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Macromolecules
NOTE

Novel Blood-Compatible Polyurethane Ionomer Nanoparticles

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Introduction

Polymeric biomaterials have been widely used in biomedical applications, including disposable clinical apparatus, such as vascular prostheses, blood pumps, artificial kidney and hearts, dialyzers, and plasma separators. One of the key requirements is that these materials have to be blood-compatible because of their contact with blood. In spite of a great development in this direction, most of conventional and currently used polymers are still prone to induce clot formation, as platelets and other components of the blood coagulation system are activated.[1−4] Much attention has been paid to the thrombus formation mechanism on the surface of polymeric materials, but there were still many disputes and unknown problems.[1b] The hemocompatibility of biomaterials continues to be improved for biomedical applications.

Note that the evaluation of anticlot formation of bulk polymeric material is a fine-consuming process. Polymeric nanoparticles have been rapidly developed for catalysis, biological labeling, photonics, photography, information storage, surface-enhanced Raman scattering, and formulation of magnetic ferrofluids.[3,4] Therefore, we moved to a new path to evaluate the anticoagulation of blood cells by using polymeric nanoparticles.

First, we chose polyurethane (PU) because it is bio- and environmental-compatible.[5] PU dispersions as a special class of colloidal dispersions can be prepared from a mixture of different chemical species, in which interfacial energy can be easily manipulated. In addition to their major applications in coatings, these PU dispersions have recently found their applications in biological fluids, pharmaceuticals, foods, and cosmetics. In the current study, we synthesized novel polyurethane ionomer nanoparticles (PUI-NPs). The activated partial thromboplastin time (APTT) and prothrombin time (PT) were used to assess their blood compatibility, which was further correlated to that of PUI film evaluated by the platelet-rich plasma (PRP) contacting experiments and scanning electron microscopy (SEM).

Experimental Section

Synthesis of PUI-NPs and Film. To prepare PUI-NPs, 4,4′-diphenylmethane diisocyanate (MDI) from Sigma-Aldrich was stored at −22 °C before use to prevent its dimerization. Poly(tetramethylene ether glycol) (PTMG) and dimethylolpropionic acid (DMPA) from Aldrich were respectively dried in a vacuum oven for 12 h and in air for 2 h at 100 °C. Ethyl acetate and pyrrolidinone were purified and dried over 4 Å molecular sieves after distillation. The blank PU film (Biomedical grade) was received from Shenzhen Best Technology Co. Ltd., China. All other chemicals were used without further purification.

As shown in Scheme 1, 4.25 g of MDI, 8.8 g of PTMG, and 0.5 μL of dibutyl tin dilaurate (DBTL) were dissolved in 40 mL of ethyl acetate and added to a 500 mL four-necked round-bottom flask equipped under mechanical stirring at 75 °C and a nitrogen atmosphere. Then, 0.88 g of DMPA dissolved in 10 mL of pyrrolidinone was gradually added under stirring. 4 h later, 3 mL of isopropanol was added as the end-capping agent. The solution mixture was further stirred at 40 °C for 30 min. Subsequently, 1 mL of triethylamine was added and stirred for a further 10 min. The solution mixture was divided into two parts. One part was added to a 50 mL mixture of water and sodium dodecyl sulfate (SDS) with a weight ratio of 96:4 under stirring, resulting in a white emulsion after 30 min, which was redispersed in 0.2 M phosphate buffered solution (PBS) (pH = 7) under ultrasonic agitation for 30 min. The volume ratio of emulsion PU and PBS is 1:10. The PUI-NPs were purified by dialysis (Spectra/Por CE, MWCO = 10 000) in distilled water over a 7 day period to remove unreacted monomers, SDS, and salts. Finally, all the solvents were removed by freeze-drying, leading to PUI-NPs in a white powder form. The PUI-NPs obtained can be redispersed in water to form a dispersion. The other part was placed into a Teflon disk with a diameter of 10 cm. After removing solvents and a thorough dialysis, a PUI film was obtained.

