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Synthesis, Characterization and Haemocompatibility of Poly(styrene-*b*-isobutylene-*b*-styrene) Triblock Copolymers

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Abstract: The synthesis of well-defined poly(styrene-*b*-isobutylene-*b*-styrene) (SIBS) triblock copolymers was accomplished by cationic sequential block copolymerization of isobutylene (IB) with styrene (St) using 1,4-di(2-chloro-2-propyl)benzene (DCC)/$TiCl_4$/2,6-di-*tert*-butylpyridine(DtBP) as an initiating system in methyl chloride (CH_3Cl)/methylcyclohexane(MeChx) (50/50 v/v) solvent mixture at −80 ℃. The triblock copolymers exhibited excellent thermoplastic and elastomeric characteristics. Tensile strengths and Shore hardness increased with increasing polystyrene (PS) content, while elongation at break decreased. The blood-compatibility of SIBS was assessed by SEM observation of the platelet adhesion, blood clotting time and haemolysis ratio. The haemolysis ratios were below 5% which met the medical materials standard. The platelet adhesion test further indicated that SIBS block copolymers had a good blood compatibility.

Keywords: poly(styrene-*b*-isobutylene-*b*-styrene), haemocompatibility, thermoplastic elastomer, living/controlled cationic polymerization.

Introduction

Biomaterials have become a very attractive research area in polymer science.[1] Most tissues other than bone and cartilage are of the soft category. So many medical device applications require the use of a biostable elastomer. These include the range of blood contacting catheters, ureteral catheters, intra-aortic balloon pumps, artificial hearts and drug delivery coating for catheters and stents.

Materials used in soft tissue replacement and reconstruction depend on the biomaterial properties. Medical-grade polyesters with Shore hardness A > 100, can hardly be considered elastomer and are used in woven or knitted structures to yield increased flexibility.[2] Polyurethane biomaterials are characterized by excellent mechanical and fatigue properties. However, they are subject to biodegradation; and the *in vivo* hydrolysis of polyurethanes may lead to potentially toxic diamines.[3,4] Silicone rubbers are classified as "thermoset rubber". They are based on poly(dimethylsiloxane), carrying functional groups for cross-linking (vulcanization) with peroxides or platinum. The reinforced medical grade silicone rubber is still weak in comparison with other reinforced rubbers. Poly(styrene-*b*-isobutylene-*b*-styrene) (SIBS) triblock copolymer is a new biomaterial. It was relatively unknown in medicine prior to the introduction of Boston Scientific Corporation's (BSC) (Natick, MA) Drug Eluting TAXUSs Coronary Stent Material in 2002. This medical device has significantly reduced the incidence of coronary

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bypass procedures and associated morbidities.[5-8] The SIBS materials whose physical properties overlap both the silicone rubbers and polyurethanes was cited as having superior oxidative, hydrolytical and enzymatical stableness over its lifespan in the body by virtue of the quarternary carbon backbone associated with the PIB phase of the polymer. These enabling properties will allow the continuing development of medical devices based on SIBS which meet demanding physical and biological requirements.

Haemocompatibility is one of the most important properties that determine the biocompatibility of biomaterials. The haemocompatibility testing is to look for possibly undesirable effects (e.g., haemolysis, thrombus formation, alterations in coagulation) in the blood, caused by a medical device or by chemicals leaching from a device. To the best of our knowledge, there has been no report on haemocompatibility of SIBS. In the present work, SIBS were synthesized by living/controlled cationic polymerization, physical properties and haemocompatibility (included rate of haemolysis, dynamic clotting time and platelet adhesion) were also investigated.

