A novel crosslinkable polymer as drug-loaded coating for biomedical device

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A novel copolymer has been synthesized by the radical polymerization of poly (ethylene oxide) methacrylate, stearyl methacrylate, hydroxypropyl methacrylate and trimethoxysilylpropyl methacrylate. The polymer was characterized by Fourier transform infrared spectroscopy, proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy and gel permeation chromatography. The crosslinkable coating was prepared by dip-coating 5 mg/ml solution in tetrahydrofuran onto glass substrate. A stable crosslinked coating was obtained after curing the coating at 70 °C for 9 h. Contact angle results indicated the possible reorganization of the surface amphiphilic molecule which interpreted the excellent biocompatibility revealed by the results of the platelet adhesion and plasma recalcification time. Rhodamine S and Cibacron Blue were used as model drugs to prepare drug-containing coating at the same conditions. Drug-releasing curves indicated that the mechanism of the release is approximately Fickian release.

1. Introduction

Synthetic polymers have been widely used in medical devices fabrication, and contribute significantly to the quality and effectiveness of the health care system. However, when the polymer is placed into a biological environment, unfavorable responses such as blood protein adsorption and platelet adhesion may be elicited and initiate fatal thrombus. Other adverse biological reactions such as unfavorable immunoresponses and inflammation are also observed at the surfaces of synthetic materials when implanted into the body [1, 2]. Therefore, it is of great interest to improve the surface properties of polymeric biomaterials to avoid these problems [3, 4].

Since current biomedical devices generally have adequate mechanical properties and are relatively inexpensive, the inadequate surface properties were prompted by various types of coating. Of the various coating materials and technology developed, the PEO based polymer appears to be attractive, as it provides a hydrophilic anti-adhesive layer due to its low interfacial energy, high mobility and the steric repulsion to protein and cells [5–7].

Another intriguing development in polymeric coatings is the application of drug-loaded polymeric coating. Controlled permeation and releasing of drugs from polymeric coated implant will concentrate the drug at the precise site where it is needed. It provides a new approach to treat device based infection, tumor and stent restenosis etc [8–10].

In this research, we explore the preparation of a novel PEO containing crosslinkable coating for blood contact device. The crosslinkable structure was specially designed to offer additional advantage over traditional methods in term of stability of the coating and more alternative to control releasing of drug. Two model drugs were entrapped into this PEO containing crosslinkable coating and their release behaviors were investigated.

2. Experiment

2.1. Materials

Poly (ethylene oxide) methacrylate (PEOMA), stearyl methacrylate (SMA), hydroxypropyl methacrylate (HPMA, supplied as a mixture of isomers) and trimethoxysilylpropyl methacrylate (TSPMA) were commercially obtained from Aldrich Chemical Co. and used without further purification. The initiator chosen was α, α′-azodiisobutyronitrile (AIBN) which was recrystallized from methanol. Rhodamine S and Cibacron Blue were used as received. Other reagents were purified by conventional methods.

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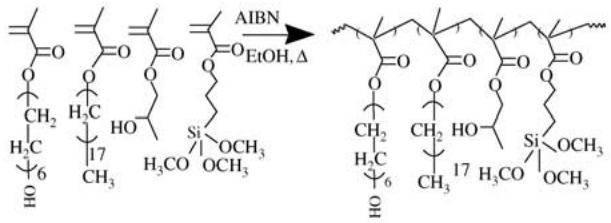

Figure 1 Reaction scheme for the preparation of crosslinkable polymers.

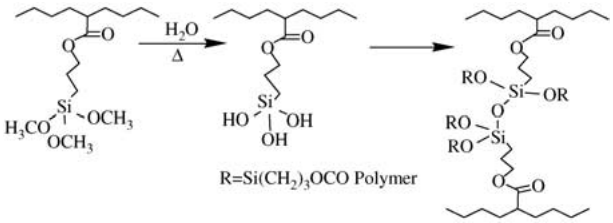

Figure 2 Scheme of the crosslinking reaction.

2.2. Synthesis and characterization of crosslinkable polymers

Crosslinkable polymers were synthesized as shown in Fig. 1.

