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Surface biocompatible modification of polypropylene by entrapment of polypropylene-block-poly(vinylpyrrolidone)

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ABSTRACT

A macromolecular surface modifier, namely polypropylene-block-poly(vinylpyrrolidone) copolymer, could diffuse preferably onto the surface and effectively increased the biocompatibility of PP. The blood and cell compatibilities of PP and PP-*b*-PVP/PP blend films were evaluated by determination of the extent of platelet adsorption, plasma recalcification time, hemolysis rate and cell proliferation using smooth muscle cells from rabbit aorta. The results revealed that the blood and cell compatibilities of PP were improved significantly by entrapment of a few PP-*b*-PVP copolymers in PP.

1. Introduction

As a polymer soluble in both water and organic solvents, poly(vinylpyrrolidone) (PVP) has been the focus of numerous applications including additives, cosmetics, coatings and biomedicines. For example, Robinson and Williams [1] reported that PVP could be simply adsorbed on silica particles to inhibit protein adsorption and remove adsorbed proteins from the particle surface. The principal reason for successful PVP applications is its excellent biocompatibility with living tissues and extremely low cytotoxicity. Belfort and coworkers [2] had also successfully photochemically modified poly(ether sulfone) ultrafiltration membrane with *N*-vinyl-2-pyrrolidone to increase the surface wettability and decrease the adsorptive fouling. On the other hand, Higuchi et al. [3] covalently conjugated PVP on the surface of polysulfone membrane with a multiple chemical process. It was reported that PVP modified polysulfone membrane gave lower protein adsorption from a plasma solution and much suppressed number of adhered platelets than original polysulfone and other surface-modified membranes.

Polypropylene (PP) has become one of the most developed polymers because of its low price, abundant resources and processing convenience. Besides, its desirable mechanical properties, wear resistance as well as its non-toxicity have propelled us to try to research and promote its potency as a kind of good biomaterial. However, PP is quite different from other developed biocompatible polymers in that its chemical composition contains only carbon and hydrogen, which results in its higher hemolysis rate, adherent platelets and platelet aggregates, and limits its potential applications in biomaterial fields such as enzyme-immobilized membrane bioreactors, bioseparations and biomedical devices. To improve its biocompatibility, water-soluble monomers such as D-gluconamidoethyl methacrylate [4], *N*-vinyl pyrrolidone [5] and sodium acrylate [6] or $COOH^+$ ion [7] have been introduced onto the surface of PP by γ-radiation or UV-induced polymerization. However, chemical grafting modification often deals with a series of complicated chemical reactions and refining process.

In our previous study [8], we had illustrated that PP-*b*-PVP used was an A-B-A triblock copolymer and had a good thermal stability determined by quantitative FTIR spectroscopy and thermal gravimetric analyzer, respectively. We also had verified PP-*b*-PVP suitable to be used as a macromolecular surface modifier to enhance effectively the hydrophilicity of PP with lasting-effective. In this work, PP-*b*-PVP was used as a functional polymer to be blended with PP to increase its surface biocompatibility. The cell and blood compatibilities of the PP-*b*-PVP/PP blend films with different contents of PP-*b*-PVP were evaluated by platelet adher-

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ent, plasma recalcification time, hemolysis rate, cell compatibility and cell proliferation of smooth muscle cells from rabbit aorta (RaSMCs).

2. Materials and methods

2.1. Materials

Polypropylene-block-poly(vinylpyrrolidone) A-B-A type triblock copolymer was synthesized through the esterification of dicarboxyl-terminated polypropylene with monohydroxyl-terminated poly(vinylpyrrolidone) [8]. The molecular weights of polypropylene and poly(vinylpyrrolidone) segments in PP-*b*-PVP were 2000 and 1500 g/mol, respectively. Smooth muscle cells from rabbit aorta (RaSMCs) used in the experiments were kindly provided by Multidisciplinary Research Center of Shantou University (Guangdong, China). DMEM medium (Gibco, USA), fetal bovine serum (FBS, Min Hai Bio-engineering, P.R. China), penicillin (Sigma, USA), streptomycin (Sigma, USA), phosphate-buffered saline (PBS, Shanghai Sangon Biological Engineering Technology & Services, China), cell counting kit (CCK-8, Beyotime, China) were used as received. Glutaraldehyde (50%, Alfa-Aesar) was diluted to 2.5% with PBS buffer before being used.

