx dense films modified by 0.75% and 3.0% SF solutions are 0.138 and 0.147, respectively, with respect to 0.119 (control, unmodified PHBHHx film) seen in Fig. 6, indicating that SF-coating enhances the initial cellular attachment on the substrates, though the difference in OD values is not significant ($P > 0.05$). Subsequently, after 1 week of cell incubation, the OD values of two modified films are much higher than that of the control, suggesting that SF coating also promotes the cell proliferation on the substrates. Meanwhile, it can be found that the more SF coated on the films, the more cells grow.

3.2.2. Cell growth on the PHBHHx porous scaffolds

From Fig. 7 (A, B, C), it is found that MTT assay demonstrates an enhancement in the cell spreading for 7 days cell culture in series of SF-modified PHBHHx porous 3D scaffolds with different pore sizes compared with that in control. The different time courses in Fig. 7 were chosen for the observation of cell proliferation stopping after long time cell culture. The initial numbers of attached cells for first three days cell culture in the scaffolds with pore sizes of 76–150 μm (Fig. 7A) and 50–75 μm (Fig. 7B) have no significant difference ($P > 0.05$) compared with that of control, and even a little fewer numbers of cells initially adhered and

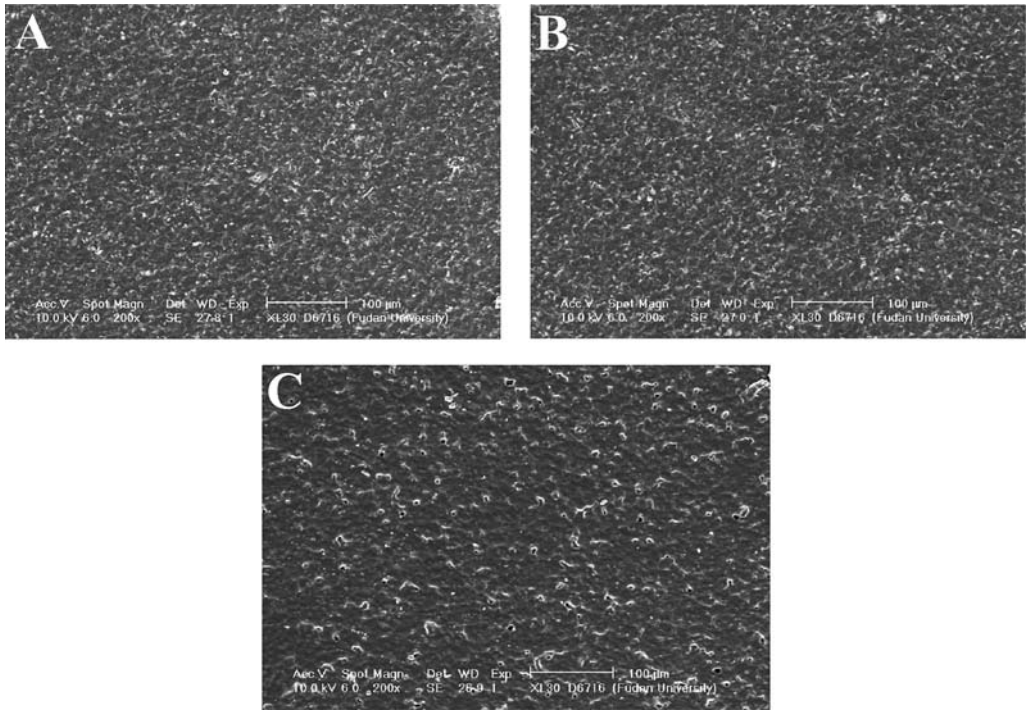

Fig. 4 SEM images of different 2D films: (A) PHBHHx 2D film; (B) and (C) are SF-PHBHHx 2D films modified with 0.75% and 3.0% SF solutions, respectively.

spread in the scaffold with pore size of 30–50 μm than those in the controls (Fig. 7C). Such a behavior of cell growth, which is not promoted by SF modification, is different from that on the 2D dense film, possibly is attributed to the SF modification somewhat affecting the surface pore size and reducing the porosity of the scaffold. However, after three to fourteen days cell culture in the modified scaffolds, number of cells shows dramatically increasing, demonstrating

