JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE **16** (2005) 277–282

The effect of formation of the liquid crystalline phase on the blood compatibility of a cholesterol modified silicone

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Cholesterol modified silicones were synthesized by grafting copolymerization of 10-Cholesteryloxydecanol onto polymethylhydrosiloxane (PMHS). Fourier transform infrared (FT-IR) spectroscopy, proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy and gel permeation chromatography (GPC) confirmed the chemical structures of polymers. Differential scanning calorimetry (DSC) and polarized optical microscopy (POM) results indicated the mesogenic properties of those polymers. The modified silicone with 45% 10-Cholesteryloxydecanyl (SC45) indicated obvious thermotropic liquid crystalline transform at about 122–124.9 °C. The thermotropic liquid crystalline phase could be retained at room temperature via a special annealing/quenching process. The anneal-quenched film (SC45C) formed continuous liquid crystalline phase, whereas the unannealed films presented amorphous structure. The blood compatibility of the coatings was assessed from SEM observation of the platelet's adhesion to coating surface and plasma recalcification time (PRT). The results revealed that the formation of the liquid crystalline phase could greatly improve the *in vitro* blood compatibility of the materials. The positive results of liquid crystalline onto haemocompatibility allow broad potential in biomaterials.

1. Introduction

Polymeric materials have been widely used for artificial organs, medical devices and disposable clinical instruments [1], which have contributed significantly to the quality and effectiveness of health care system [2]. Silicone rubber-based material is one of the most important polymeric materials and is widely used for different applications including tubing, catheters, vascular graft, plastic reconstruction, encapsulation of electronic components and voice prostheses [3]. Among all these applications, silicone rubber showed outstanding flexibility and transparency as well as high structural resistance toward heat, ozone and chemical [4]. However, Silicone rubber-based materials are still prone to initiate the formation of clots, as platelets and other components of the blood coagulation system are activated. Despite decades of research, non-thrombogenic surfaces for blood-contacting polymers remain an elusive goal.

A potential solution to the problem of thrombogenic polymers may now be realized by creating liquid crystalline polymers that imitate the movable morphology of the natural biomembrane surface. Hall *et al.* [5] pointed that cell membrane, polypeptide, nucleic acid, inner surface of blood vessel and other biomembrane in the body, especially the surface of cell membrane contacting with blood frequently, are all mobile liquid-liquid crystal. Zhou *et al.* [6, 7] reported recently that the liquid crystal in the polymer-liquid crystal composite membrane appears to be beneficial in improving the blood compatibility and reducing the thrombogenicity.

It is well known that cholesterol is one of the most common membrane sterols in animals and plays important roles in regulating membrane fluidity and self-association of molecules in biological systems [8, 9]. In fact, cholesterol containing liquid crystalline materials have attracted more and more attention in biomaterials field [6, 7, 10]. This research is based on two facts: firstly, cholesterol has high thermodynamic affinity for the cell membranes and the ability to change the membrane's permeability and fluidity [11]; secondly, the mesogenic character of cholesterol known for many of its derivatives [12].

In this paper, a cholesteryl-modified silicone was synthesized by grafting copolymerization of 10-Cholesteryloxydecanol onto polymethylhydrosiloxane. A

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special annealing/quenched thermal treatment was used to prepare two couples of liquid crystalline samples and its controls. The effect of the liquid crystalline phase on the blood compatibility of cholesterol-modified silicones was then investigated via the platelet adhesion and plasma recalcification time.

2. Experiment

2.1. Materials

Cholesterol modified silicone (SC45, SC15) were synthesized by grafting copolymerization of 10-Cholesteryloxydecanol onto polymethylhydrosiloxane. 10-Cholesteryloxydecanol and polymethylhydrosiloxane (Table I) were dissolved in toluene (30 ml), a catalytic amount (2 ml) of hydrogenhexchoroplatinate (IV) hydrate/THF solution (the molar ration of Pt/THF was $1/10^3$) was added to the above solution under nitrogen, and the reaction temperature was 50 °C (see Fig. 1). After 72 h, excessive methanol was added into the reaction system and the reaction continued for another 6 h to terminate the active silicon-hydrogen bond. On completion, the reaction was concentrated using a rotary evaporation followed by pouring the solution into large amount of ethanol to precipitate the polymers. The products were washed with ethanol repeatedly, and then dried under vacuum.