2.2. Preparation of tested films

In a typical procedure, 0.0017 g of PP-*b*-PVP triblock copolymer prepared was added to 0.1796 g of host PP in a 25 ml flask equipped with a magnetic stirring bar in 20 ml xylene. After heating the suspension to 120 °C using an oil bath, a uniform solution of PP and PP-*b*-PVP triblock copolymer was formed by continuous stirring for 1 h. The solution of the PP-*b*-PVP triblock copolymer entrapped in PP was immediately poured into a flat-bottomed dish maintained at 120 °C in an explosion-proof oven, which allowed the solvent to evaporate. The dish was removed from the oven and allowed to cool to room temperature. It was then placed in a vacuum oven for 30 min to remove the last traces of the solvent. The polymer film was peeled off the glass dish and cut into rectangular strips to be analyzed. The thicknesses of films were about 80–100 μm.

Similar procedures were used to prepare other loadings of PP-*b*-PVP in PP varying the quantity of PP and functional copolymer as necessary. In our work, the contents of the modifier were 1%, 2%, 3%, 4% and 5%.

2.3. Blood compatibility

2.3.1. Platelet adhesion

Whole rabbit blood mixed with 3.8% sodium citrate was centrifuged at 1500 rpm for 10 min at 4 °C to obtain platelet-rich plasma (PRP), which was used in platelet adhesion test. The polymer films in the glass dish were sterilized with 75% ethanol, then were washed three times with PBS and equilibrated in PBS overnight. After being heated to 37 °C, 1 ml PRP was added to the films, and which were then incubated at 37 °C for 1 h. Films were rinsed three times with PBS to remove the platelets which were not attached to the film surface. The platelet-attached films were fixed, dehydrated and dried for SEM examination. Two parallel films were performed for each polymer. Four different regions were randomly counted on each film and a mean value was calculated.

2.3.2. Plasma recalcification time

Plasma recalcification time (PRT) is an indicator of intrinsic coagulation cascade activation, and therefore is a useful marker of biomaterial induced coagulation activation [9]. Whole rabbit blood mixed with 3.8% sodium citrate solution was centrifuged at 3000 rpm for 10 min to separate the blood cells, and the remaining platelet-poor plasma (PPP) was used for the PRT experiments. The polymer films (0.5 cm × 0.5 cm) equilibrated in 0.9% NaCl solution for 24 h, and then the PPP (0.2 ml) was placed on the films and incubated statically at 37 °C. A 0.025 M $CaCl_2$ aqueous solution (0.2 ml) was added to the PPP and the plasma solution was monitored for clotting by manually dipping a stainless-steel wire hook coated into the solution to detect fibrin threads. Clotting times were recorded at the first signs of any fibrin formation on the hook. The experiment was repeated in quadruplicate and a mean value was calculated.

2.3.3. Hemolysis rate

Whole rabbit blood, heparinized by adding a mixture of 1% heparin saline solution and 2% potassium oxalate, was diluted with 0.9% NaCl solution to prepare the suspension of erythrocytes. The polymer films (0.5 cm × 0.5 cm) were added in a tube with 10 ml 0.9% NaCl solution as a control. After incubating all tubes at 37 °C for 30 min, 0.2 ml suspension of erythrocytes was added, respectively, and incubated at 37 °C for 60 min. Then blood cells were removed by centrifugation at 2500 rpm for 5 min and the upper clear solution of the blood mixture was characterized at an absorbency wavelength of 545 nm with a spectrophotometer (721#, made in China). The sample for positive control was prepared by mixing 10 ml distilled water with 0.2 ml blood sample, while the sample for negative control was just the mixture of 10 ml 0.9% NaCl and 0.2 ml blood sample. Experiments were performed in triplicate and a mean value was calculated.

