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Improvement of cytocompatibility of electrospinning PLLA microfibers by blending PVP

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Abstract In this study, microfiber films were used as scaffolds for the purpose of vascular tissue engineering. The microfiber films were prepared by electrospinning of poly (l-lactide) (PLLA) and polyvinyl pyrrolidone (PVP). PLLA and PVP with different ratios were blended with dichloromethane as a spinning solvent at room temperature. The properties of the composite microfiber films were investigated by differential scanning calorimetry (DSC), scanning electron microscopy (SEM) and contact angle measurement. The SEM images showed that the morphology of the microfiber films was mainly affected by the weight ratios of PLLA/PVP. The DSC results demonstrated that PLLA and PVP mixed uniformly. And the hydrophilicity of the films measured increased along with the decrease of the PLLA/PVP ratio. Vascular smooth muscle cells (VSMCs) were used to test the cytocompatibility. Cell morphology and cell proliferation were measured by SEM, laser scanning confocal microscopy (LSCM) and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay after 2, 4, 6 days of culture. The results indicated that the cell morphology and proliferation on the composite films were better than that on the pure PLLA film. Furthermore, morphology and proliferation of VSMCs became better with decreasing of the weight ratio of PLLA/PVP. In addition, adhesion of platelet on the films was observed by SEM. The SEM images showed that the number of adhered platelets decreased with increment of PVP content in the films. The electrospinning microfiber composite films of PLLA and PVP would have potential use as the scaffolds for vascular tissue engineering.

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1 Introduction

The area of tissue engineering has been driven by bio-inspired design of biomaterials to recreate the natural three-dimensional environment for the better of growth cell and tissue [1]. Recently, electrospinning has aroused much interest as an attractive technique for producing polymer fibers with diameter in the range from several micrometers to tens of nanometers [2]. Because of the unique properties of the electrospinning fibers, such as high specific surface area and high aspect ratio, they have potential to find a wide variety of applications in biomedicine fields [3, 4]. Numerous polymers, such as collagen, poly(e-caprolactone), PCL/poly(ethylene glycol), poly(l-lactide) (PLLA), polyglycolide, poly(L-lactide-co-caprolactone) and poly (hydroxybutyrate-co-hydroxyvalerate) have been successfully electrospinning into microfibers [5–12]. PLLA has been widely used in various biomedical applications [13] due to its biodegradability, biocompatibility, nontoxicity, good mechanical properties. However, the hydrophobic nature of PLLA can become a serious problem in a predominantly hydrophilic bio-environment [14].

An easily achieved approach to increase the hydrophilicity of the scaffold is incorporation of a hydrophilic polymer into the spinning solution [12]. Polyvinyl pyrrolidone (PVP), a synthetic and water-soluble polymer, is used as a blood plasma expander and a vitreous humor substitute for its good biocompatibility [15, 16]. Because of

its special molecular structure, PVP has many outstanding properties. First of all, PVP is remarkable for its capacity to interact with a wide variety of hydrophilic and hydrophobic materials. Second, it has similar properties to those of a protein for its structure of pyrrolidone. For example, it can be precipitated by most protein precipitators. Third, it has little immunogenicity and antigenicity, and it has a favorable chelating ability and is used in the drug and food field as a chelating agent. Therefore, it would be interesting to combine PVP in PLLA to increase its hydrophilicity and biocompatibility. Currently, many studies have attempted to investigate the composite of PLLA, such as with collage, heparin, etc. However, there is little report about the composite of PLLA with PVP.

In this study, composited microfiber films were obtained by electrospinning of PLLA and PVP. The composite of PLLA and PVP blended were testified by differential scanning calorimetry (DSC). The morphology and hydrophilicity/hydrophobicity of the microfiber films were studied by scanning electron microscopy (SEM) and contact angle measurement. And the cytocompatibility of vascular smooth muscle cells (VSMC), such as cell morphology and cell proliferation, was investigated by SEM, laser scanning confocal microscopy (LCSM) and MTT assay. Furthermore, blood compatibility was evaluated initially by platelet adhesion.