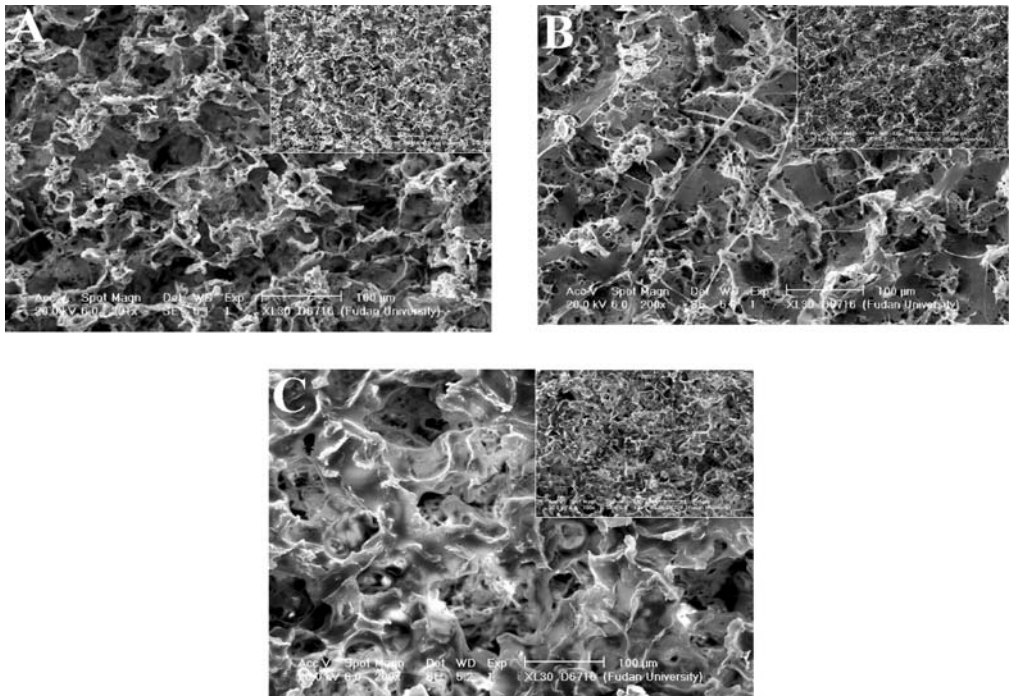

Fig. 5 SEM images of different surfaces of PHBHHx 3D scaffolds porosed by NaCl particle with size between 50–75 μm: (A) pure PHBHHx; (B) modified by 0.75% SF solution; (C) modified by 3.0% SF solution. The inserted illustrations are images with wide surface ranges.

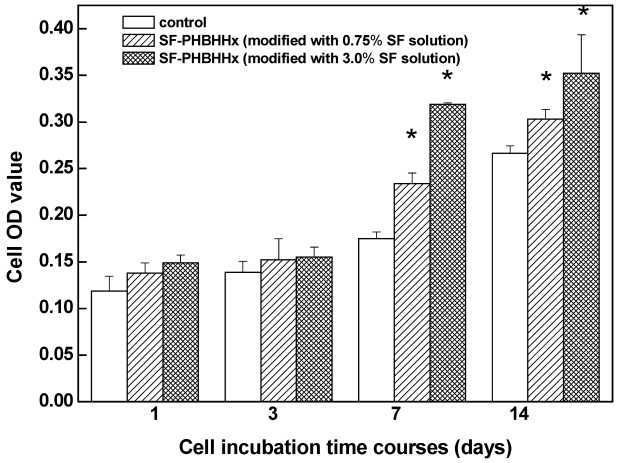

Fig. 6 Cells OD values on a series of SF-modified 2D PHBHHx films at different culture time, with pure PHBHHx film as control (n = 4). (*: $P < 0.05$ compared to the control).

the introduction of SF greatly promotes the cell growth in the scaffolds eventually. Fig. 7 also shows that the scaffolds modified with fewer amount of SF provide the circumstance of cell growth better than that modified with more amount of SF. Although the scaffold with higher SF content is more hydrophilic, being favorable to the cell attachment on the scaffold surface, it reduces the porosity of the scaffold, probably limiting the cell proliferation. That is, for the optimum of cell growth in a PHBHHx 3D scaffold, it is necessary to consider a compromise between higher hydrophilicity and lower porosity both induced by more SF coated on the scaffold. Under our exampled scaffolds, lower SF concentrated solution is more ideal to modify the 3D PHBHHx scaffold for both cells attachment and proliferation. Practically, the cells proliferation will stop automatically when cells contact each other and then converge [50], for example, after three weeks cell culture seen in Fig. 7C.