Experimental

Materials. Titanium tetrachloride ($TiCl_4$) (Beijing Chemical Co., 99.9%), 1,1-diphenylethylene (DPE) (Alfa, 98%), 2,6-di-*tert*-butylpyridine (DtBP) (Alfa, 97%) were used as received. 1,4-Di(2-chloro-2-propyl)benzene (DCC) was synthesized by reacting 1,4-di(2-hydroxy-2-propyl) benzene (Alfa, 98%) with dichloro-sulfoxide at ice water temperature under nitrogen atmosphere in methylene chloride ($MeCl_2$).[9] Styrene (St,Yanshan Petrochemical Co. 99.9%) was fleshly vacuum distilled from calcium hydride before use. Methyl chloride (MeCl, Yanshan Petrochemical Co., 99.99%) and isobutylene (IB, Yanshan Petrochemical Co., 99.99%) was dried in the gaseous state by passing them through in-line gas-purifier columns packed with $CaSO_4$/drierite. They were condensed in the cold bath of a glovebox prior to polymerization. Methylcyclohexane (MeChx, Aldrich, anhydrous grade), anhydrous methanol (MeOH, Beijing Chemical Co., 99.8%), sodium chloride (NaCl, Beijing Chemical Co., AR), calcium chloride ($CaCl_2$, Beijing Chemical Co., AR) and alcohol (Beijing Chemical Co., anhydrous) were used as received.

Synthesis of SIBS. All polymerizations were carried out under a dry nitrogen atmosphere in a stainless steel glovebox using CH_3Cl/MeChx (50/50 v/v) solvent mixtures. IB was first polymerized in the DCC/$TiCl_4$/DtBP/−80 ℃ initiating system for 90 min and then St stock solution was added. The polymerizations were terminated by prechilled methanol after 3.5 h. After the evaporation of volatiles, the polymer was dried in a vacuum oven at 40 ℃ to a constant weight.[10]

Compression Molding. All triblock copolymers were compression molded into ~1 mm thick sheets at 150 ℃ for 3 min in an electrically heated hydraulic press at a pressure of 10 MPa, following preheating for 1 min at 150 ℃ before applying pressure. After molding was completed, the samples were cooled to room temperature before releasing the pressure.

Haemocompatibility.

Blood Samples and Other Reagents: Anticoagulant citrate dextrose (ACD) blood was purchased from the Experimental Animal Center of Peking University People's Hospital. To obtain platelet-rich plasma (PRP), the ACD blood was centrifuged at 1500 rpm for 10 min and then diluted by normal saline (PRP/normal saline=1:4.5). $CaCl_2$ solution was used as a coagulant for blood clotting test.

Haemolysis Ratio (HR) Test:[11] The SIBS membranes were cut into small bars (0.5 cm×0.5 cm) and put into a vial. After rinsing the bars three times with distilled water and normal saline (distilled water: sodium chloride=100 : 0.9), respectively, 10 mL normal saline was poured into the vials and then they were kept in a shaking bath at 37 ℃. After incubation for 60 min, 0.2 mL diluted ACD whole blood (8 mL ACD whole blood was diluted by 10 mL normal saline) was dropped into the vial allowing the sample to be soaked in the solution of ACD blood and saline at 37 ℃ for 60 min. Distilled water and normal saline were used as positive and negative controls, respectively. After incubation, each fluid was transferred to a suitable tube and centrifuged at 2500 rpm for 10 min. The haemoglobin released from haemolysis was measured by the absorbency (abs.) of the supernatants at 540 nm using a spectrophotometer UV (UV-2450).

The haemolysis ratio (HR) may be obtained from the equation (1):

$$HR\% = \frac{\text{Sample abs.} - \text{Negative abs.}}{\text{Positive abs.} - \text{Negative abs.}} \times 100\% \quad (1)$$

Blood Dynamic Clotting Test.[12,13] The SIBS film materials (0.5 cm×0.5 cm) were put into beakers to preheat for 5 min at 37 ℃. Then the 0.25 mL ACD whole blood was dropped on the surface of the SIBS film, followed by the addition of 0.02 mL $CaCl_2$ solution (0.2 mol/L) to the blood sample. The beakers containing blood sample were incubated in a thermostat at 37 ℃. The blood clotting test was carried out by spectrophotometric measurement of the relative absorbency of blood sample that had been diluted to 50 mL with distilled water at 540 nm at intervals of predetermined time after addition of $CaCl_2$. The absorbancy of solution of 50 mL distilled water and 0.25 mL ACD whole blood at 540 nm was assumed

to be 100, which was used as reference value. That is to say, the blood clotting index (BCI) of biomaterials can be quantified by of following equation:

$$BCI\% = \frac{I_s}{I_w} \times 100\% \quad (2)$$

Where I_s is the absorbency of blood which had been in contact with sample, I_w is the absorbency of solution of distilled water and ACD blood, respectively.