Cell Electrophysiological Recordings. Experiments were performed on HEK293 cells that stably expressed with the wild-type a human ether-ά-go-go related gene (hERG) containing green fluorescent protein (provided by Prof. Zhao Zhang, College of Life Sciences, Nanjing Normal University, China). Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM, Hyclone) supplemented with 10% (v:v) fetal bovine serum and 1% (v:v) penicillin and streptomycin at 37 °C in an humidified atmosphere of 5% CO_2.

hERG currents were recorded by the patch clamp technique in the whole-cell mode (Axopatch-200B amplifier controlled by pCLAMP 9.2, Axon Instruments). The PUI-NPs solutions were freshly prepared with the physiological solution. The electrophysiological recordings were performed using previously described standard methods.[6]

In Vitro Coagulation Time Tests. The nephelometry measurements, including APTT and PT, were performed with the coagulation instrument Sysmex CA-1500 (Sysmex Corp., Japan) that measured the change of luminosity when light traversed the plasma sample.

Platelet Adhesion Test. The disk-shaped blank PU and PUI films (15 mm in diameter) were placed into a 24-well cell culture plate. To equilibrate the film surface, 1 mL of PBS [pH 7.4; ionic

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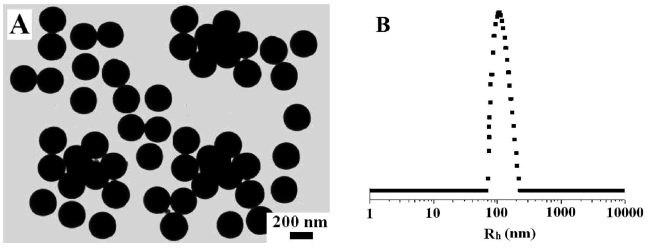
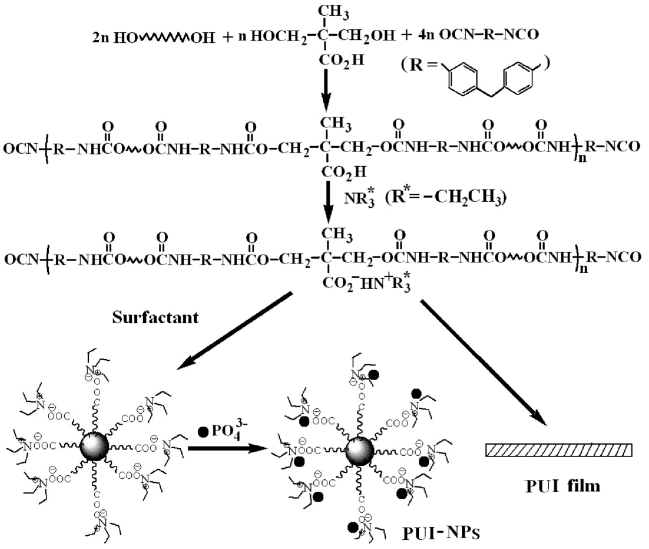


Figure 1. (A) TEM image of PUI-NPs measured by JEOL JEM 2011 interface high-resolution transmission electron microscopy. (B) Particle size distribution measured by a Microtrac UPA 250 dynamic light scattering all for five analyses.

Scheme 1. Elementary Steps for the Synthesis of PUI-NPs and Film



strength, 0.15 M] was added into each well and allowed to remain for 12 h. After removing PBS, 1 mL of freshly prepared PRP of human blood was poured on the film and allowed to remain for 90 min at 37 °C, where PRP was prepared from citrated fresh human blood (Blood Center of Jiangsu Province, China), and the platelet number (1.0×10^9 unit/mL) inside was determined with a Coulter counter (Sysmex KX-21, East Asia Co., Japan). After PRP was removed with an aspirator, the film was rinsed three times with 1 mL of PBS. Further, 1 mL of 2.5 vol % glutaraldehyde in PBS was poured into each well, which was maintained at the room temperature for 1 h to fix the platelet on the film. The film was washed with PBS again and then subsequently dehydrated by systemic immersion in a series of ethanol−water solutions [50, 60, 70, 80, 90, 95, and 100% (v/v)] for 30 min each time and allowed to evaporate at the room temperature. The film surface was gold-sputtered.