2.4. Cell compatibility

Cell compatibility assay was performed according to the published method [10]. RaSMCs was incubated in DMEM supplemented with 10% (v/v) FBS, 50 U/ml penicillin and 50 U/ml streptomycin at 37 °C in 5% CO_2 incubator. All tested films were sterilized first using 75% ethanol/water solution overnight. Subsequently, the films were dipped in PBS three times 0.5 h each. DMEM with 10% (v/v) FBS was exchanged for another night. RaSMCs with a density of 1.0×10^4/well were seeded on different tested films in a 24-well tissue culture plate at 37 °C in the 5% CO_2 incubator. After incubation, all films were dipped in 2.5% glutaraldehyde buffer solution overnight. The fixed tested films were washed three times with PBS for 15 min each and then dehydrated, followed by drying for SEM examination. In order to reveal the morphology of cells cultured on the films, cells cultured for 3 days on the films were examined by SEM.

2.5. Cell proliferation

A cell count kit-8 was employed in this experiment to quantitatively evaluate the cell viability. 360 μl DMEM with 40 μl CCK-8 was added to each pre-cultured film and then the plates were incubated in 5% CO_2 incubator for 3 h at 37 °C. The absorbance at 450 nm was determined using the microplate reader (Multiskan MK33, Thermo Electron Corporation, China).

2.6. SEM examination

Cell-seeded and platelet-attached films were washed twice using PBS and immersed in PBS containing 2.5 wt% glutaraldehyde (pH = 7.4) overnight. They were then dehydrated in an ethanol-graded series (from 30%, 50%, 70%, 90%, 95% to 100%) for 15 min, respectively, followed by lyophilization. The samples were then mounted on aluminium stumps, coated with gold in an auto fine coater (JFC-1600, JEOL, Japan) for 10 min, following by examination under a scanning electron microscope (JSM-6360LA, JEOL, Japan).

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2.7. Statistical analysis

All quantitative results were obtained from triplicate samples or more. Data were expressed as the mean ± SD. Statistical comparisons were performed using Student's test. *P*-values <0.05 were considered statistically significant.

3. Results and discussion

Material surface properties such as charge density, chain mobility, hydrophilicity, have been shown to effect the composition and conformation of the adsorbed protein layer [9]. Most of polymer materials are poor biocompatibility. They can induce surface thrombus and activate platelets, which result in the adsorption of certain plasma proteins and the adhesion of platelets when they are used as biomaterials to contact with blood or body fluid. To overcome these defects, it is necessary to modify the surface of polymers to enhance their surface biocompatibilities. Appropriate surface modification of existing polymeric biomaterials possessing

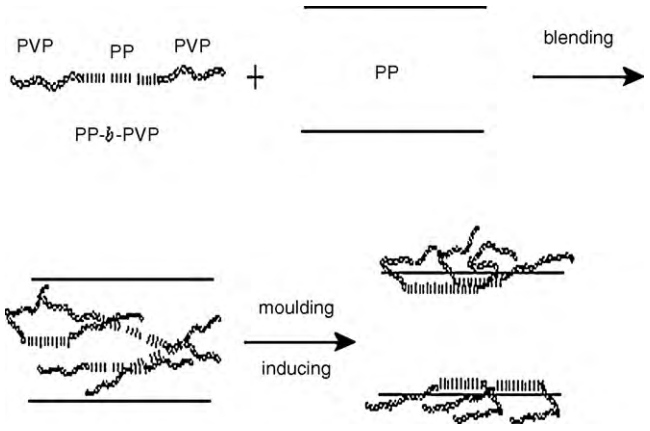

Fig. 1. Model of surface modification by blending.