Platelet Adhesion Test:[14] The degree of platelet adhesion and spreading on the different films was investigated based on the observations using scanning electron microscopy (SEM). In our work, the SIBS films (about 0.5 cm×0.5 cm) were washed three times with distilled water and then soaked in PRP for 1 h at 37 ℃. The platelets in PRP were allowed to adhere and spread on the SIBS films surfaces and non-adherent platelets were removed by washing the surface with phosphate-buffered saline (PBS, pH=7.4), and fixed with 2.0% (w/v) glutaraldehyde solution. These samples were later dehydrated by critical point drying using CO_2 as a transitional fluid. The samples were sputter coated with gold, and examined with a SEM 505 scanning electron microscope.

Measurements. Molecular weights and polydispersity index (PDI) were measured with a size-exclusion chromatography system equipped with a model 510 HPLC pump, a model 410 differential refractometer (Optilab REX, Wyatt Technology Inc., America) and online multiangle laser light scattering (MALLS) detector (laser wavelength=690 nm), (MiniDawn, Wyatt Technology Inc., America) and four ultra-styragel GPC columns connected in the following series: 500, 10^3, 10^4 and 10^5 Å. Samples were eluted in tetrahydrofuran (THF) at a flow rate of 1.0 mL/min at room temperature. The *dn/dc* values of triblock copolymers were calculated by assuming 100% recovery of the injected mass.

UV spectra were obtained using a UV spectrometer (UV-2450, Shimadzu Co., Japan) scans at 350~600 nm wavelength absorbance.

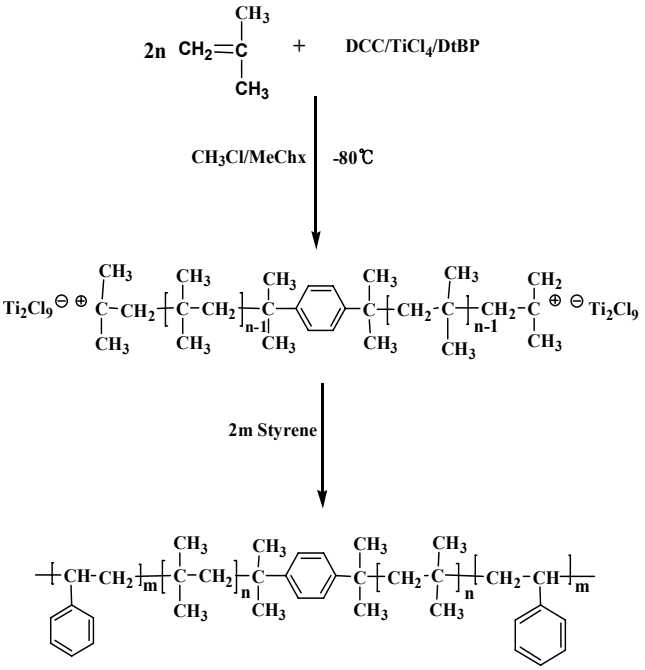
Phase separation structures of the polymers were measured using a AFM analysis system (SPM-9500J3, Shimadzu Co., Japan) and analyzed with the digital image processing software. Thin films of the block copolymers were prepared by spin coating 5 wt% solutions in toluene onto silicon wafers by using a headway resist spinner at 6000 rpm for 30 s. Analyses were performed at 25 ℃.

The vacuum-sputtered samples with Au were characterized by SEM 505 scanning electron microscope (SEM-505, Shimadzu Co., Japan). The samples were magnified 2000 times.

Tensile testing were performed on microdumbbell samples (thickness 1.0 mm) using a universal testing machine (RG 2000~100, Shenzhen reger instrument Co., Ltd. China), with a constant crosshead speed of 100 mm/min. Hardness testing were measured by Shore hardness tester (LX-A, Shenzhen reger instrument Co., Ltd. China).