2 Experiments

2.1 Materials

PLLA (MW = 100,000) was provided by Jinan Medical Devices Research Center in China. PVP (MW = 40,000) was purchased from Sigma. All reagents were analytical grade and used without further purification.

2.2 Electrospinning process

The PLLA powder (2 g) was dissolved in 20 ml dichloromethane in a flask, and the PLLA solution was mixed with PVP at PLLA/PVP weight ratios of 100/0, 100/3, 100/5, 100/9 and then stirred for 8 h at room temperature, respectively.

The schematic setup of the electrospinning process used in this study was similar to those depicted elsewhere [17–19]. In brief, the above mixed solution was placed into a plastic syringe (10 ml) with a stainless steel needle with an inner diameter of 0.57 mm. The positive electrode of a high voltage power supply (GF-II High Static Voltage) was connected to the stainless steel needle by copper wires. The voltage was 20 kV, and the tip-to-collector distance was fixed at 20 cm. The electrospinning was performed at room temperature. A grounded collection drum was used as the collector and the fibrous nonwoven mats were collected on the surface of collection drum and dried at temperature of 60°C for 40 min.

2.3 Characterization of electrospinning films

The morphology of microfiber films collected on the collection drum was studied using SEM (JSM-6460, Japan) after gold coating. The properties of the microfiber films were analyzed by DSC (DSC2910, TA, USA) and contact angle measurement (JC2000, Shanghai, China).

2.4 Cell culture

The VSMCs were cultured at 37°C in a humidified atmosphere of 5% CO_2 in air, in 25-cm^2 tissue culture flasks containing 5 ml Dulbecco's modified Eagle medium (DMEM, Gibco, USA) supplemented with 10% newborn calf serum (Gibco), 50 U/ml penicillin and 50 U/ml streptomycin.

A density of 5×10^4 cells/cm^2 were seeded onto the microfiber films, which were sterilized by ^{60}Co γ-irradiation at a dose of 15 kGy, in a 24-well plate for MTT assay, SEM observation and LCSM (LCSM, Bio-Rad Radiance 2100, AG-2Q, Bio-Rad, UK). The assays were performed at 2, 4 and 6 days, respectively.

2.5 Cell morphology

After 2, 4 and 6 days culture, the microfiber films were washed with PBS to remove the non-adherent cells and then fixed with 2.5% glutaraldehyde for 20 min at room temperature, dehydrated through a series of graded alcohol solutions, and then air-dried overnight. All samples were sputter coated with gold before SEM observation.

Cells cultured on the films were also examined by LCSM after being through washed with PBS and stained with propidium iodide (PI, Sigma, St.Louis, MO, USA) to visualize the cells. Cells were incubated in 5 μg/ml PI solution diluted with PBS buffer for 30 min at 37°C. Then the samples were washed with PBS buffer for three times. The mixture of 50% glycerin and 50% PBS buffer was added to keep the samples wet during the examining period. According to the emission wavelength of PI, the filter was set 570 LP and the laser was 543 nm in the fluorescence mode. In the reflection mode, the laser was 488 nm and the filter was set 488/4 nm.

2.6 MTT assay

The MTT (Sigma) assay was used to determine the level of cellular energy metabolism and indicate the condition of

cell proliferation indirectly. After VSMCs were cultured in 48-well plate for 2, 4 and 6 days, the cell proliferation was evaluated by MTT assay. Fifty micro liters of MTT (5 mg/ml) was added to each well and incubated at 37°C for 4 h in humidified atmosphere of 5% CO_2 in air. At the end of the assay, the blue formazan reaction product was dissolved by adding 200 µl dimethyl sulfoxide (DMSO) and 100 µl solution was transferred to a 96-well plate. The solution of each sample was placed in a microtiter plate and the absorbance at 490 nm was measured on a SS-3000 Immunoanalyser. The absorbance at 490 nm can be quantified to cell number.

