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Glucose-sensitive and blood-compatible nanogels for insulin controlled release

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ABSTRACT: For insulin delivery, many reported glucose-sensitive materials are designed to response to the glucose in the blood. However, few particular studies on their blood compatibility have been reported. In this article, for controlled insulin release in diabetes therapy, a glucose-sensitive nanogel was prepared through thermally initiated precipitation polymerization using the aminophenylboronic acid-containing monomer to copolymerize with methacrylic acid. The obtained nanogels showed the uniform and spheroidal morphology as observed by SEM, and their sizes in aqueous solution are dependent on the concentration of glucose. Through *in vitro* and *in vivo* insulin release tests, it was found that nanogels showed the glucose-dependent insulin release and prolonged effect of lowing blood glucose level. The blood compatibility of nanogels has also been explored through various assays including the hemolysis, activated partial thromboplastin time, prothrombin time as well as the thromboelastography. All results indicated that the obtained glucose-sensitive nanogels showed good blood safety. Moreover, their low cytotoxicity suggested a potential application in diabetes therapy. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43504.

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INTRODUCTION

Glucose-sensitive systems have received significant attention in recent years due to their applications in drug delivery, biosensing, bio-separation, etc.[1–3] Especially in diabetes therapy, glucose-sensitive drug delivery systems have drawn great attention. Glucose-sensitive carriers can response to glucose in the blood by swelling so that the loaded insulin can be released.[4–6] Luo *et al.* prepared a glucose-sensitive layer-by-layer film for insulin long-term release. Insulin participates in the supramolecular assembly and the obtained film could sustainably release insulin for 1 month.[7] Liu *et al.* synthesized the block copolymer containing phenylboronic acid to form the glucose-sensitive micelles, which displayed a reversible response to the glucose concentration and then the repeated on–off insulin release by glucose level.[8] Yang *et al.* prepared a glucose-sensitive hydrogel based on the dynamic covalent chemistry and inclusion complexation. The synthesized PEG-PVA block copolymer could interact with α-cyclodextrin, resulting in a supramolecular hydrogel for controlled insulin release.[9]

Among these carriers, nanogel has been applied widely in drug delivery due to their rapid response to environmental changes.[10–12] Because of its nanosize, nanogel is more sensitive to specific stimuli, and also makes it possible to simple administration through injection.[13] Moreover, compared with liposome, micelles, or polymer carries, nanogels are more stable in circulation which reduces the drug leakage and side effects.[14–16] Particularly, nanogels have been reported to deliver insulin and show the good effect. For example, Zhao *et al.* prepared the glucose-sensitive nanogel through a thiolene copolymerization. The obtained nanogel showed obvious glucose sensitivity and good biocompatibility.[17] Wu *et al.* prepared an injectable nanogel for the controlled release of insulin. *In vivo* assay indicated that insulin-loaded nanogels could reduce the blood glucose level in diabetic rats and maintain the baseline level for almost 2 h.[18]

However, most reported nanogels need complicated preparation process, and their morphology and homogeneity are not easy to control. Moreover, though all reported glucose-sensitive

materials are designed to response to the glucose in the blood, few particular studies on the blood compatibility of the materials have been reported.

Our previous work has prepared the PMAA nanogels through the precipitation polymerization, and found that PMAA could form the nanosized and uniform spheroidal nanogels.[14] In this work, a glucose-sensitive nanogel with uniform and spheroidal morphology was prepared via one-step thermally initiated precipitation polymerization. A monomer containing aminophenylboronic acid group, which could reduce immune reaction *in vivo*,[19] was used to copolymerize with methacrylic acid. The used methacrylic acid could increase the hydrophilic property of the obtained nanogels and also decrease the pK_a value of aminophenylboronic acid group, which could contribute the glucose-sensitive nanogels to work at physiological pH. *In vitro* and *in vivo* assays confirmed that the obtained nanogels release insulin sustainedly and reduce blood glucose levels in diabetic rats. In particularly, the blood compatibility and toxicity of nanogels have also been studied by a series of blood assays and *in vitro*/*vivo* assays.