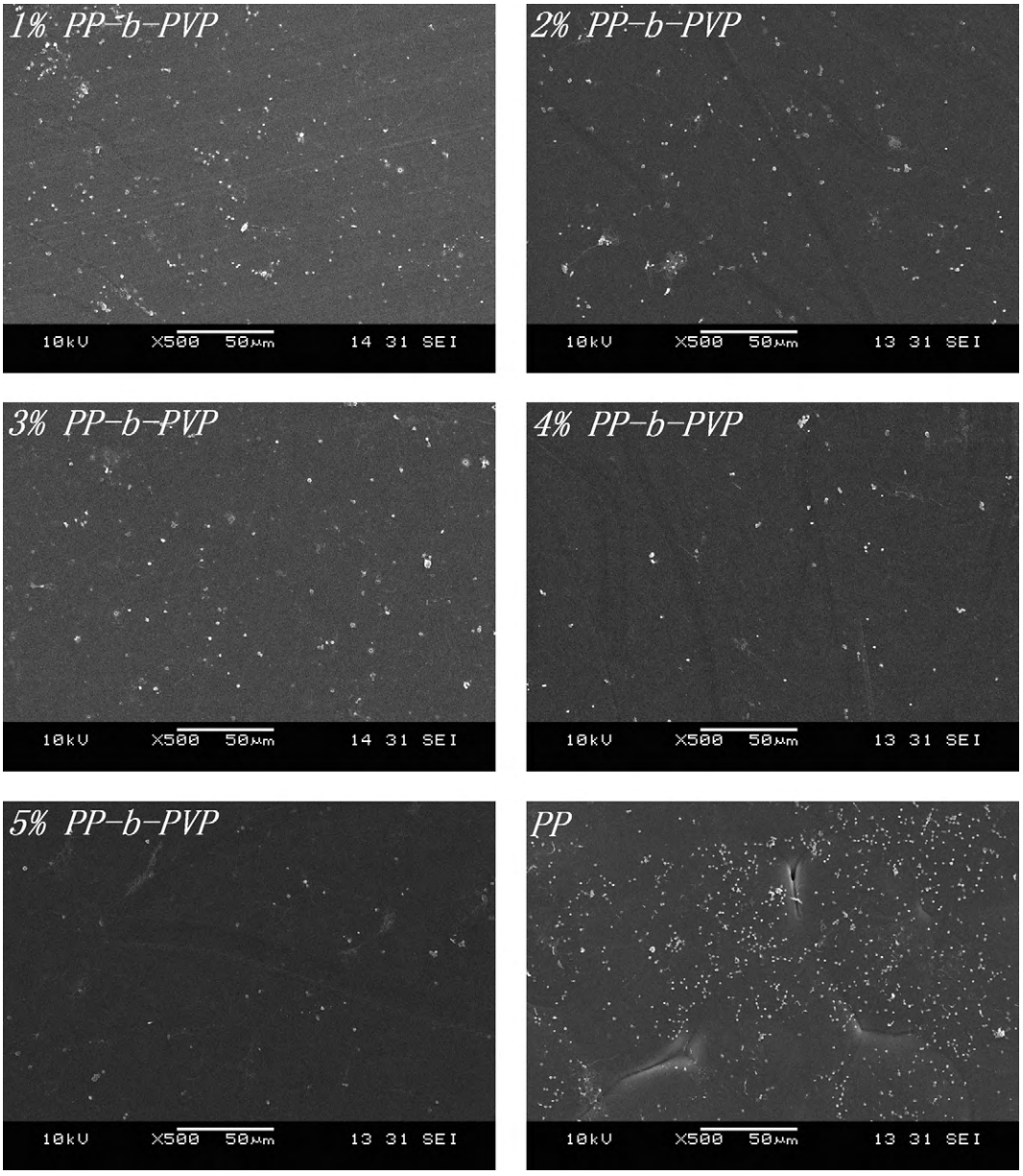

Fig. 2. SEM images of platelet adhesion on tested films.