2.7 Platelet adhesions

The films of PLLA and PVP, weight ratios of 100/0, 100/3, 100/5 and 100/9, were prepared by solution casting method on a glass plate for platelet adhesion study. The films with diameter of 1 cm were incubated in PBS (pH 7.4) for 1 h for use. Fresh human blood, anticoagulated with acid citrate dextrose, was centrifuged at 2,500 rpm for 5 min to obtain the platelet rich plasma (PRP). The films were laid flat in 48-well plate and submerged with PRP and left at 37°C for 30 and 120 min in an incubator, respectively. After washed gently three times with PBS to remove the non-adhering platelets, they were fixed with 2.5% buffered gluteraldehyde overnight in the refrigerator at 4°C, and then dehydrated through a series of graded alcohol solutions, and then air-dried overnight and observed by SEM.

3 Results and discussion

3.1 Characterization of microfiber films

3.1.1 DSC analysis

Figure 1 illustrated the DSC thermograms of the microfiber films of PLLA/PVP with different weight ratios. The glass transition temperature (T_g) and melting temperature (T_m) of the pure PLLA were about 63 and 176°C, which were similar to the earlier reported [20]. However, the T_g of the pure PVP was near to 165°C. Both T_g and T_m of the microfiber film with different PVP content were in accordance with the pure PLLA microfiber film. This indicated that the crystalline microstructure of the composite microfiber films developed well. And there was an exothermic peak after the T_g, which was recognized as a cold crystallization temperature. And the exothermic peak shifted toward the higher temperature when the weight ratio of PLLA and PVP was 100/9 in the Fig. 1. This demonstrated that the PVP in the blends leaded to

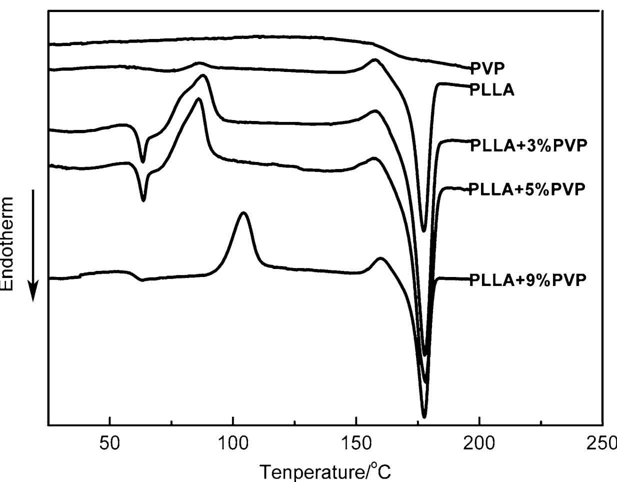

Fig. 1 DSC curves of the microfiber films with different weight ratios of PLLA/PVP

molecular re-orientation in the microfiber films and strong intermolecular force [20–22].

3.1.2 Morphology of microfiber films

An interesting feature of the microfibers was that they had pore structure on their surfaces, and the diameter of the pores was about 300 nm (Fig. 2a–c). This morphology was believed to the result of the rapid evaporation of the highly volatile solvent and solidification out of the spinning jet. Other studies had observed the direct formation of pores in electrospinning PLA, PMMA and polystyrene [23]. Figure 2 also indicated that it was possible to vary the pore size and density by adjusting the PVP content. In Fig. 2a, the number of the pores on the PLLA microfibers was about 11/µm^2. When the weight ratio of PLLA/PVP was up to 100/9, the pores disappeared. Furthermore, the depth of the pores became lower with increase of PVP content. Another interesting feature of the microfiber films was found that the mean diameter of single microfibers decreased from 6 to 2 µm with increase of PVP content from 0 to 9% (Fig. 2a–d). The observed results might be attributed to the change of viscosity and conductivity which were influenced by the PVP content. It was similar to the results of other articles in which the authors added PVP to PVA [24, 25].

3.1.3 Contact angles measurement

The contact angles of distilled water on the surface of the electrospinning microfiber films were measured and displayed in Table 1. This measurement was used to evaluate the hydrophilicity of the electrospinning membranes with different PVP contents. The contact angle results were in good agreement with the literature values of 74°, [26]