TABLE I Copolymerization data of the polymers, SC45 and SC15

Sample	Molar ratio of Monomer[a]/PMHS[a] in Feed	Molar percent[b] of attached monomer in polymers (%)	Mw[c]	MWD[c]
SC45	1:1	45	3.1×10^4	2.02
SC15	1:4	15	2.7×10^4	1.46

[a]Monomer and PMHS mean 10-Cholesteryloxydecanol and polymethylhydrosiloxane (PMHS), respectively;
[b]Calculated from ^{1}H-NMR;
[c]Mw and MWD are molecular weight and molecular weight distribution, respectively and they were calculated from GPC results.

Fourier transform infrared (FT-IR) spectroscopy (E.S.P., MAGNA-IR560, Nicolet Instrument) of the cholesterol modified silicone showed absorption at 1267 cm^{-1} which is designated to Si—CH_3 stretching in the main chain. The absorption at 2840 cm^{-1} was the contribution of aliphatic C—H stretching from the side chain. The disappearances of absorption at 3451 from O—H stretching and absorption at 2140 from Si—H stretching confirmed the success and completion of the reaction.

Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy (500 MHz Bruker) of SC15, as a representative the copolymers, are shown in Fig. 2. The ^{1}H-NMR spectrum showed peak at 0.14, which was

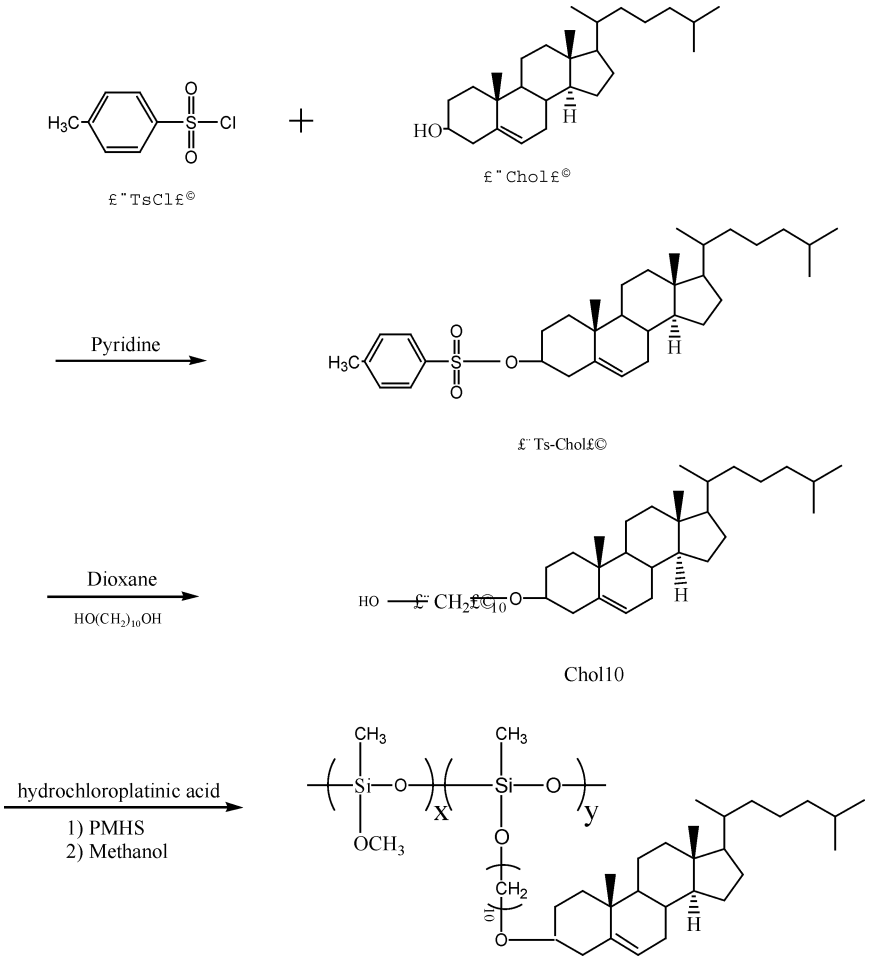

Figure 1 Synthesis pathway of monomer and copolymers.