3.2.3. *Cell morphologies*

Fig. 8 and Fig. 9 show the morphologies of fibroblasts cultured on 2D film and porous 3D PHBHHx scaffold, respectively, modified with 0.75% SF solution compared with control. It is observed that the shape of cell on both modified 2D film (Fig. 8B) and 3D scaffold (Fig. 9B) looks like sphere after 1 day cell culture. But it also shows that the cells on the modified 2D film are more flattened (Fig. 8B) than that on the control (Fig. 8A). In 3 days cell culture, all the cells elongate with shuttle-like shape (Fig. 8C, Fig. 9C and 9D). For 7 days cell culture, the cells become confluent in both modified 2D film (Fig. 8D) and 3D scaffold (Fig. 9E and 9F). It is also noted that the cells form a cell layer on the modified 2D film (Fig. 8E), fully covering the surface of substrates after 14 days cell culture. This phenomenon prevents in some

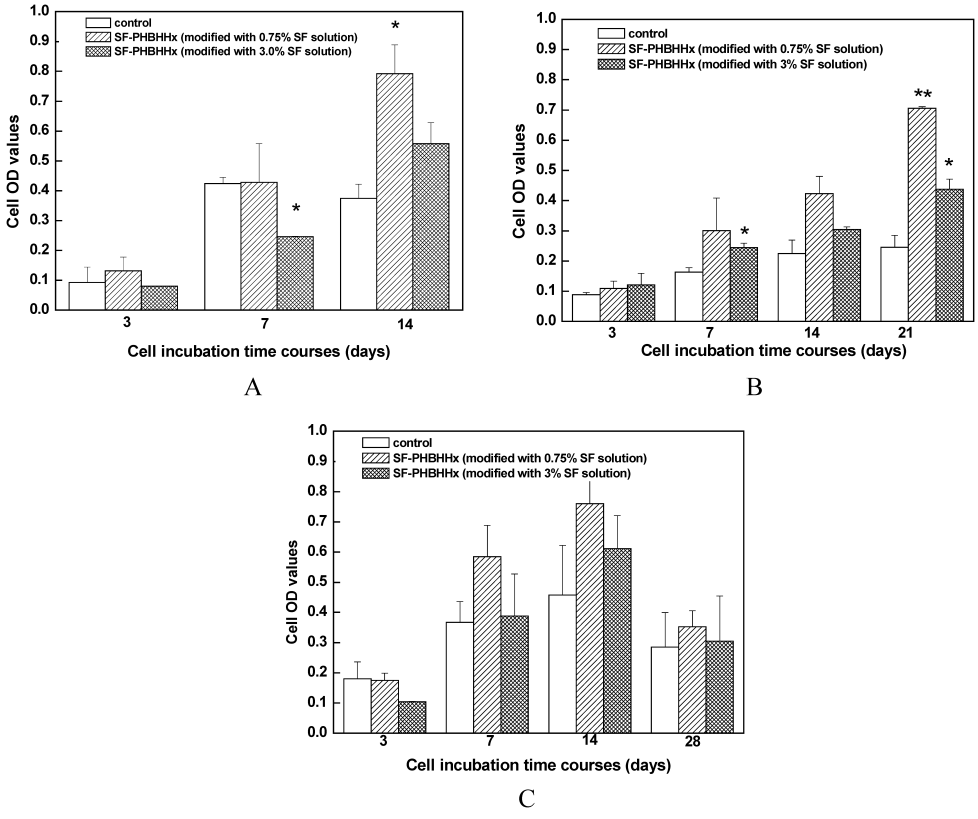

Fig. 7 Cells OD values in a series of SF-modified 3D scaffolds at different culture time courses, with pure PHBHHx 3D scaffold as control (n = 4). The scaffold pore sizes are 76–150 μm (A), 50–75 μm (B) and <50 μm (C), respectively. (*: $P < 0.05$; **: $P < 0.01$ compared to the control).