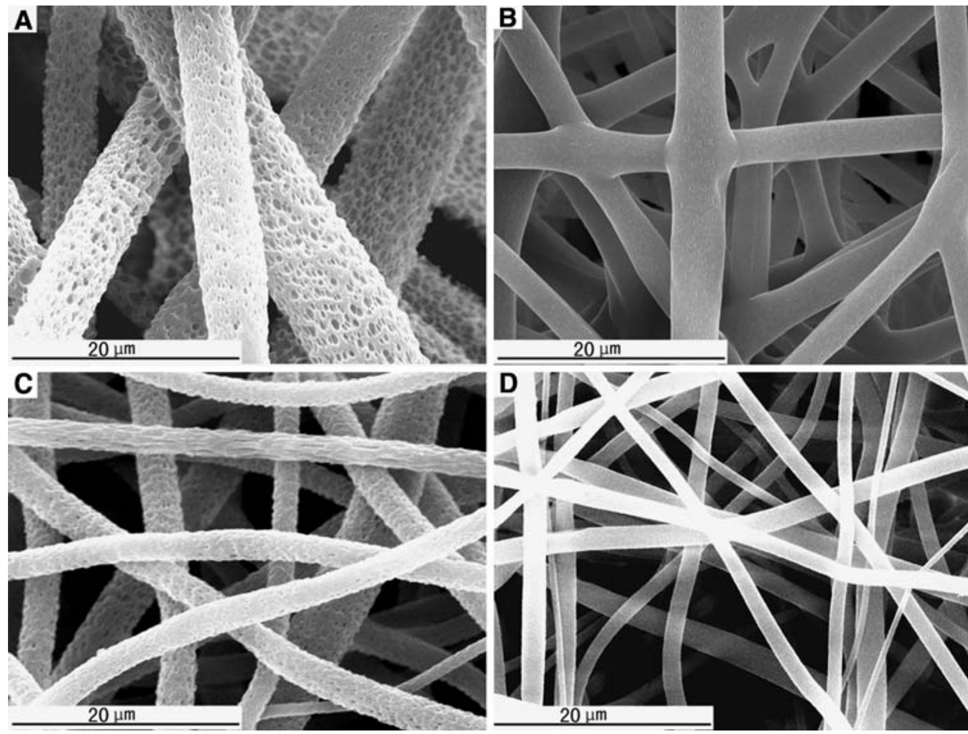

Fig. 2 SEM micrographs of microfiber films: **a** PLLA, **b** PLLA + 3% PVP, **c** PLLA + 5% PVP, and **d** PLLA + 9% PVP

Table 1 The values of contact angle of PLLA and PVP in different weight ratios of 100/0, 100/3, 100/5, 100/9

	PLLA	PLLA + 3% PVP	PLLA + 5% PVP	PLLA + 9% PVP
Contact angle (°)	74.4 ± 1.3	69.5 ± 1.3	66.1 ± 1.0	62.8 ± 1.2
Images				

whereas we found of 74.4 ± 1.3°. The contact angle results were considered that they decreased with the increase of PVP content as expected from the hydrophilicity of the PVP.

3.2 Biological evaluations

3.2.1 *Cell distribution and morphology*

The VSMCs were seeded on the electrospinning microfiber films and cultured for 2, 4 and 6 days. The morphology of VSMCs during the culture was observed by an invert phase-contrast microscopy. From the observation, the microfiber with the increase of PVP could enhance cell attachment and spreading. The cell distribution and morphology in 6 days was observed by the LCSM and SEM, as shown in Figs. 3 and 4, respectively. LCSM observation of VSMCs cultured with the increase of PVP in microfiber films (Fig. 3) testified that VSMCs spread not only in large number but also distribute evenly. The distance between the slices in the pictures in Fig. 3 was different in vertical. The distance between the slices of VSMCs cultured on PLLA film (Fig. 3a) and the PLLA adding 3% PVP film (Fig. 3b) was 8 μm and the total growth depth of the VSMC was 64 μm, whereas the distance between the VSMCs cultured on PLLA adding 5 and 9% PVP films were 12 μm (Fig. 3c, d), and the total growth depth of the VSMCs was 96 μm. The growth depth of VSMCs in the films was different which indicated better biocompatibility in 3-dimension space. Furthermore, the SEM images (Fig. 4) were analyzed that the VSMCs reached out "pseudopods" and had better morphology in 2-dimension space. In the microfiber films that added PVP content from 3 to 9%, the "pseudopods" was more obvious, progressively. In Fig. 4a, b, only two cells adhered to less microfibers. However, in Fig. 4c, d, a mount of cells