The desired amounts of PEOMA, SMA, HPMA, TSPMA and 2% (w/w) AIBN, namely 1.071, 2.188, 0.462, 0.130 g, respectively, were dissolved in 50 ml solvent (isopropanol/THF = 9 : 1 (V/V)) and the solution was poured into polymerization tubes. Oxygen in the tubes were eliminated by bubbling argon through the solution and the tubes were then shaken at 60 °C for 24 h. The reaction mixture was cooled to room temperature and concentrated by rotary evaporation followed by pouring the solution into large amount of cooled dry diethyl ether to precipitate the polymers. The precipitates collected were dried in vacuum at 30 °C and stored in desiccator for later use.

Fourier transform infrared (FT-IR) spectroscopy, proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy and gel permeation chromatography (GPC) were performed to verify the synthesis and purification of tetrapolymer. FT-IR spectra were recorded on an E.S.P., MAGNA-IR560, Nicolet Instrument. ^{1}H-NMR of the tetrapolymer in solution was performed on a 500 MHz Bruker instrument. The tetrapolymer was analyzed in pure $CDCl_3$ with reference to tetramethylsilane.

The molecular weight of tetrapolymer was determined by GPC (Baseline 810 Method, with refraction index detector of R401 DET, n1-no, Waters) using THF as an eluent with a polystyrene standard.

2.3. Preparation of casting films

Glass slides were used as substrates for polymer growth. The slides were prepared by cutting as provided specimens into rectangular slides (40 × 7 mm) with a glass cutter. Cleaning and oxidation were performed using a 0.25 M solution of potassium dichromate in a 2.0 M sulfuric acid at 80 °C for 24 h. After rinsing with deionized water, clean and hydrophilic substrates were obtained.

After the tetrapolymers were completely dissolved in THF, the process of dip-coating was performed on a lifter. With constant speed, the substrates were vertically dipped into the coating solution and then taken out. The same process was repeated eight times after 10 min intervals. The coating thickness was approximately controlled by the lifter velocity and coating times. The sample was then placed into an oven at 90 °C for 9 h to allow the polymer coating to dry evenly and cure as shown in Fig. 2. The surface analysis was carried out with the measurement of ATR-FT-IR (E.S.P., MAGNA-IR560, Nicolet Instrument).

Water contact angle measurements were performed on a KRUSS DSA10-MKII machine with a NRL goniometer using both sessile drop and captive bubble methods. In the sessile drop technique, the solid sample examined was placed in a controlled atmosphere chamber, which was partially filled with the liquid used for the contact angle measurements (water). The chamber with solid sample remained closed 0.5–1 h to achieve equilibration between the phases. A drop of water (1.0 μl) was introduced onto the solid surface through a microsyringe. Then the contact angles were measured with the NRL goniometer on both sides of the water drop at the time of 30 s.

In the captive bubble technique, the solid sample examined was placed in a rectangular glass chamber on two stable supports with flat surfaces. The glass chamber with sample was filled with water. A small air bubble was made at a tip of the U-shaped needle using a microsyringe. The gas bubble was attached to the solid surface. Then the contact angles were measured with the NRL goniometer on both sides of the gas bubble at the time of 30 s.

As with the third technique, the change of contact angle dependent on time tested was also used to detect the surface reorganization of the solid samples on real-time. This technique is similar with the sessile drop, except that the measurement started as soon as the water came into contact with the solid surface. The contact angle was recorded every 1 s in 30 min.

The results are shown in Table I. For the sessile drop and captive bubble methods, the given values are the average values from 10 parallel data of each sample. The data from the sessile drop method were the advancing contact angle θ_{adv} at the 15th second after water droplet contacted the original air interface of the samples. The data from the captive bubble method were fairly close to the equilibrated receding water contact angle θ_{rec} [11, 12]. The change of contact angle dependent on time testing was performed by sessile drop to record the change of contact angle on time. Both the static contact angle and the change of contact angle dependent on time were used to characterize the change of hydrophilicity of the coatings under different environments (air/polymer interface and water/polymer interface).