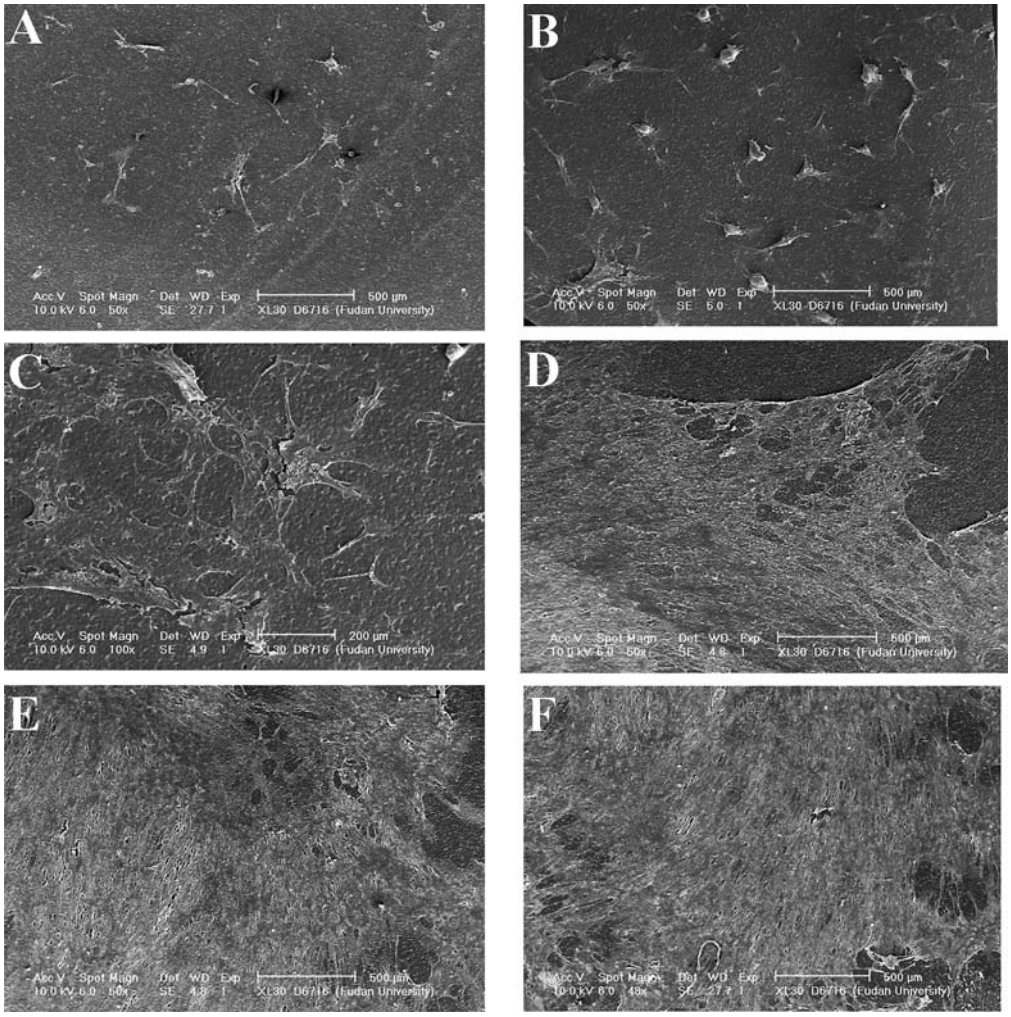

Fig. 8 Representative SEM images of cells cultured on PHBHHx 2D films: (A) cell adhesion on the SF-unmodified 2D film for 1 day; (B) cell adhesion on the SF-modified 2D film for 1 day; (C) cell spreading on the SF-modified 2D film after 3 days; (D) cell confluence on the SF-modified 2D film after 7 days; (E), (F) cell layers covering the whole surface of both the SF-modified and control films, respectively, after 14 days.

extent the cell further proliferation. It clearly rationalizes the facts that the viable cell numbers in the substrates become fewer after 28 days culture than that after 14 days culture seen in Fig. 7C. In addition, we find that the studied cells finish their proliferation within 7 days (Fig. 8 and 9) during which the scaffold basically maintains its original weight without influence of its degradation on the cell proliferation, therefore, the observation for the cell cultured in longer time (such as longer than 28 days) may not be necessary in present work.

From SEM, it is proved that cells can attach and proliferate well on both PHBHHx and SF-modified PHBHHx scaffolds. And SF-modified scaffold is more favorable to the fibroblasts growth, suggesting that the SF-modified PHBHHx 3D scaffold is a promising material applied into the tissue engineering, such as tissue engineered heart valve and lung.