MATERIALS AND METHODS

Materials

Methacrylic acid (MAA) was purchased from Sigma and distilled prior to use. *N*-acryloyl-3-aminophenylboronic acid (AAPBA) and ethylene glycol dimethacrylate (EGDMA) were purchased from TCI and recrystallized before use. The 2,2′-azobisisbutyronitrile (AIBN) was purchased from Damao Chemical Reagent Factory (Tianjin, China) and recrystallized from ethanol. Glucose, insulin and fluorescein isothiocyanate (FITC) was purchased from Aladdin Chemistry (Shanghai, China). Cell counting kit-8 (CCK-8) assay and Dulbecco's modified Eagle's medium (DMEM) were purchased from Qiyun Biology Technology (China). All other chemicals were of analytical grade and used directly. Blood from healthy consented volunteers was collected in sodium citrate tube with a blood/anticoagulant ratio of 9:1. Reagents for conventional coagulation assays were provided by the First Affiliated Hospital of Jinan University (Guangzhou).

Preparation and Characterization of Glucose-Sensitive Nanogels

Glucose-sensitive nanogels were prepared through the thermally initiated precipitation polymerization. For Glu(2/3) sample instance, 0.411 mmol MAA and 0.616 mmol AAPBA were dissolved in 50 mL acetonitrile, and then 2.398 mmol EGDMA and 0.19 mmol AIBN were added. The mixture was reacted at 60 °C for 24 h under N_2 atmosphere. After the reaction, the mixture was centrifuged, and the product was washed with ethanol to remove the unreacted monomers. The obtained nanogels were then lyophilized with a yield of 86%.

The nomenclature of nanogel (Glu(a/b)) was as follows: *a/b* represents the molar ratio of MAA/AAPBA. For example: Glu(2/3) denotes that the product was prepared at an molar ratio of MAA/AAPBA equals to 2:3, with 1 vol % initial monomers concentration and 70 mol % EGDMA content.

The morphology of products was observed by the scanning electron microscope (SEM, PHILIPS XL-30). Each sample was coated with aurum by sputter coating at 30 mÅ for 90 s. The diameter of nanogels was studied by the dynamic laser light-scattering (DLS, Malvern, Zetasizer Nano ZS). The elemental analysis was carried out to determine the AAPBA monomer contents in the obtained nanogels. The pK_a of the nanogels has been analyzed by Henderson–Hasselbalch analysis.[20]

Insulin Loading and *In Vitro* Release

For loading and release tests, insulin was labeled by FITC according to the reported method.[21] The labeled insulin was loaded into nanogels through an immersing method. Briefly, 20 mg nanogels were well dispersed into 25-mL phosphate buffer solutions (PBS, pH = 7.4) which contained 0.3 mg mL^{-1} insulin, and the suspension was kept in a vapor-bathing constant temperature vibrator at 37 °C for 24 h. After that, the insulin-loaded nanogels were collected by centrifugation and washed with PBS. The drug loading content (LC) was calculated by the results from UV–vis spectroscopy. As determined, the LC was 14.8% and that was to say the loading amount was 0.148 g g^{-1}.

For the release test of insulin, 15 mg FTIC-insulin-loaded nanogel was dispersed in 3 mL PBS, and then the dispersive nanogel was divided into three equal aliquots. Each sample was transferred into a dialysis bag (MWCO = 30,000) and immersed into 10 mL PBS containing different concentration of glucose. The samples were incubated in vapor-bathing constant temperature vibrator with 60 rpm min^{-1} at 37 °C. At predetermined time points, 2.0 mL of released solution was taken out and 2.0 mL of fresh PBS containing different glucose concentration was added back. The amount of FITC-insulin was analyzed by a UV spectrophotometer at 498 nm absorption wavelength. The percentage of accumulated amount of FITC-insulin released was calculated from a standard curve ($R^2 > 0.999$). All results were repeated in triplicate.

In Vivo Assay

The male Wistar rats (180–220 g) with diabetes were obtained according to the reported method and the rats were considered diabetic when their blood glucose level was higher than 16.7 × 10^{-3} mol L^{-1}.[22] The Institutional Administration Panel for Laboratory Animal Care approved the experimental design. The university guidelines for care and use of laboratory animals were strictly followed. All rats were housed and fed in the Experimental Animal Center of Jinan University and were specific pathogen free. Rats were randomly divided into four groups and each group contained five rats. Insulin-loaded nanogels were delivered into rates through hypodermic injection, and insulin content was 30 IU kg^{-1}. The rats injected with pure insulin and blank nanogels without loading insulin were also tested. Their blood glucose levels (BGLs) were measured through the blood which was obtained from the tail vein by a one-touch blood glucose monitoring system (OneTouch® UltraEasy™, LifeScan, Milpitas, CA).