2.4. Platelet adhesion test

Platelet-rich plasma (PRP) was obtained from the Central Blood Bank in Hangzhou. The samples pre- and post-treated in water for 24 h and dried were used. At room temperature, 20 μl of the fresh PRP was dropped onto

TABLE I Static contact angle data of substrate and the coatings[a]

	Sessile drop (θ_a) (deg)	Captive bubble (θ_b) (deg)	Contact angle hystereses (θ_{adv}[b] − θ_{rec}[c]/(deg))
Glass control	9.80 ± 0.83	5.80 ± 0.83	4.00 ± 0.83
Uncrosslinked coating	92.80 ± 1.50	38.00 ± 1.21	54.80 ± 1.50
Crosslinked coating	102.00 ± 0.63	42.30 ± 0.67	59.70 ± 0.67

[a]Prepared from the tetrapolymer.
[b]$\theta_{adv} \approx \theta_a$.
[c]$\theta_{rec} \approx \theta_b$.

samples. After the samples were kept in touch with PRP for 30 min, they were gently rinsed with PBS and treated with 1% solution of glutaraldehyde for 2 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.

2.5. Blood-clotting assay

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 tube was then placed into an oven at 90 °C for 9 h to allow the polymer coating to dry evenly and cure within the tube. Ten samples made from each polymer were prepared and compared to 10 uncoated controls.

Fresh frozen 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 tube was immersed kept in a thermostatic water bath at 37 °C whilst undergo shaking.

2.6. Drug-releasing curve determination

Tetrapolymer coatings entrapped with model drug were prepared by coating the mixture solution of the synthesized polymer and model drug onto substrates and then curing the polymer at 90 °C for 9 h. The coatings were then transferred into a vial containing 30 ml deionized water. The vial was shaken in a horizontally moving shaker at 37 °C (± 0.5 °C). The coating were then transferred into another vial containing 30 ml fresh deionized water after shaking for 10 min. Transfers were made to equal volumes of water at desired time. The amount of model drug contained in each vial was then determined by a microplate reader (Model 550). Firstly, a group of standard known concentrations of 250, 250 × 1/2, 250 × 1/4, 250 × 1/8, 250 × 1/16, 250 × 1/32 µg/ml were prepared in water. The solutions were then analyzed by the microplate reader. The absorbancies of Rhodamine S and Cibacron Blue at 550 nm were then detected. The concentration versus absorbancies was drawn as a calibration curve. The concentration of the model drug in unknown samples was then estimated from their absorbancies at the same wavelength.

3. Results and discussion

3.1. Characterization of tetrapolymer

The FT-IR spectrum of the tetrapolymer is shown in Fig. 3. In the FT-IR spectrum of the tetrapolymer, the peaks at 2924.0, 2852.6 and 1465.3 cm^{-1} are attributed to -CH_2- (SMA and main chain); the peaks at 1151.6, 1086.8, 966.4 cm^{-1} are attributed to C-O(H) (HPMA), Si-O (TSPMA) and -O-CH_2CH_2-O- (PEOMA), respectively.

The ^{1}H-NMR spectrum of the tetrapolymer is shown in Fig. 4. The ^{1}H-NMR ($CDCl_3/CD_3OD = 1:1$) spectrum of the tetrapolymer shows peaks at 3.6 (d) belonging to OCH_2CH_2O (PEOMA), 3.9 (f) to COO-CH_2 (HPMA), 3.7 (e) to Si-OMe_3 (TSPMA), 0.8 (b) to CH_3 (SMA), 0.6 (a) to CH_2-Si (TSPMA) and 1.3 (c) to CH_2 (Chain and SMA).

The integral intensities of those ^{1}H-NMR peaks at $\delta = 0.6, 0.8, 3.6, 3.9$ were used to calculate proportions of each monomer in the tetrapolymer.

The average molecular weight (Mw and Mn) of the polymers were characterized by GPC using a polystyrene standard, from the relationship between retention time and molecular weights derived for narrowly distributed standard polystyrene.