Results and Discussion

Figure 1 shows that PUI-NPs are narrowly distributed with an average hydrodynamic diameter of 234 nm and a polydispersity index of 0.068. The gel permeation chromatographic (GPC, Agilent 1100 series equipped with PLgel 5 μm mixed-c 79911GP-MXC chromatographic column; the mobile phase is tetrahydrofuran, and its flow speed was set at 0.6 mL min^{-1} at 35 °C) analysis of PU chains inside these particles has a weight-average molar mass (M_w) of 2.29×10^4 g/mol and a polydispersity index (M_w/M_n) of 1.68.

PUI-NPs with PO_4^{3-} as their counterions has a ζ-potential of −3.2 mV measured by a BI-9000AT ζ-potential analyzer. It has been known that a negatively charged surface normally leads to better hemocompatibility.[1b,7]

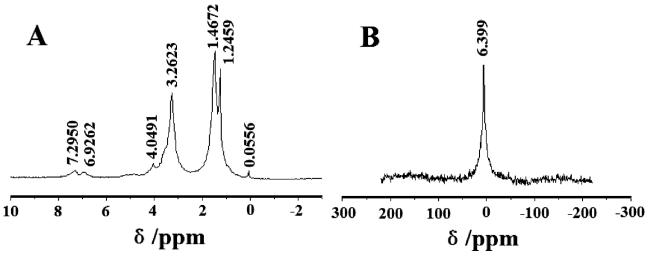


Figure 2. (A) Solid-state 1D ^1H NMR. (B) Solid-state ^{31}P NMR spectrum of PUI-NPs.

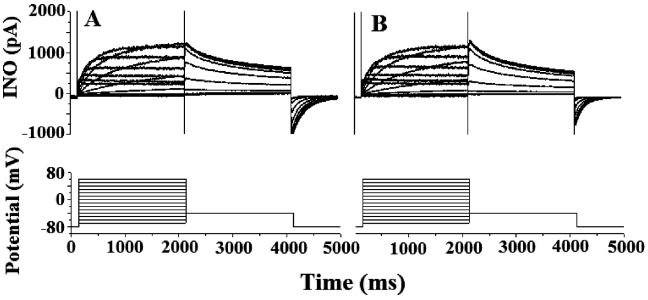


Figure 3. Representative hERG current traces (A) in the absence of the PUI-NPs and (B) in the presence of the PUI-NPs (20 μg/mL).

The chemical structure of the PUI-NPs was also analyzed by solid-state 1D ^1H and ^{31}P NMR spectroscopy. Typically, the ^1H NMR spectrum and the peak assignments of sample PUI-NPs are present in Figure 2A.[8] The strong proton signal at δ 3.26 ppm was attributed to all the methylene protons of $N(CH_2CH_3)_3$ and PTMG (O=C−$CH_2CH_2CH_2$$CH_2$C=O). Signals at δ 1.47 or 1.25 ppm were assigned to methyl protons of $N(CH_2CH_3)_3$ or $-CCH_3$, respectively (Scheme 1). The peak area ratio between methyl protons of $N(CH_2CH_3)_3$ and that of $-CCH_3$ is ca. 3:1, indicating that triethylamine exist in PUI-NPs. The urethane linkage in the product was also traced through ^1H NMR. Imino group −OOCNH− from reacted cyanate group assigned to δ 4.05 ppm. The two signals appearing at 7.29 and 6.93 ppm were from the arene protons. One signal observed at δ 6.40 ppm in the 1D ^{31}P NMR experiment (Figure 2B) was assigned to PO_4^{3-}.[9] All these results revealed that MDI-diisocyanate reacted with DMPA and PTMG through the coupling reaction, and the polyurethane was formed. Moreover, PO_4^{3-} was introduced into PUI-NPs by the self-assembled method. The characterization of triethylamine and PO_4^{3-} in PUI-NPs by NMR after being treated with 0.2 M PBS might indicate that this ionomer is stable.