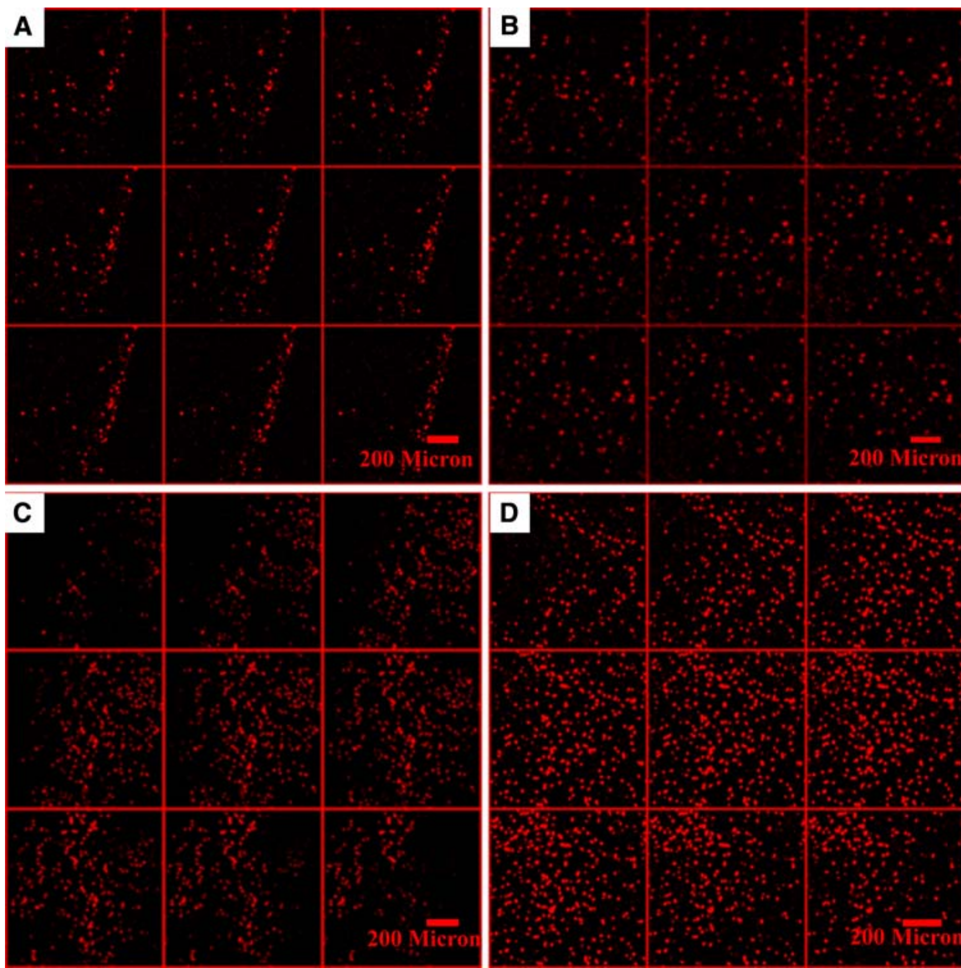

Fig. 3 LCSM observation of VSMCs cultured on films for 6 days: **a** PLLA, **b** PLLA + 3% PVP, **c** PLLA + 5% PVP, and **d** PLLA + 9% PVP

adhered to more microfibers. Moreover they formed a relatively thin and continuous monolayer.

LCSM images implied that the cell distribute evenly with increase of PVP content. And the SEM images showed the cell morphology become better with PVP content increase.

3.2.2 Cell proliferation

The cell growth and proliferation was measured by MTT assay of VSMCs cultured on the different electrospinning microfiber films with 2, 4 and 6 days culture, shown in Fig. 5. The proliferation of VSMCs cultured on each microfiber films increased with the increasing of culture time and the content of PVP. In the study, the cell proliferation of VSMC cultured on PLLA/PVP microfiber films was obviously more than that on PLLA microfiber film. In addition, the cell proliferation of VSMCs was improved with different content of PVP. In Fig. 5, the cell proliferation and growth were best on the PLLA/PVP of 100/9 films in 6 days. The absorbance at 490 nm was almost 1.00.