Blood Compatibility Evaluation

The Morphology of Red Blood Cells (RBCs). The RBCs were incubated with a series of nanogel samples for 10 min, and then

the suspensions were centrifuged gently. The obtained RBCs were fixed using 4% paraformaldehyde overnight. The fixed RBCs were planted on glass slides and dehydrated with 70, 85, 95, 100% (v/v) ethanol for 10 min respectively. The dried RBCs were coated with gold and observed by SEM.

Hemolysis Assay. For the hemolysis study, 50 μL RBC suspension(16% v/v in PBS) was added into 1 mL nanogels solution, and then incubated for 3 h. RBC suspensions incubated with PBS and distilled water were used as negative and positive controls, respectively. After that, the RBC suspensions were centrifuged for 5 min. The supernatants (200 μL) were measured at 540 nm with a 96-well plate reader (Multiskan MK3, Thermo Scientific, USA). The percentage hemolysis was calculated by the following formula[23]: hemolysis (%) = [(OD of the test sample − OD of negative control) × 100]/OD of positive control.

Activated Partial Thromboplastin Time (APTT) and Prothrombin Time (PT). APTT and PT assays were carried out by a SF-8000 automatic coagulation analyzer (Beijing Succeeder Company, China). A 20 μL nanogel solution was mixed with 180-μL platelet-poor plasma at 37 °C, PBS was used as control group. In tests, the effects of the samples on clot formation were detected for up to 100 s according to the setting of the analyzer. Each experiment was carried out in triplicate.

Thromboelastography (TEG). The effects of different nanogel samples on blood coagulation were studied at 37 °C by a thromboelastograph hemostasis system 5000 (TEG, Haemoscope Corporation, USA). In brief, 360 μL whole blood was mixed with 40 μL nanogel solutions in PBS in a tube containing kaolin, and then 340 μL suspension was transferred into TEG cup. Subsequently, the TEG analysis was initiated by adding 20 μL of 0.2 mol L^{-1} $CaCl_2$ solution. PBS was used as control group.

Cytotoxicity

The cytotoxicity of the nanogels on NIH-3T3 (mouse embryonic fibrolast cell line) cells was studied by CCK-8 assay. Briefly, NIH-3T3 cells (5000 cells/well) were seeded in 96-well tissue culture plates and incubated for 24 h at 37 °C and 5% CO_2 in DMEM culture medium (with high glucose and 10% fetal bovine serum supplemented). After that, the growth medium was replaced with a fresh medium that contained the blank nanogels. PBS was set as control group. After 24 or 48 h, the media was carefully removed and the cells were carefully washed with PBS. Then, the cells were replenished with100 μL DMEM containing 10 μL CCK-8 solutions and incubated for 2 h. The absorbance of supernatant was measured at 450 nm by a microplate reader (Multiskan MK3, Thermo scientific, USA).

RESULTS AND DISCUSSION

Preparation of Glucose-Sensitive Nanogels

PMAA could form the nanosized and uniform spheroidal nanogels,[14] however, AAPBA could not form the nanogels using the precipitation polymerization. So, the glucose-sensitive nanogels were prepared via a thermally initiated precipitation copolymerization using MAA and AAPBA monomers. To change the molar ratios of MAA and AAPBA, two glucose-sensitive nanogel samples, Glu(1/4) and Glu(2/3), were obtained, and the PMAA nanogel without glucose-sensitivity was also prepared as control.

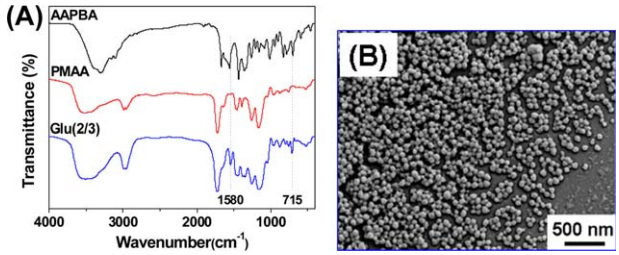

Figure 1. (A) FT-IR spectra of AAPBA monomers, PMMA nanogel and Glu(2/3) nanogel; (B) SEM image of Glu(2/3) nanogel. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