In order to assess the effect of the PUI-NPs on the cell membrance and the safety of new biomaterials, the currents of the K+ channel were examined using a whole-cell patch clamp method.[6] The recordings of the hERG current in the absence and presence of the PUI-NPs were obtained (Figure 3). It was observed that the currents in the presence of the PUI-NPs are not obviously changed comparing with that in the absence of the PUI-NPs. The results show that the PUI-NPs do not damage to the cell, implying that the HNR_3^+ cations are not exchanged by cations in cell perfusion solutions.

Clinically, the abnormality of blood plasma is often detected by both activated partial thromboplastin time (APTT) and prothrombin time (PT). Recently, they are also used to evaluate in vitro the antithrombogenicity of a given biomaterial.[10] Table 1 shows that, for a given volume of PUI-NPs dispersion both APTT and TT, measured by Sysmex CA-1500 (Sysmex Corp., Japan), increase with the PUI-NPs concentration, indicating that the PUI-NPs are antithrombogenic and exhibit a high anticoagulant activity. It is considered that the improved antithrombogenicity

Table 1. Activated Partial Thromboplastin Time (APTT) and Prothrombin Time (PT) of Citrated Normal Human Plasma Samples after They Are Treated with Different Concentrations of PUI-NPs[a]

	clotting time (s)	
concentration of PUI-NPs (mg/mL)	APTT	PT
0	37.0 ± 2.47	13.6 ± 0.87
10	43.8 ± 2.52	14.0 ± 1.43
15	49.4 ± 3.14	14.2 ± 1.28
20	51.4 ± 2.89	14.3 ± 1.21
25	61.4 ± 3.67	14.5 ± 1.31

[a] The results are average values of three measurements.

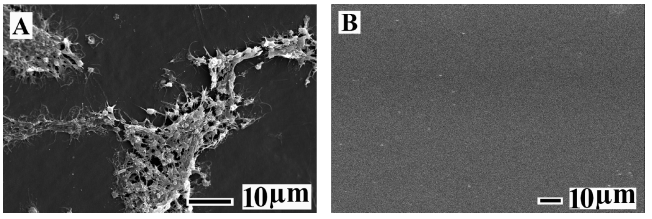


Figure 4. SEM graphs of film surface after it is contacted with platelet-rich plasma for 90 min: (A) blank PU film and (B) PUI film.

can be attributed to the ionomer structure of PUI-NPs. The mechanism of nonthrombogenic of PUI-NPs may be similar to that of zwitteronic molecules.[2] A more clear mechanism will be further investigated by our group in the future.

In order to correlate the blood compatibility of PUI-NPs to a PUI film, we further shilled the platelet adhesion on the PUI film after it was in contact with platelet-rich plasma (PRP) by SEM.[11] Figure 4A shows that after 90 min contact with the PRP, the blank PU film is high platelet adhesive and most of the adhered platelets were distorted with pseudopodia. Surprisingly, there are nearly no adhered platelets on the PUI surface (Figure 4B), revealing that the PUI film has an excellent antiplatelet adhesive property. This is consistence with previous anticoagulant results of PUI-NPs.

Conclusions

Narrowly distributed novel polyurethane ionomer nanoparticles (PUI-NPs) can be prepared by emulsion polymerization. A combination of activated partial thromboplastin time (APTT), prothrombin time (PT), and platelet-rich plasma (PRP) adhesion tests reveals that PUI-NPs have good blood compatibility. Our result also shows that there is a good correlation between a macroscopic film and microscopic PUI-NPs, which enables us to use nanoparticles to quickly test whether a given polymeric material is blood-compatible.

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