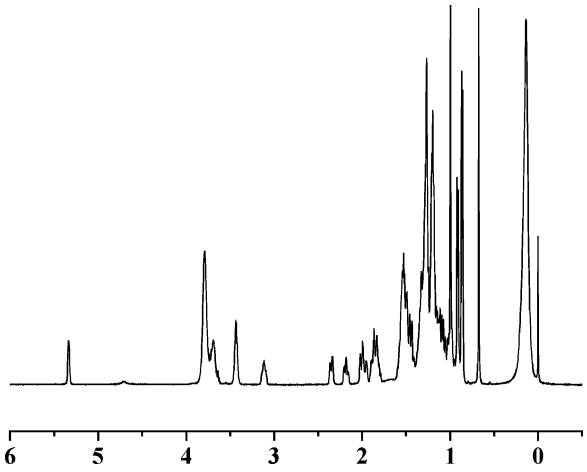

Figure 2 ^{1}H-NMR spectum of polymer, SC15.

attributed to the $-CH_3$ of the main chain. The peak at 0.68 was the contribution from $-C(CH_3)-$ of the cholesterol. The peak at 5.34 came from the $-C(CH_2-)=CH-$ of the cholesterol. The peak at 3.63–3.79 came from the $-Si-O-CH_2-$ and $-Si-O-CH_3$. Other peaks, 0.86–2.40, 3.12, and 3.43–3.46 were the contributions from the H of the attached monomer.

From the integral intensities at $\delta = 0.14$ and 0.68, the molar percent of the attached 10-Cholesteryloxydecanol in the copolymers was calculated (Table I).

GPC (Baseline 810 Method, with refraction index detector of R401 DET, n1-no, Waters) were used to determine the molecular weight of copolymers. Differential scanning calorimetry (DSC, Perkin-Elmer DSC7) was used to characterize the mesogenic properties of those polymers.

2.2. Sample preparation

The glass slides were prepared by cutting as provided specimens into square slides (25 × 25 mm) with a glass cutter and cleaned with a 1:1 (V/V) solution of 2.0 M sulfuric acid and 30% hydrogen peroxide at 80 °C for 30 min. After rinsing with deionized water, clean substrates were obtained.

The polymer films were prepared by spin coating from a 5 mg/ml solution of the polymer in toluene onto clean glass substrates. The process of spin coating was performed on a spin-coater at the speed of 3000 r/min for 30 sec. The samples were then placed into a vacuum oven at 90 °C for 9 h to allow the polymer coating to dry evenly. The anneal-quenched samples were annealed under vacuum at 140 °C for 9 h and then quenched to room temperature rapidly. Polarized optical microscopy (POM, OLYMPUS BX51) was used to observe the liquid crystalline characteristic of these coatings.

2.3. Platelet adhesion test [13]

Platelet-rich plasma (PRP) was obtained from the Central Blood Bank in Hangzhou. At room temperature, 20 μl of the fresh PRP was dropped onto the samples. After the samples were kept contacted with PRP for 30 min, they were gently rinsed with PBS and treated with 1% solution of glutaraldehyde for 0.5 h. After rinsing with tri-distilled water three times, the samples were dehydrated by systematic immersion in a series of ethanol/water solutions: 60, 70, 80, 90, 95 and 100 vol% and allowed to dry in a desiccator. The samples were observed on an Invert Fluorescence Microscope (Olympus IX70) and the average number of platelets in five regions were recorded for each sample. The morphology of the adherent platelets was observed using scanning electron microscopy (SEM) (Jeol, JSM-35C) following gold sputtering on the sample surface. Each material was measured 5 parallel samples for reproducibility. Sixteen different fields were randomly counted for each sample.

2.4. Blood-clotting assay [13]

The polymer that was to be evaluated was dissolved in ethanol at a concentration of 10 mg/ml. In order to coat a glass tube, the tube was filled with the polymer solution, left for 1 min before the solution was poured out of the tube again, whilst rotating the tube between the fingers. The samples were then placed into a vacuum oven at 90 °C for 9 h to allow the polymer coating to dry evenly. The anneal-quenched samples were annealed under vacuum at 140 °C for 9 h and then quenched to room temperature rapidly.