Results and Discussion

Synthesis of the SIBS Triblock Copolymers. Scheme 1 outlines the overall synthetic strategy for the preparation of SIBS triblock copolymers. First, IB was polymerized by the DCC/$TiCl_4$/DtBP initiating system to yield living PIB (M_n~74000 g/mol and PDI<1.15). After 90 min, styrene in MeChx (50/50 v/v) was added. The most important aspects of the polymerization have been found to be the maintenance of living polymerization characteristics throughout the block copolymerization and an efficient crossover from the polyisobutylene blocks to the styrene blocks. So the living characteristics of styrene block copolymerization was studied. Figure 1 shows the M_n and PDI values of obtained SIBS triblock copolymers as a function of the feed ratio of styrene to DCC ($[St]_0/[DCC]_0$). The M_n values linearly increases in proportion to the $[St]_0/[DCC]_0$ ratio, and the PDI remains narrow over the whole $[St]_0/[DCC]_0$ range. The experimental results verify that there is good control over the molecular characteristics of the synthesized triblock copolymers. Figure 2 shows the RI traces of $^+PIB^+$ segment and of these triblock copolymer formed at different $[St]_0/[DCC]_0$ ratios. The shift in the peak



Scheme 1. Synthesis of SIBS triblock copolymers.

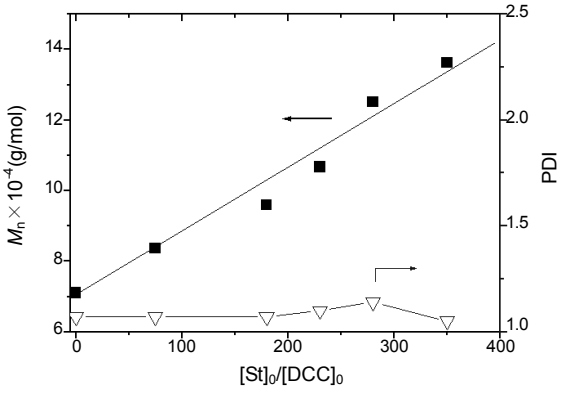


Figure 1. M_n and M_w/M_n values of SIBS triblock copolymers obtained as a function of the feed ratio of styrene to DCC.

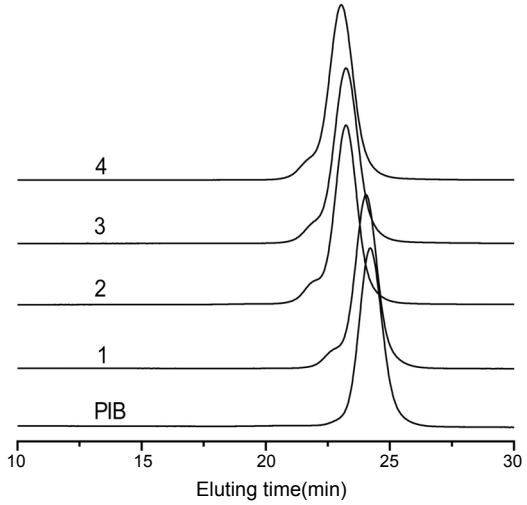


Figure 2. SEC traces (RI) of the PIB homopolymer and SIBS block copolymer synthesized at −80 ℃. 1) PS content=30 wt%, M_n=8.37×10^4; 2) PS content=35 wt%, M_n=9.59×10^4 ; 3) PS content=40 wt%, M_n=10.68×10^4; 4) PS content=45 wt%, M_n=12.51×10^4; [IB]=1.5 M, [DCC]=1×10^{-3} M, [DtBP]=2×10^{-3} M, [$TiCl_4$]=2.4×10^{-2} M.

position of the triblock copolymers toward higher molecular weights (lower retention times) relative to the $^+$PIB$^+$ segment indicates signification styrene incorporation.

Morphology of Triblock Copolymers. We investigated the morphology of thin films of SIBS triblock copolymers by AFM in the tapping mode to avoid damaging the soft surfaces. Figure 3(a) shows the image of a PIB homopolymer film in comparison with the 3b image of SIBS film. The white areas in the latter image are assigned to discreet hard PS phases distributed in the continuous PIB phase. The PS domain size is 40~60 nm. The block copolymer microphase separates on a nanometer scale due to the inherent thermodynamic and chemical incompatibility of the individual polymer blocks.