By the elemental analysis, the contents (w/w) of AAPBA in Glu(1/4) and Glu(2/3) was determined as 19.2 and 14.3%, respectively. According to the feed ratio of MAA/AAPBA/EGDMA, the theoretical AAPBA contents in Glu(1/4) and Glu(2/3) was calculated as 24.1 and 18.6%, which was comparable to the feed ratio of the obtained nanogels, suggesting the similar monomer reactivity ratios between MAA and AAPBA.[24]

The obtained nanogels were first characterized by FT-IR and SEM observation as shown in Figure 1. It was seen that PMAA nanogel and AAPBA monomer displayed their characteristic absorption bands respectively in Figure 1(A). Particularly, AAPBA showed the bands of aromatic group at 1580 and 715 cm^{-1}.[25] The typical spectrum of Glu(2/3) showed all characteristic absorption bands of both PMAA and AAPBA, confirming that Glu(2/3) contained AAPBA group which could interact with glucose to result in a glucose-sensitivity. Figure 1(B) showed the SEM image of Glu(2/3) nanogel. It was seen that the obtained Glu(2/3) showed a uniform and symmetrical spherical structure with the diameter of about 100 nm. The nanogels sizes in aqueous PBS have been tested by DLS, and the results showed that the Glu(2/3) diameter was 740 nm and Glu(1/4) diameter was 650 nm. It was found that the sizes in PBS were much bigger than that by SEM observation. This was resulted from their good swelling property in aqueous solution, which was important in drug loading and controlled release.

To confirm the glucose-sensitivity under physiological conditions of the obtained nanogels, DLS was used to record the changes of nanogel size with the glucose concentrations in PBS. As shown in Figure 2, PMAA nanogels without AAPBA showed neglectable change in size, while both Glu(1/4) and Glu(2/3) showed an obvious glucose-sensitivity. Only 0.01 mol L^{-1} glucose made the sizes of Glu(1/4) and Glu(2/3) increase by 120 and 70%. Moreover, Glu(1/4) and Glu(2/3) swelled obviously with the increase of glucose concentration, and the higher AAPBA content resulted in higher sensitivity to glucose. It was reported that phenylboronic acid (PBA) is a kind of Lewis acid with the equilibrium between an uncharged form and a charged form. The charged PBA can interact with *cis*-diol compounds such as glucose to form the hydrophilic phenylborates, which increases the swelling ratios of materials, resulting in the release of payload. This provides a strategy for designing insulin carriers. However, PBA only works in alkaline media but not at physiological pH due to its high pK_a value.[26] The reason was

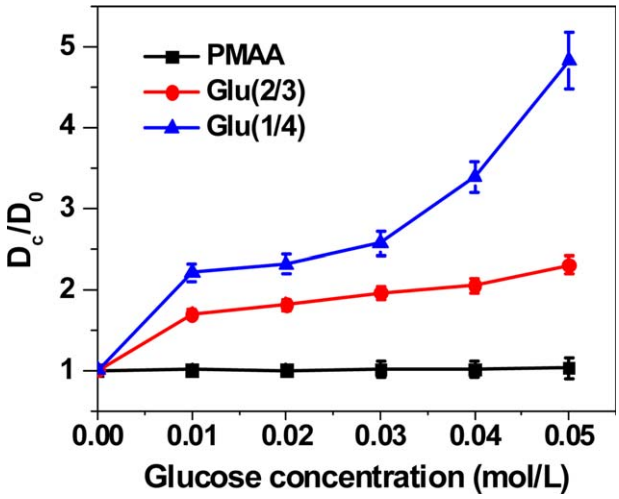

Figure 2. The changes of nanogel sizes with glucose concentrations in PBS at 25 °C ($n = 3$). (D_c denotes the nanogel size at a certain glucose concentration; D_0 denotes the nanogel size when the glucose concentration was zero.). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

only a small part of the phenylboronic acid moieties were ionized under physiological conditions (pH = 7.4), leading to poor water solubility of the PBA-containing copolymer and thus lowering the binding affinity to glucose. In this work, MAA monomer was used to co-polymerize with AAPBA, in which PMAA could increase the hydrophilic property of the obtained nanogels, and also amino group on the phenyl ring could decrease the apparent pK_a value of PBA moiety. The value of pK_a for nanogels was determined as 8.0 by Henderson–Hasselbalch analysis, which showed a significant decrease in pK_a compared with reported PBA-containing copolymer. This may be resulted from the existence of MAA monomer and EGDMA crosslinker, suggesting a promising application under the physiological conditions.