The FT-IR spectrum indicated that the synthesized copolymer contains -CH_2-, C-O(H), Si-O and -O-CH_2CH_2-O- groups, which belong to SMA and main chain, HPMA, TSPMA and PEOMA, respectively. The composition of the copolymer, calculated from the ^{1}H-NMR spectrum, was found to be $SMA_{51.2}PEOMA_{8.8}HPMA_{33.9}TSPMA_{6.1}$. Molecular weights of the copolymer, estimated from the GPC

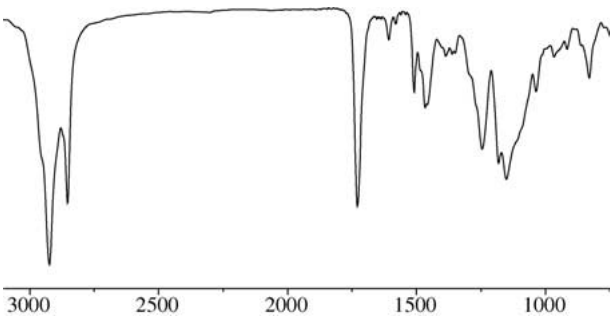

Figure 3 FT-IR spectrum of tetrapolymer.

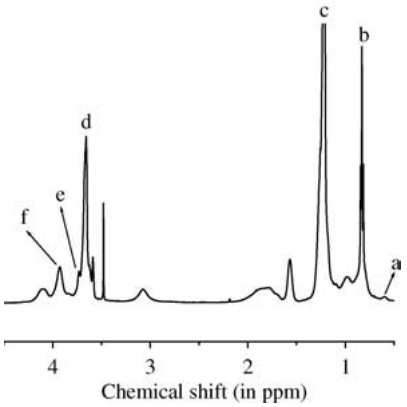

Figure 4 ^{1}H-NMR spectrum of tetrapolymer in $CDCl_3$. (a) -CH_2-Si-; (b) -CH_3 (SMA); (c) -CH_2- (SMA); (d) -O-CH_2CH_2-O (PEOMA); (e) Si$(OCH_3)_3$ (TSPMA); (f) $COOCH_2$ (HPMA).

spectrum, were found to be 25 000 (Mn) and 54 000 (Mw) and the Mw/Mn to be 2.16. The results from FT-IR, ^{1}H-NMR and GPC indicated the tetrapolymers had expected structure as shown in Fig. 1.

3.2. Characterization of the coating

3.2.1. ATR-FT-IR

The PEO based coating were prepared by dip-coating and curing at 90 °C for 9 h. The ATR-FT-IR spectra for the pre-crosslinked and post-crosslinked coatings are presented in Fig. 5.

The pre-crosslinked coating produced two peaks at 1001.1 and 777.6 cm^{-1}, in contrast to the post-crosslinkable coating which produced two peaks at 1008.8 and 787.3 cm^{-1} due to the crosslinked Si-O-Si bond.

3.2.2. Contact angle test

Two methods named sessile drop and captive bubble were performed to measure the static contact angle of the coating. The results are shown in Table I. The given values are the values from 10 parallel data of each sample. The contact angle of the control substrate (glass), pre- and post-crosslinking of the coating by means of sessile drop are 9.8°, 92.8° and 102.0°, respectively, whereas the corresponding captive bubble data were tested to be 5.8°, 38.0° and 42.3°, respectively.

It was reported that the data from the sessile drop method were the advancing contact angle θ_{adv} at the 15th second after water drop let contacted the original air

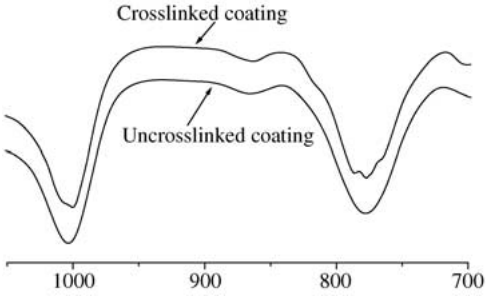

Figure 5 ATR-FT-IR spectra of the pre- and post-crosslinked coatings.