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the desired physical properties is beneficial in improving biocompatibility without altering the bulk properties of the biomaterials. As far as we know, three general categories such as physical adsorption, grafting coupling and surface grafting polymerization have been used to enhance the surface biocompatibility of polymer materials [11]. However, grafting coupling and grafting polymerization often result in the remainders of chemicals, which is fatal for biomaterials. Homopolymer additives used in biocompatible modification of PP by blending are clung to the host polymer by simple physical absorption and can easily be extracted by blood or body fluid. In our prior studies, we had verified that PP-*b*-PVP triblock copolymer was suitable to be used as a macromolecular surface modifier and could diffuse preferably onto the surface of PP to enhance its hydrophilicity effectively by ATR-FTIR, contact angle measurement and scanning electron microscopy. For example, the water contact angle of film was reduced from 99.51° of PP to 55.49° of modified PP at loading of 5% PP-*b*-PVP triblock copolymer [8]. We also had found that macromolecular surface modifier with host-philic segment had adequate affinity for host polymer to avoid to be extracted by ethanol, water or acetone, which endowed the modification with lasting-effective [12,13]. The strategy is illustrated in Fig. 1. PP-*b*-PVP triblock copolymer could diffuse preferably onto the surface of PP by the inducement of high energy interface. The host-philic segment in macromolecular surface modifier endows the modifier to interact strongly to host polymer, while the biocompatible segment located on the surface can enhance the surface biocompatibility of modified PP. Based on this strategy, the surface biocompatible modification of PP can be achieved by simply blending with functional macromolecular surface modifier.

3.1. Blood compatibility

3.1.1. Platelet adhesion

Platelet adhesions as well as plasma recalcification time and hemolysis rate are often used to evaluate the blood compatibility of biomaterials. Fig. 2 is the SEM micrographs of the studied films contacted with platelets for 1 h, the statistical numbers of platelets adhered on the films are also shown in Fig. 3. We can see that the amount of adhered platelets on unit films surface decreased sharply by blending with small amount of PP-*b*-PVP. The adhered platelets would further decrease with the increase of modifier concentration. At higher modifier concentration, only few platelets adhered to the films surface. These results indicated that the hemocompatibility of PP can be improved obviously by introducing of PVP, which may be ascribed to the hydrophilicity and the biocompatibility of PVP segment. It has been suggested that the behavior of platelet adhesion on polymeric materials depends strongly on the surface characteristics such as wettability, hydrophilicity/hydrophobicity balance,

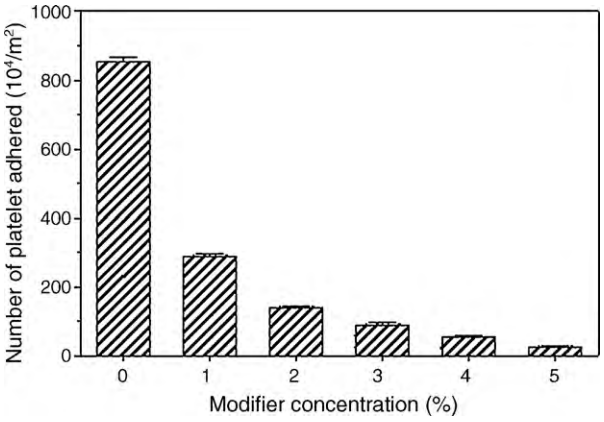

Fig. 3. Number of platelets adhered on tested films for 1 h at 37 °C. Data are presented as mean ± standard deviation of four different experiments.

surface free energy, chemistry, charge density, roughness, micropore diameters and mechanical characteristics [14]. In general, material hydrophilicity plays an important role in blood compatibility. A hydrophilic surface absorbed fewer platelets, and thus blood coagulation time would be longer on a hydrophilic surface compared with a hydrophobic one [15]. In our previous work [8], we had verified that PP-*b*-PVP copolymer could diffuse preferably onto the surface and effectively increased the hydrophilicity of PP by the results of ATR-FTIR and contact angles measurements. When the films surface contacts with platelets, the PVP segments can be hydrated, these hydrated segments on the surface have exerted hydrodynamics and steric hindrance effects to the approaching of platelets, which greatly reduces the adhesion and aggregation of platelets.

The shape of platelet adhering on the film surfaces is also one of the characteristics of biocompatibility. It was clearly observed that the platelets on the PP extended their pseudopods, which indicated the activation of the platelets, and the aggregation of platelets on film surface (see Fig. 4). This is the most important factor to restrict PP hollow fiber membrane application in the artificial lung oxygenator. In comparison, the platelets adhered to the surfaces of modified PP film with 5% modifier kept their original spherical shape. There were fewer platelets adhered on the modified polypropylene film without platelets aggregation. This result means that the surfaces do not activate the platelets. From the adsorption and the shape of the adhered platelets, we can conclude that the platelet compatibility of the polymer changes drastically at loadings of 5% modifier.