3.2.3 Platelet adhesions

The results of platelet adhesion test had been shown in Fig. 6. It showed the SEM images of the platelet adhered on surfaces of the PLLA containing 0 and 5% of PVP for 30 min (Fig. 6a–d) and 120 min (Fig. 6e–h). After 30 min adhesion, the number of adhered platelets per square on PLLA film (Fig. 6a) was more than that on PLLA-5% PVP film (Fig. 6c). About 14 platelets adhered to the PLLA film in Fig. 6b, while only one platelet adhered on the PLLA-5% PVP film in Fig. 6d. In addition, the platelets reached out “pseudopods” and aggregated together on the PLLA surface (Fig. 6b). However, the platelets on the PVP blend surfaces exhibited rotundity and complete platelets (Fig. 6d). After 120 min adhesion, the number of the platelets on both the surfaces was increase than that after 30 min adhesion (Fig. 6e, g). From Fig. 6f, h, we can see the number of platelets increased to 23 and 1, respectively. With the extension of time, the platelets reached out more “pseudopods” and aggregated more together on PLLA film (Fig. 6f) than that on PLLA-5% PVP film (Fig. 6h). The result indicated

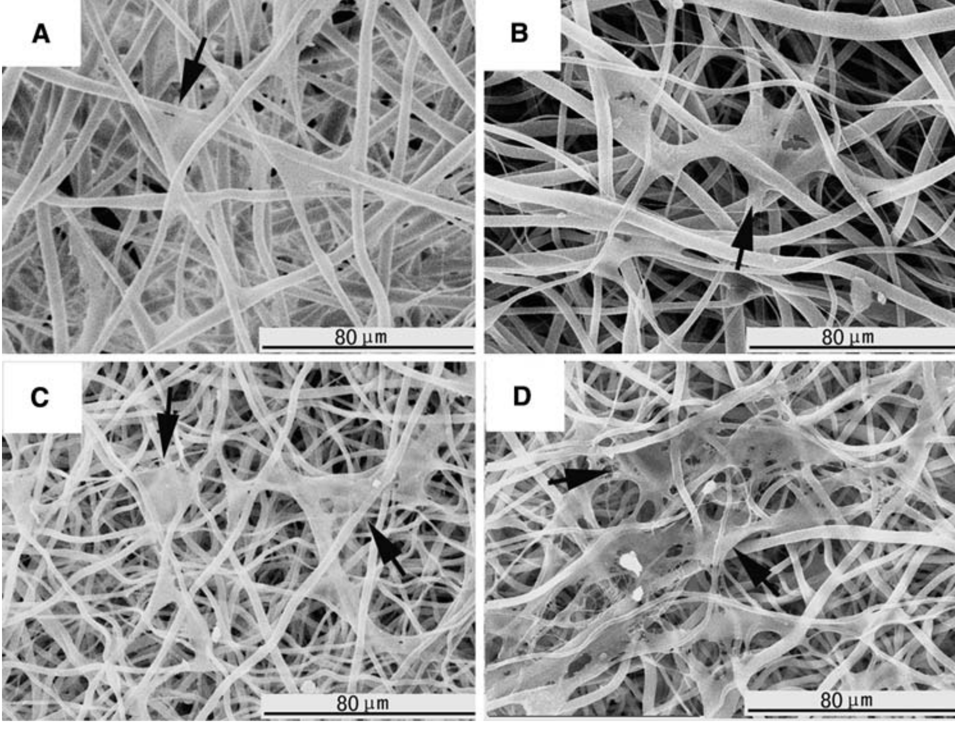

Fig. 4 SEM images of VSMCs cultured on films for 6 days: **a** PLLA, **b** PLLA + 3% PVP, **c** PLLA + 5% PVP, and **d** PLLA + 9% PVP