Fresh plasma (500 μl) was added to each sample tube and allowed to stand for 2–3 min at 37 °C before the addition of 500 μl of 0.025 M $CaCl_2$ solution, also at 37 °C, at which point a stopwatch was started. The mouth of the tube was then sealed tightly with parafilm. The stopwatch was stopped when fibrin clotting was first visible and the time recorded. The tests were performed at 37 °C under agitation and a minimum of ten measurements was used to calculate the average clot time.

2.5. Statistical analysis

Data are expressed as the mean ± standard deviation (SD). Statistical analysis was performed using two-populations Student's t-test. The significant level was set as $P < 0.05$.

3. Result and discussion

Cholesterol was attached on to polymethylhydrosiloxane through the reaction between hydroxyl of 10-Cholesteryloxydecanol and silicon-hydrogen bond (Fig. 1 and Table I). It was specifically designed to obtain a thermotropic liquid crystalline silicone. The cholesterol end group is expected to be able to form a liquid crystal due to its mesogenic character. The advantage of using 10-Cholesteryloxydecanol is due to the flexible decamethylene to provide the mobility and architecture to form liquid crystalline phase.

3.1. Observation of the liquid crystalline character of the coatings

A DSC thermogram was used to investigate the liquid crystalline character of the copolymers. The DSC of the polymers was done according to the following three

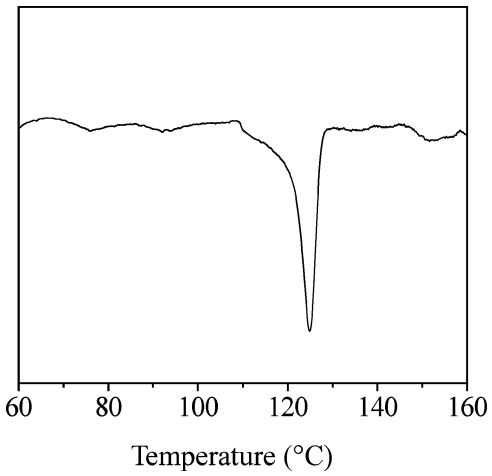

Figure 3 DSC thermogram of polymer, SC45 (heating rate: 10 °C/min).

steps: (1) Heating from 30 °C to 160 °C at 10 °C/min; (2) Cooling from 160 °C to 30 °C at 10 °C/min and (3) Heating from 30 °C to 160 °C at 10 °C/min. The DSC thermogram of polymer for the first heating circle, SC45 are shown in Fig. 3. The results indicated that the transition temperature of SC45 was 124.9 °C and SC15, 122 °C. This result is consistent with a previous report [20]. While in the following two steps, the second step and the third step, the enthalpy changes of the transition were significantly decreased (data not shown). We considered that this phenomenon came from the possibility that the liquid crystalline phase was retained in the cooling circle because of the high rate (cooling rate: 10 °C/min) of the cooling process. And we deduce that after the quenching treatment, whose cooling rate is much higher than that of cooling process in DSC, the quenched materials will show no transition.

Thermotropic liquid crystalline polymers can be quenched from the liquid crystalline fluid to room temperature, where it forms a liquid crystalline glass [14]. This property is critical for thermotropic liquid crystalline polymers to be used as biomaterials since all biomaterials will be used at the temperature of human body. The Polarized optical microscopy of both the annealed and unannealed coatings is showed in Fig. 4. The result indicated that neither SC45 nor SC15 contained liquid crystalline phase. But the anneal-quenched sample with 15% cholesteryloxydecanol (SC15C) formed discrete island-like liquid crystalline, while the anneal-quenched sample with 45% cholesteryloxydecanol (SC45C) showed continuous liquid crystalline respectively.

3.2. Blood compatibility evaluation

3.2.1. Plasma recalcification time (PRT) characterization

On the original air equilibrated surface, blood-clotting assay was carried out. When the tested system, containing anti-coagulated human plasma was added into Ca^{2+} (Factor IV), the endogenetic blood coagulation system will start to activate the prothrombin (Factor II) converting into thrombin, and then thrombin will initiate the formation of insoluble fibrin from fibrinogen. The duration of this procedure was measured as plasma recalcification time (PRT).