4. Conclusions

This work investigated the hydrophilicity and biocompatibility of SF-modified PHBHHx matrices. The small water contact angle on the SF-modified PHBHHx surface indicates the improvement in the hydrophilicity of PHBHHx matrices. And more numbers of cells attach on both SF-modified PHBHHx 2D dense films and 3D porous scaffolds compared with the controls, suggesting the enhancement of biocompatibility. Our results show that SF, a natural biopolymer with a good biocompatibility, could be used as a preferable candidate to modify the synthetic polymers in order to improve the cell growth in them, and further possibly promote the new tissues formation. However, it is also found that the higher concentrated SF coated on the PHBHHX 3D scaffold is not so favorable to cell growth as the lower one although the higher SF content would lead to a better hydrophilicity

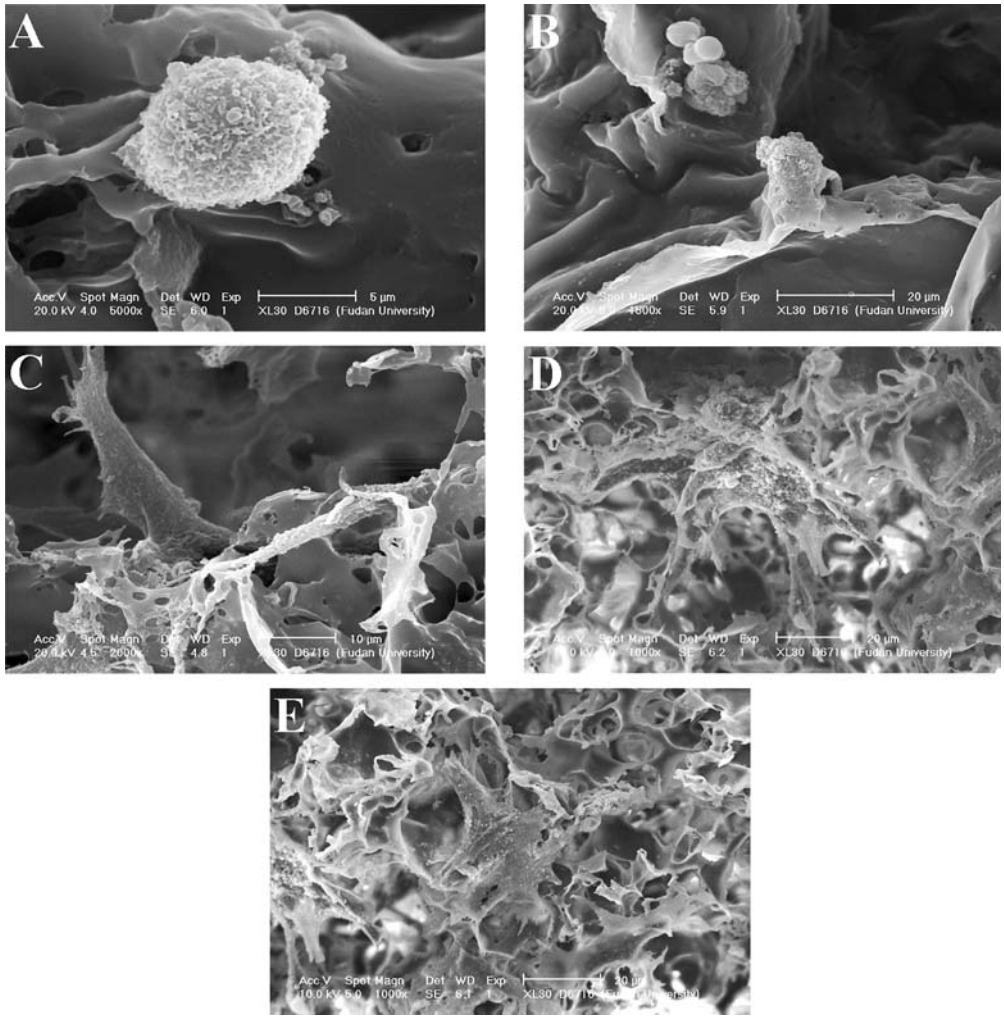

Fig. 9 Representative SEM images of cells cultured in PHBHHx 3D scaffolds: (A) and (B) are the cells attached on the control and SF-modified 3D scaffolds for 1 day, respectively; (C) are the cells spreading on the surface and in the pores of SF-modified 3D scaffolds after 3 days; (D) and (E) are the cell confluence on the surface and in the pores of SF-modified 3D scaffolds, respectively, after 7 days.

for the cell growth on the 2D substrate surface. There is a compromise condition apt to both cell attachment on the surface and proliferation in the porous 3D scaffold, which are controlled by the SF content and porosity of scaffold. This important finding would give one a guidance to design a biocompatible porous scaffold more efficiently.

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