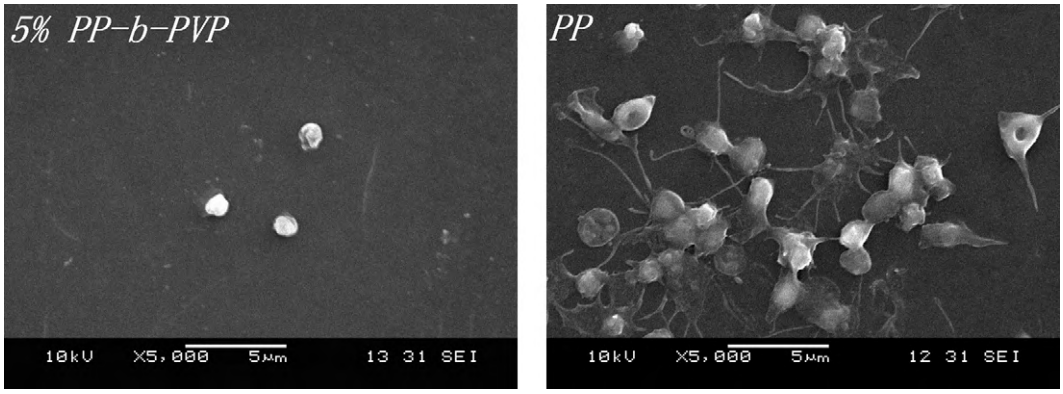

Fig. 4. SEM images of platelet morphology on tested films.

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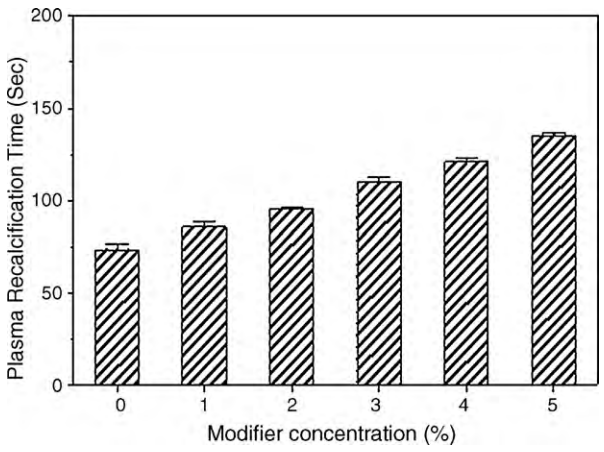

Fig. 5. Plasma recalcification time of tested films. Data are presented as mean ± standard deviation of four different experiments.

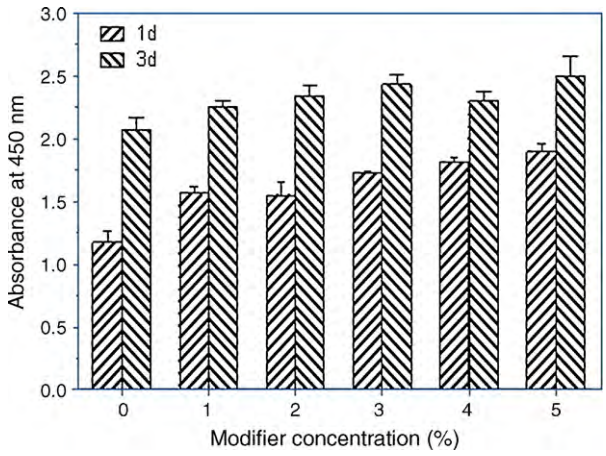

Fig. 6. Proliferation of RaSMCs on tested films for 1 day and 3 days. Data are presented as mean ± standard deviation of three different experiments.