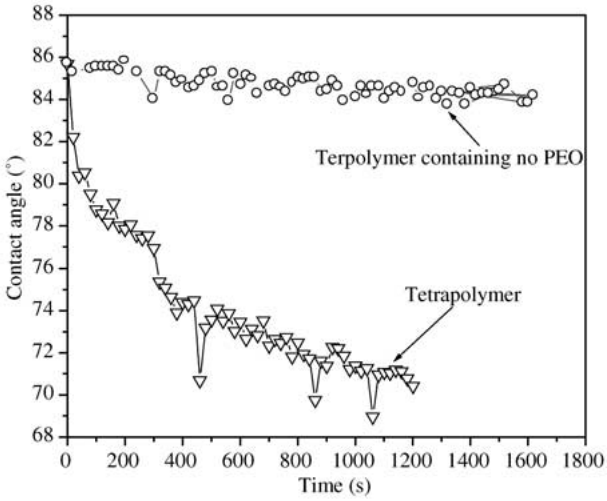

Figure 6 The change of contact angle dependent on time test recording the change of contact angle versus time using sessile drop method.

interface of the samples, while the data from the captive bubble method were fairly close to the equilibrated receding water contact angle θ_{rec} [11, 12]. In this experiment, the contact angle hystereses of tetrapolymers (54.8° and 59.7° for pre- and post-treated samples, respectively) were larger than that of those control samples (4° for control glass).

The change of contact angle dependent on time test by the means of sessile drop was used to monitor the changes of the contact angle in real-time. The initial contact angle results within first 30 min are shown in Fig. 6. The results reveal that the polymer with hydrophilic group (PEO) shows obvious decrease of contact angle with time, whereas the polymer without PEO did not exhibit this character.

Both the contact angle hystereses and the change of contact angle dependent on time testing suggest that the hydrophilicity of the coating was greatly improved after contacting with water.

This is not surprising if we examine the data with respect to the surface migration behavior on the copolymer/air interface. As reported in previous papers [13, 14], an interface will always attempt to achieve the lowest interfacial energy. If a polymer system contains more than one component, migration of the components, when localized at the interface, will result in a minimum interfacial energy.

The critical surface tensions of n-Octadecane and PEO are 27.87 [15] and 45.9 in 10^{-3} N/m, respectively [16]. Therefore, it is expected that the stearyl will migrate and accumulate at the polymer/air interface. In an aqueous environment, the PEO molecules will achieve their minimum free energy by binding water molecules to the ether groups (exothermic) and diffusing into the water phase to achieve as random (high entropy) conformations as possible [17]. A more hydrophilic surface will be then obtained in the blood contact condition.

3.3. Blood compatibility evaluation

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 coagula-

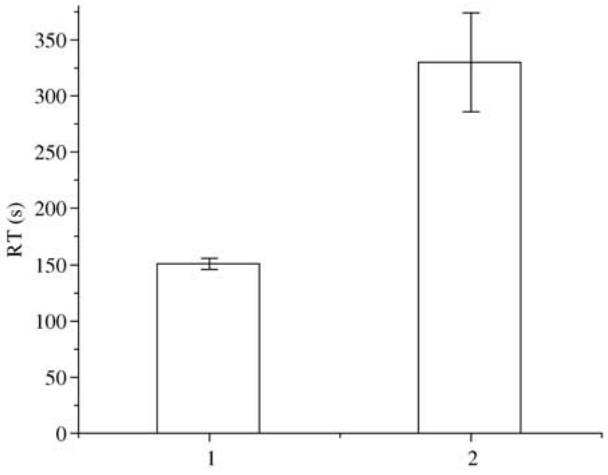

Figure 7 Columnar scheme of recalcium time: (1) Control glass; (2) Crosslinked coating prepared from the tetrapolymer.

tion 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).

As shown in Fig. 7, PRT of the coating was prolonged compared with the case of control sample which results from the anti-coagulated surface coating system. Fig. 8 shows typical images of surface-blood-contacting samples obtained from the Invert Fluorescence Microscope. Fig. 9 is the sketch map of the number of platelets on each sample.