Fig. 5 showed the PRT of the coatings SC45, SC45C, SC15 and SC15C. The result revealed that the PRT of the coatings SC45, SC45C, SC15 and SC15C were 270, 394, 328 and 321 sec respectively.

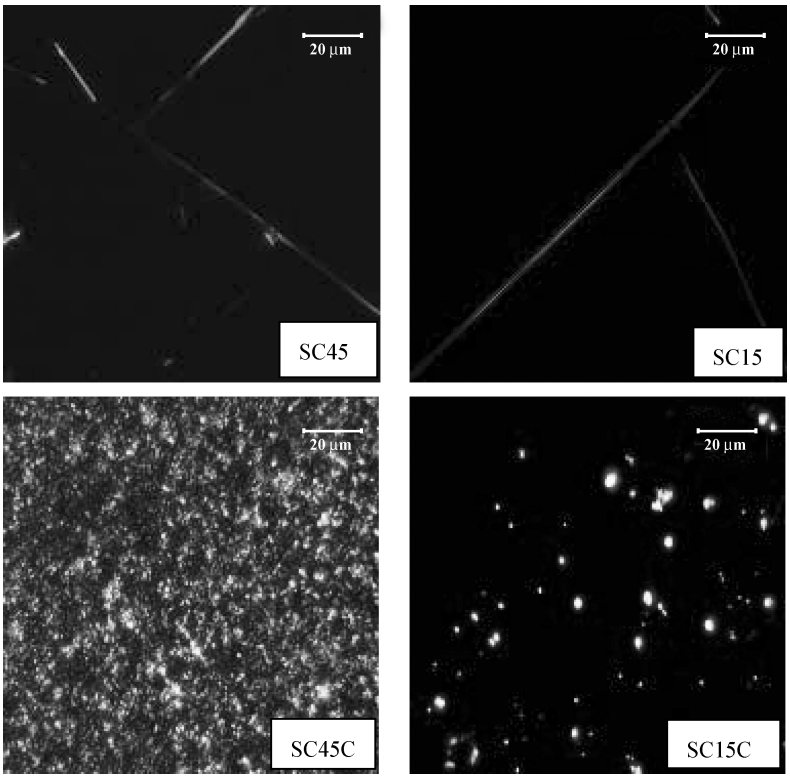

Figure 4 Polarized optical microscopy of SC45, SC15, SC45C and SC15C.

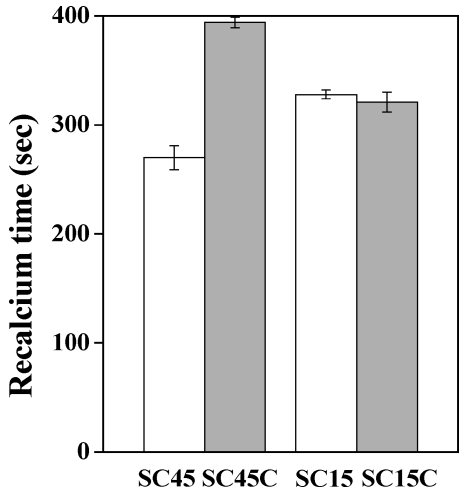

Figure 5 Bar chart of recalcium time: SC45, SC45C, SC15 and SC15C.

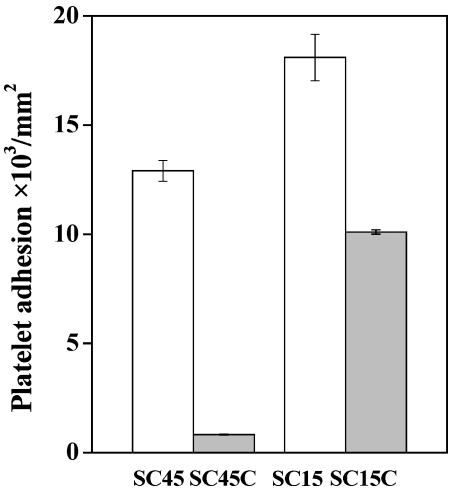

Figure 6 Bar chart of platelet adhesion on SC45, SC45C, SC15, and SC15C.