3.1.2. Plasma recalcification time

Plasma recalcification time (PRT), a measurement of intrinsic coagulation cascade activation, indicates the time required for fibrin clot formation once calcium has been introduced into sodium citrate anticoagulated plasma. Contact activation of the intrinsic cascade in plasma will vary with the type of surface, and thus plasma recalcification can be used as an indicator of blood–biomaterial interactions [9]. Longer PRT often means excellent biocompatibility of surface. The plasma recalcification times of the surface-modified PP films are shown in Fig. 5. The PRTs of PP surface were slightly increased by the immobilization of PP-*b*-PVP with 1% loadings of PP-*b*-PVP. However, the PRTs were significantly prolonged through increasing the loadings of the copolymer. Nearly double PRT can be achieved on the surface of modified film at loading 5% PP-*b*-PVP, indicating that the blood compatibility of PP can be enhanced effectively by introducing PVP on the surface of PP.

3.1.3. Hemolysis rate

The blood compatibility of modified PP is evaluated by hemolysis tests, which is related to the interaction of modified PP with healthy rabbit's blood. The absorbency at a wavelength of 545 nm of the samples was characterized with a spectrophotometer. The extent of hemolysis is manifested as hemolysis rate (%) using the following equation [16]:

$$\text{hemolysis rate}\,(\%) = \frac{\text{OD}_{\text{sample}} - \text{OD}_{\text{negative}}}{\text{OD}_{\text{positie}} - \text{OD}_{\text{negative}}} \times 100$$

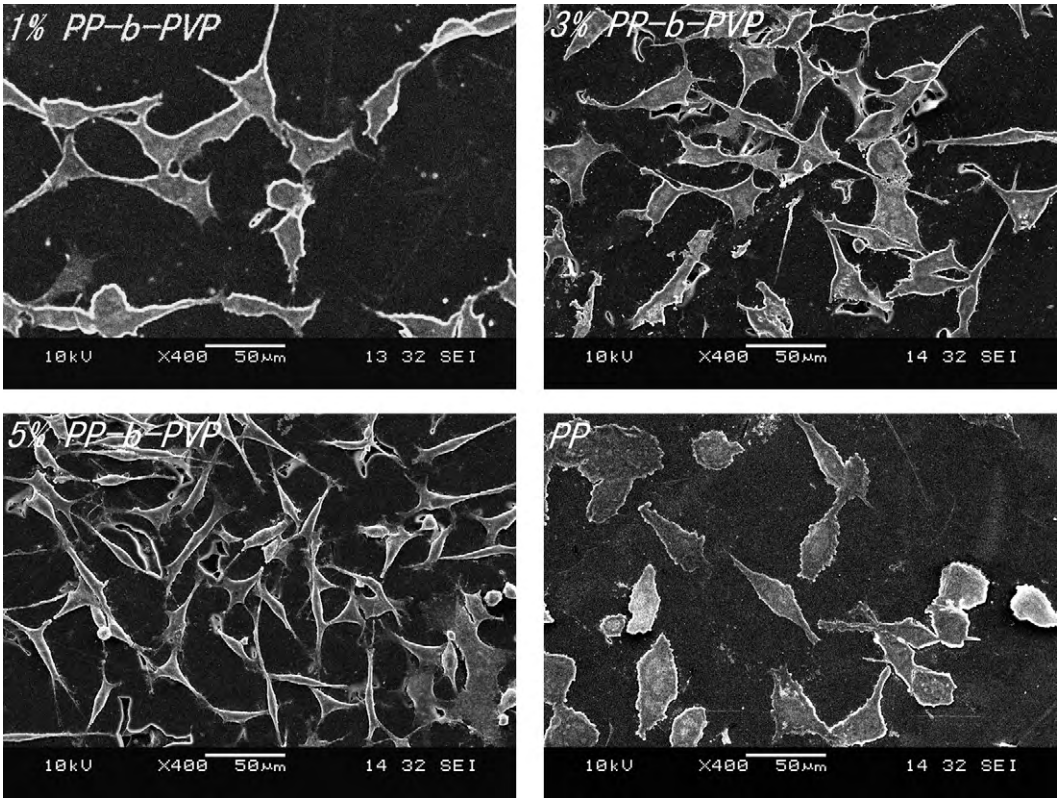

Fig. 7. SEM images of RaSMCs cultured on tested films for 3 days.