Mechanical Properties. Processability is an important consideration in medical-device manufacturing. SIBS triblock copolymers with 10~45 wt% PS display thermoplastic elastomeric (TPE) properties. That is to say, they behave like crosslinked rubbers at room temperature, whereas melt at temperatures above the T_g of the glassy PS block and

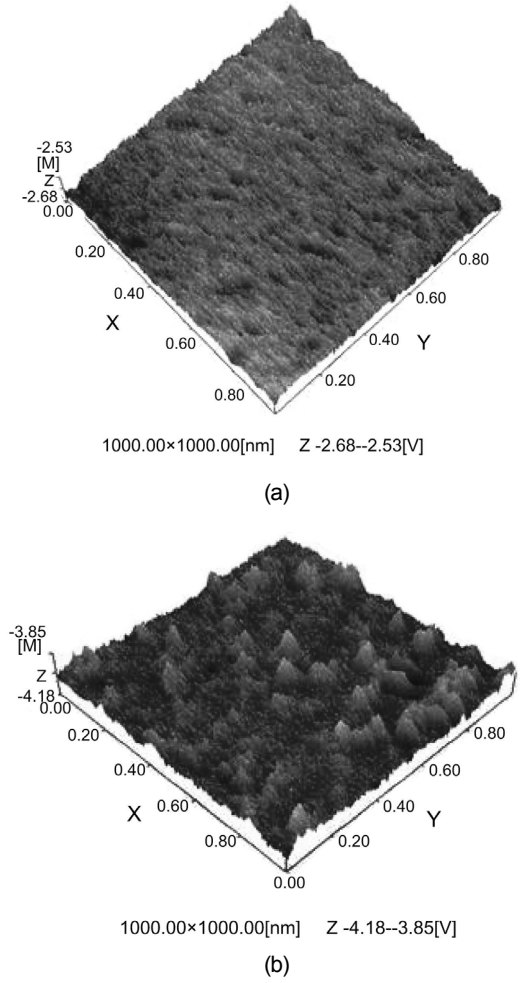


Figure 3. Three-dimensional AFM height images of PIB (a); SIBS block copolymer (b).

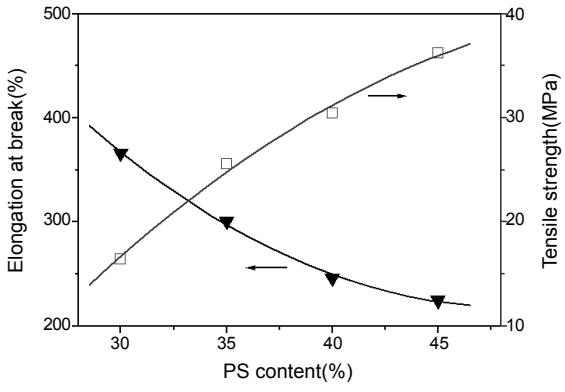


Figure 4. The effect of SIBS triblock composition on stress-strain values.

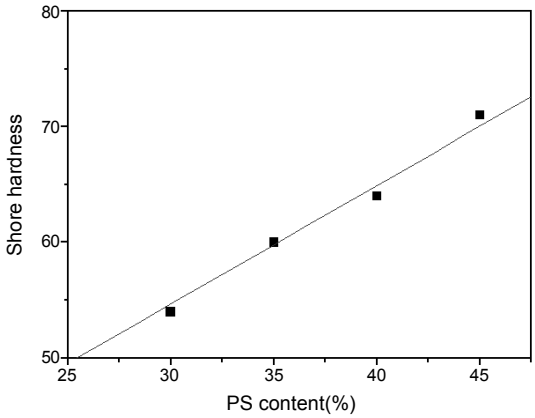


Figure 5. Plot of styrene mass percent fraction in SIBS versus Shore hardness.

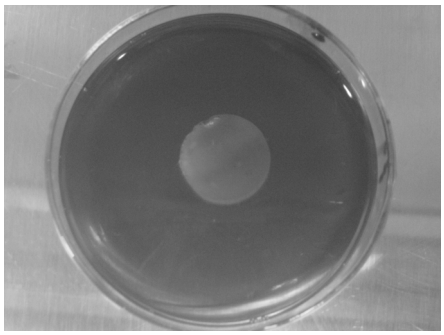


Figure 6. Haemolytic phenomenon of SIBS and rabbit blood.

therefore can be processed like plastics. The ratio of soft and hard segments of SIBS determines the overall properties. Figure 4 shows the effect of SIBS triblock composition on stress-strain values. The results indicate that tensile strengths increase with increasing PS content in the triblocks, while elongation at break decrease. It is interesting to note that Shore hardness increased almost linearly with increasing PS content (Figure 5). The Shore hardness of SIBS is lower than 70, and the tensile strengths are larger than that of silicone rubber (ranges from 6 to 12 MPa).[1] These suggest that SIBS is a good soft biomaterial.