Samples were contacted with the blood from the same donor and under the same conditions as the control. The uncoated sample (A) surface was covered with cellular matter; fibrin clots and activated platelets. For the

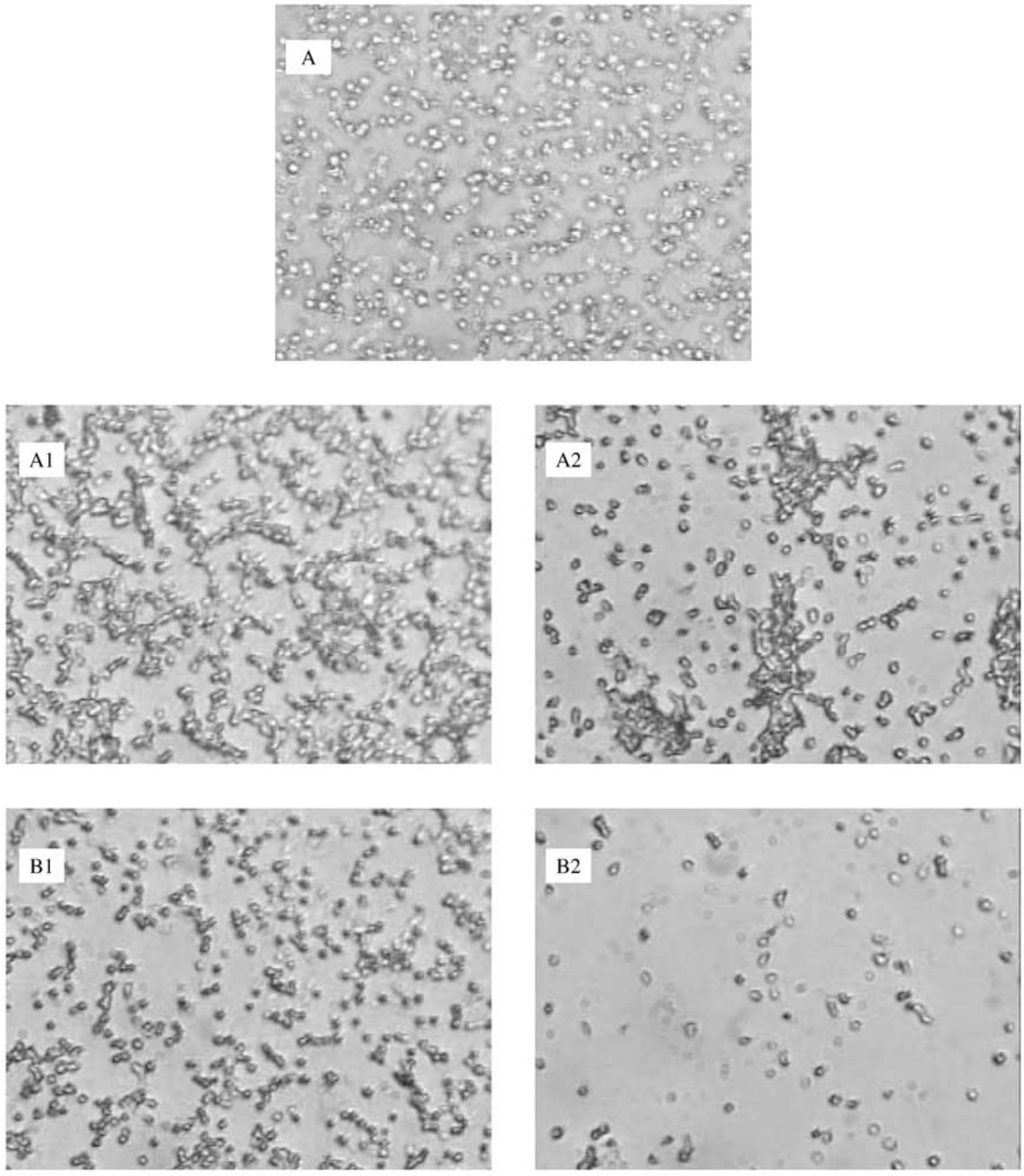

Figure 8 Typical images of platelet on (A1) glass control before treatment; (A1) Terpolymer coating with no PEO group before treatment; (B1) Tetrapolymer coating with PEO group before treatment; (A2) Terpolymer coating with no PEO group after treatment; (B2) Tetrapolymer coating with PEO group after treatment.