It was found that the PRT of the anneal-quenched coating SC45C was distinctly longer than that of the unannealed SC45 ($P = 0.0006$). The anneal-quenched with continuous liquid crystalline can prolong the PRT significantly.

But the anneal-quenched sample with discrete island-like liquid crystalline (SC15C) show no difference with its amorphous control (SC15) ($P = 0.286$). The high content of the liquid crystalline phase is necessary to prolong the PRT of the coating.

3.2.2. Platelet adhesion characterization

Fig. 6 is a bar chart showing the number of platelets on each sample. Fig. 7 shows typical images of surface-blood-contacting samples obtained from the SEM. Samples were contacted with the blood from the same donor and under the same conditions as the control.

The number of platelets adhered to the coatings SC45 and SC45C were 12900 and 816 per mm^2, respectively. The results clearly showed that the number of platelets adhered to the anneal-quenched SC45C coating is much lower than that of the unannealed coating SC45 ($P = 0.0002$). Fig. 7 indicated that most of the platelets adhered to the unannealed coating SC45 completely gathered together, whereas the adhesive platelets on the anneal-quenched SC45C maintain their discoidal shape with low spreading area. The extent of platelet adhesion and deformation on the anneal-quenched coating SC45C coating is clearly lower than that of the control of SC45. In addition, the number of platelets adhered to the coatings SC15 and SC15C were 18100 and 10100 per mm^2, respectively. After annealing, the number of platelets adhered to the coating SC15C decreased ($P = 0.003$).

As descried in above, the cholesterol modified silicones were prepared via covalent binding of cholesteryl onto PDMS via decamethylene as a "flexible spacer". The modified silicone with 45% 10-Cholesteryloxydecanol formed a continuous liquid crystalline phase via an annealing/quenching process, which showed much less adhesion of platelets and the platelets activation than the unannealed amorphous control. In comparison to unannealed films, the annealed films with continuous cholesterol liquid crystalline structure could prolong plasma recalcification time significantly. The formation of a liquid crystalline phase on the coating was favorable to improve the *in vitro* haemocompatibility of the materials. The positive results of biomimetic liquid crystalline for enhancing haemocompatibility provides broad potential in surface tailoring of biomaterials.

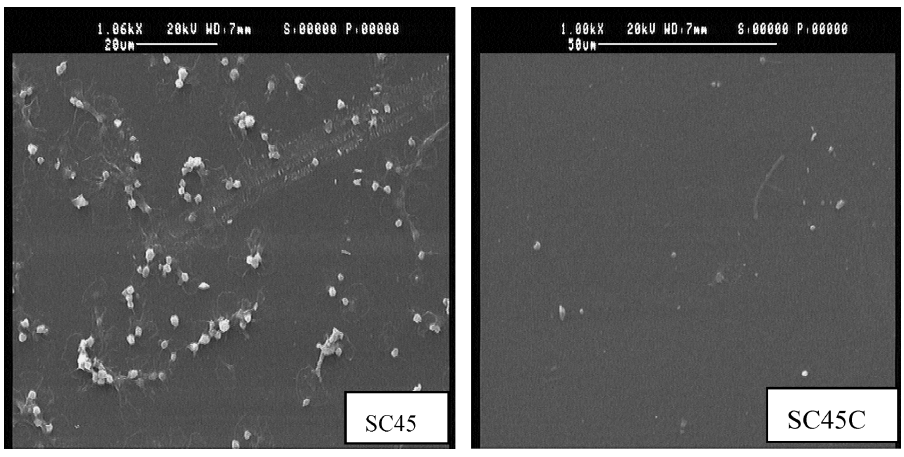

Figure 7 Typical images of platelet on SC45 and SC45C.

Acknowledgment

This research was financially supported by Major State Basic Research Foundation of China (grant No. G1999054305), Natural Science Foundation of Zhejiang Province (501114) and and the Natural Science Foundation of China (NSFC-20174035).

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Received 9 February
and accepted 17 June 2004