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Table 1
Hemolysis test of modified of modified PP films.

Percentage of PP-*b*-PVP (%)	Hemolysis rate (%)
1	2.0
2	1.8
3	1.4
4	0.9
5	0.7

The hemolysis rate of PP is 2.9%.

where OD_{sample} is the absorbency of the test films, $OD_{positie}$ is the absorbency of the positive control and $OD_{negative}$ is the absorbency of the negative control. The data are listed in Table 1. By entrapment of a few PP-*b*-PVP additives, the hemolysis rate of modified PP film surface significantly lowered, especially at higher loadings of PP-*b*-PVP. The hemolysis of modified PP would decrease with the increasing of the content of PP-*b*-PVP. Only 0.7% hemolysis rate was observed at loadings of 5% PP-*b*-PVP, which was much lower than the medical adsorbent standard used in China (<5%) (equivalent to ISO 10993.4:2002), indicting that the blood compatibility of PP film was effectively enhanced by blending with PP-*b*-PVP macromolecular modifier.

3.2. Evaluation of cell compatibility of the tested films

The growth and proliferation of the RaSMCs, which was acquired from the blood vessels of rabbit, on the tested films were used to investigate the cell compatibility of modified PP. The results are shown in Fig. 6. The RaSMCs began to show remarkable growth and proliferation after 1 day and 3 days incubation on modified films, based on the CCK-8 assay. This demonstrated that all modified PP films possessed the ability to maintain the cell viability, growth, and the non-toxicity of the modified PP films to RaSMCs. Meanwhile, modified PP films showed the least cell viability among the tested materials in all time period. After 1 day incubation, CCK-8 assays of RaSMCs on tested films showed that cell proliferation on all modified films was 40–70%, higher than that on PP. After 3 days incubation, RaSMCs spread very well on all the tested films, but the growth and proliferation of RaSMCs on all the modified PP films were still better than those on PP.

SEM revealed a better cytomorphology of RaSMCs on modified PP than that on PP (Fig. 7). The cell morphology changed obviously from a non-contractile phenotype to a quiescent contractile phenotype during the cultivation process, suggesting a conversion of the cells grown on the modified PP films from pathological to physiological states [17,18]. Furthermore, the cell growth on 5% PP-*b*-PVP became much smaller than their size on other tested films, adhering at only few points to the surface and showing more cohesion to each other. This change characterized that RaSMCs change to contractile-like phenotype [19]. It has been reported that the polymer properties including chemical composition, surface morphology, surface energy and hydrophobicity play an important role in regulating phenotype of cell growth [15,20], and the interaction between cells and a material surface contributes partially to material biocompatibility [21,22]. Usually, a hydrophilic interface is regarded to be beneficial for cell adhesion and proliferation [23]. Meanwhile, low crystallization degree and rapid crystallization rate usually generate a smooth surface which improves the attachment and growth of some mammalian cells [24]. Modification of PP by introducing of PVP segment on the PP surface resulted in lower crystallinity and hydrophobicity of PP surface, and promoted a phenotypic change of RaSMCs from a contractile to a synthetic phenotype. This, in turn, may well explain the observed typical contractile-like phenotype of RaSMCs on modifier films.

4. Conclusion

PP-*b*-PVP can be used as a macromolecular surface additive to enhance the blood and cell compatibilities of PP by blending. The hemocompatibility of PP can be improved obviously by entrapment of PP-*b*-PVP in PP. Compared with those of PP, lower platelet adhesion and hemolysis rate, longer PRT times were found in modified PP film. The results of proliferation of RaSMCs also revealed that the modified PP films possessed excellent RaSMCs viability. Both blood and cell compatible determination demonstrated that the modified PP was biocompatible and made it desirable candidates for biomaterials. The results were ascribed to the hydrophilicity and biocompatibility of PVP segment located on the surface of PP.

Acknowledgements

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