Evaluation of Haemocompatibility.

The Haemolysis Ratio (HR): The haemolysis ratio represents the extent of red blood cell broken by the sample contacting with blood. The more the number of the broken red cells, the greater the value of HR is. That is to say, the smaller the HR value, the better the blood compatibility of the biomaterial. Generally speaking, the HR value of biomaterials must be below 5% for medical applications. Figure 6 shows haemolytic phenomenon of SIBS in rabbit blood. Table 1 shows effect of SIBS triblock composition on haemolysis ratio. It can be seen that all the HR are much lower than 5%, conforming to the HR value of biomaterials. And the HR values exhibit

Table 1. Haemolysis Ratios of SIBS Triblock Copolymers

Sample	Abs.	Haemolysis ratio/%
Positive	0.894	100
Negative	0.012	0
SIBS-30	0.031	2.60
SIBS-35	0.018	2.28
SIBS-40	0.014	2.24

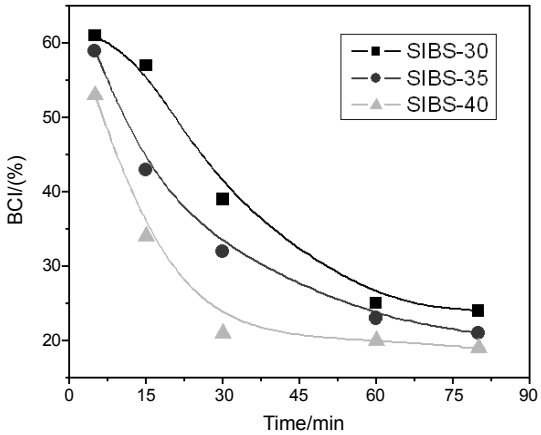


Figure 7. Dynamic clotting time of SIBS block copolymer.

no evident change with PS content. As we have known, the HR values are similar to that of polyurethane (HR value is 2.5) [11] and larger than that of polysiloxane (HR value is 0.72).[15]

Blood Dynamic Clotting Time: The blood clotting test reflects the variation of antithrombogenic activity with the increase in blood-sample contacting time. In this study, the antithrombotic activity is qualitatively expressed by a relative parameter BCI (see equation (2)). Generally speaking, the better the blood compatibility of the sample, the larger the BCI value. Figure 7 suggests the effect of PS content on the BCI. It is clearly indicated that the BCI declines with the increase in PS content at the same contacting time. Figure 7 also suggests that the contacting time of blood sample affect the value of BCI under same conditions. The general trend is that the BCI declines with the increase of blood-sample contacting time. It is to be noted that the BCI declined swiftly with the lengthening of the contacting time in SIBS-40. While, the BCI decreased more slowly in SIBS-30. The trend of BCI also states clearly the haemocompatibility. That is to say, the increase of PS content in polymer is not beneficial for improving the blood compatibility of polymer.

Platelet Adhesion Test: *In vitro* platelet adhesion testing was performed to investigate the quantity, morphology, aggregation and pseudopodium of the adherent platelets. The adhesion and aggregation of platelets are important steps in the process of thrombus formation, which occurs when activated platelets secrete substances that enzymatically