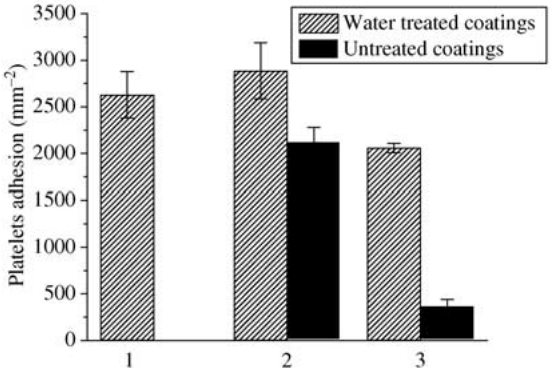

Figure 9 Columnar scheme of platelet adhesion: 1, 2, 3 are control glass, terpolymer with no PEO group, tetrapolymer with PEO group, respectively.

coatings containing no PEOMA, the results were similar for pre- (A1) and post- (A2) treated samples. As for The PEO containing samples, The post-treated one (B2) was rather clear and very little unactivated platelets existed on the sample contrasting to the pre-treated one (B1).

This could be explained by the more hydrophilic surface of the treated coating as could be seen from the results of contact angle.

The favorable biocompatibility of the crosslinked coating is indicated by the results of recalcium time and platelet adhesion.

3.4. Drug release characteristic of coatings

Plasma recalcification time testing and platelet adhesion testing have indicated that the tetrapolymer coating has good blood compatibly, which will satisfy the primary request for the biomedical use of the biomaterials contacting with blood. In addition, controlled permeation and releasing of drugs from polymeric coated implant provides a new approach to treat device based infection, tumor and stent restenosis etc. Therefore, it is interesting to explore the drug release characteristic of the coatings. We chose Rhodamine S and Cibacron Blue, both of which are easily obtainable and easy to be characterized quantitatively, as model drugs to perform the precursory work.

Fig. 10 demonstrated the releasing curves of model drugs, Rhodamine S and Cibacron Blue from the crosslinked coatings of tetrapolymer.

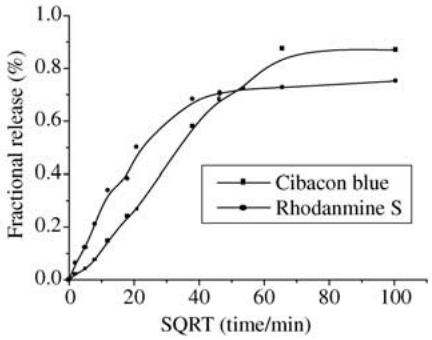

Figure 10 Drug-releasing curves of model drugs, Rhodamine S and Cibacron Blue.

A simple semi-empirical equation was introduced to represent the drug release process of swelling polymer [18]:

$$\frac{M_t}{M_\infty} = kt^n$$

The diffusion kinetic constant k defines the characteristics of a polymer network system. The diffusion coefficient n represents the mechanism of drug transportation; $n = 0.50$ for the Fickian release and $n = 1.00$ for Case-II release. Between these two limiting cases, where $0.50 < n < 1.00$, the intermediary case is classed as anomalous release.

The n value is ≈ 0.5 for Rhodamine S before 70% total release amount $(n = 0.46 \pm 0.02)$ and for Cibacron Blue before 85% total release amount $(n = 0.54 \pm 0.03)$ as can be concluded from the linear increase of release amount on $t^{1/2}$. These results suggest that the mechanism of the release is approximately Fickian release. $T_{50\%}$ of Rhodamine S is 8.3 h and that of Cibacron Blue is 19.2 h. The longer $T_{50\%}$ may come from the relatively larger molecular weight of Cibacron Blue (738) than that of Rhodamine S (374.5). More detail work about the release of sirolimus from the crosslinked coating is underway.

Acknowledgment

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