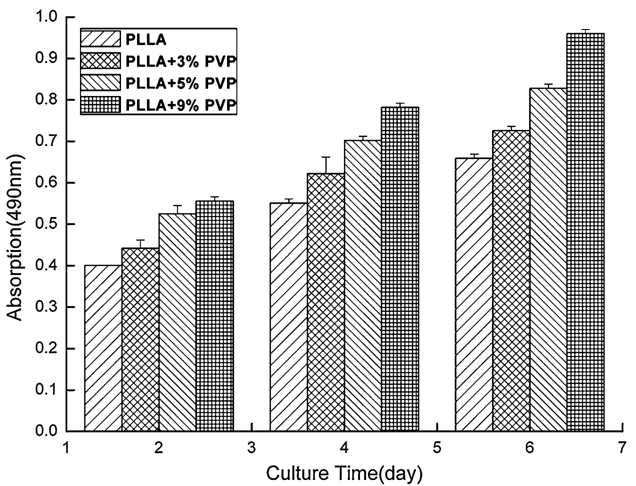

Fig. 5 MTT assay of cell viability from VSMCs cultured on films

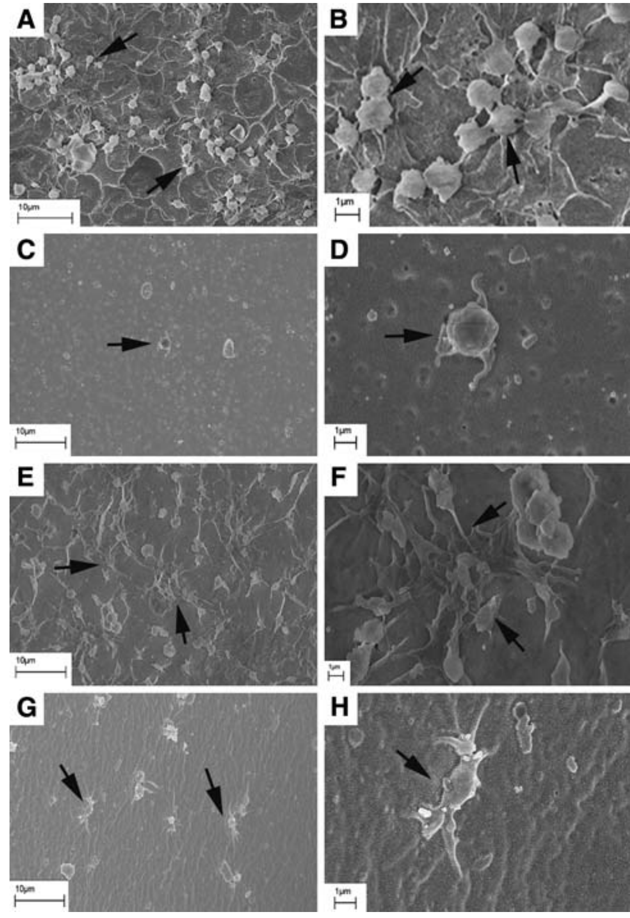

Fig. 6 SEM photographs of testing platelet adhesion on microfiber films: **a** PLLA, **b** PLLA + 3% PVP, **c** PLLA + 5% PVP, and **d** PLLA + 9% PVP

that pure PLLA film could active platelets more effectively than PLLA-5% PVP film. This is due to the different chemical composition at the interface. The results of our research and the activation process of the human platelet were same as the report of Yangzhe Wu [27]. It was clear from the magnified images that with PVP added in PLLA, the blood compatibility of the PLLA surface had enhanced.

PVP has contributed in improving the hydrophilicity of microfiber films due to its physical and chemical nature provided with the contact angle measurement. It is becoming a trend that the hydrophilicity of microfiber films get better with the increase of PVP content.

However, maybe there exists a threshold. Furthermore, we could adjust the content of PVP for different application.

4 Conclusions

In this study, PVP were successfully introduced to the PLLA microfiber films by electrospinning technique to improve the hydrophilicity of electrospinning microfiber films. The films obtained with blending with PVP was beneficial to improve the VSMCs compatibility on PLLA substrates, and the platelet adhesion test showed that the microfiber films had excellent blood compatibilities with the addition of PVP in PLLA. In our research, VSMCs growth may be affected by the PVP content and the changes of microfiber morphology. These will be made further research in our future work. However, the composite electrospinning microfiber films might be potential applications in blood vessel tissue engineering.

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