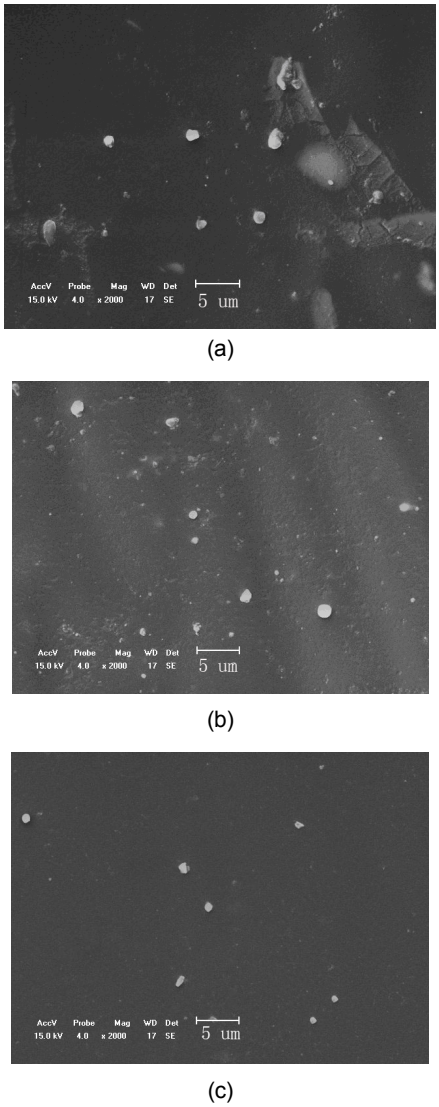


Figure 8. SEM images of platelets attached to the surfaces of the SIBS with different PS content after 60 min incubation: (a) PS content=30 wt%; (b) PS content=35 wt%; (c) PS content=40 wt%.

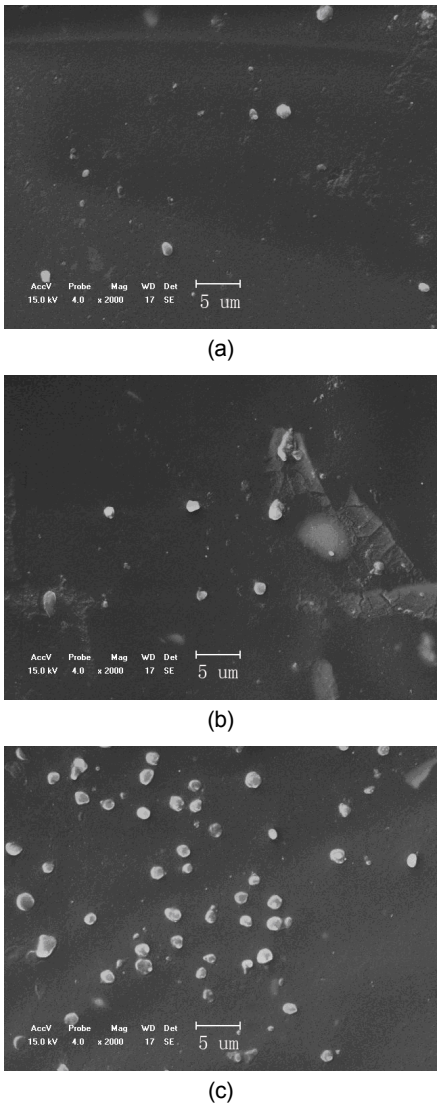


Figure 9. SEM images of SIBS material at different adhesion time: (a) incubation 30 min; (b) incubation 60 min; (c) incubation 180 min.

activate otherwise inactive clotting factors, culminating in the conversion of the soluble fibrinogen protein into an insoluble mesh of fibrin polymer. Figure 8 shows the SEM pictures of platelets attached to the surfaces of the SIBS with different PS content after 60 min incubation at 37 ℃. We found that PS content in SIBS had no evident effect on the platelet adhesion. But the incubation time is an important factor in the platelet adhesion test. As shown in Figure 9(a), (b), (c), the number of platelet on surface materials increased with extending incubation time, and these have no aggregating platelets on SIBS surface. After 180 min, the morphology of the platelets adherent on the surface is regular. However, the morphology of adhered platelets on polyurethane-based biomaterial undergoes a relatively low degree of variation.[16] So it also clearly proves the SIBS has better anti-clotting property than that of polyurethane-based biomaterial.

Conclusions

The synthesis of SIBS had been accomplished in DCC/$TiCl_4$/DtBP initiating system at −80 ℃ using living cationic polymerization, which has moderate molecular weight and narrow molecular weight distribution. With PS content increased, the elongation at break of SIBS had fallen, the hardness and tensile strength of SIBS increased. Compared to PIB, SIBS triblock copolymers exist phase separation structure. All haemolysis ratio values of SIBS are below 5% which suit for medical material application. The dynamic

clotting time of SIBS block copolymers show that the anti-clotting properties of SIBS-30 is superior to that of SIBS-35 and SIBS-40. In the platelet adhesion test, the incubation time is an important factor. With the incubation time increased, the number of platelet on surface materials increased; and these have no aggregating platelets on SIBS surface.

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