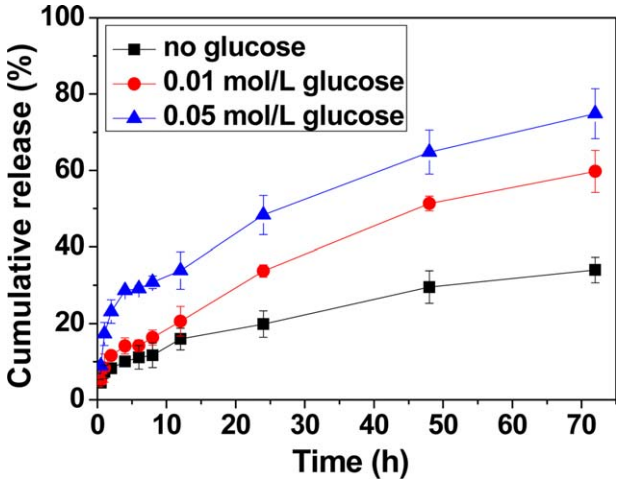

Figure 3. Cumulative FITC-insulin release profiles from Glu(2/3) nanogels under different glucose concentrations (37 °C, PBS, $n = 3$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

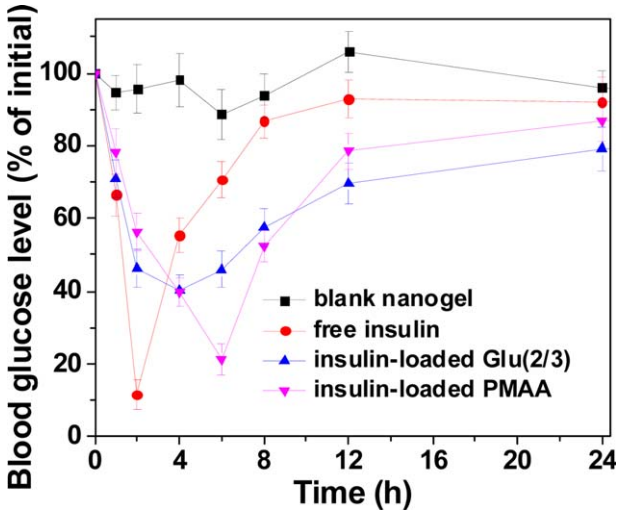

Figure 4. The profiles of rats blood glucose concentrations after injected with different samples. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

In Vitro and *In Vivo* Release

Because of the crosslinked structure, nanogels could release the loaded drugs in controlled manner and reduce the drug leakage in the circulation process.[27] Figure 3 showed the FTIC-insulin release profiles from Glu(2/3) at different glucose concentrations. There is no initial burst release for Glu(2/3) in PBS, and FITC-insulin was released lasting for more than 72 h. Because of the interactions between Glu(2/3) and glucose, FITC-insulin released faster when glucose was added into the release medium. The release rate was dependent on the glucose concentrations, a higher glucose concentration resulted in a faster FITC-insulin release.

In vivo assay was studied by recording the BGLs of diabetic rats after injecting with insulin-loaded nanogels, and the result was shown in Figure 4. For blank nanogels without insulin loaded, the BGL of diabetic rats kept close to the baseline over the course of the assay period. This result demonstrated that the

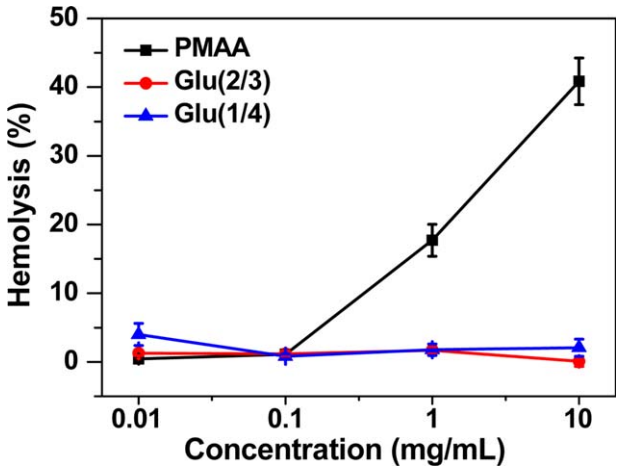

Figure 5. Effect of the nanogels with different concentrations on the hemolysis. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

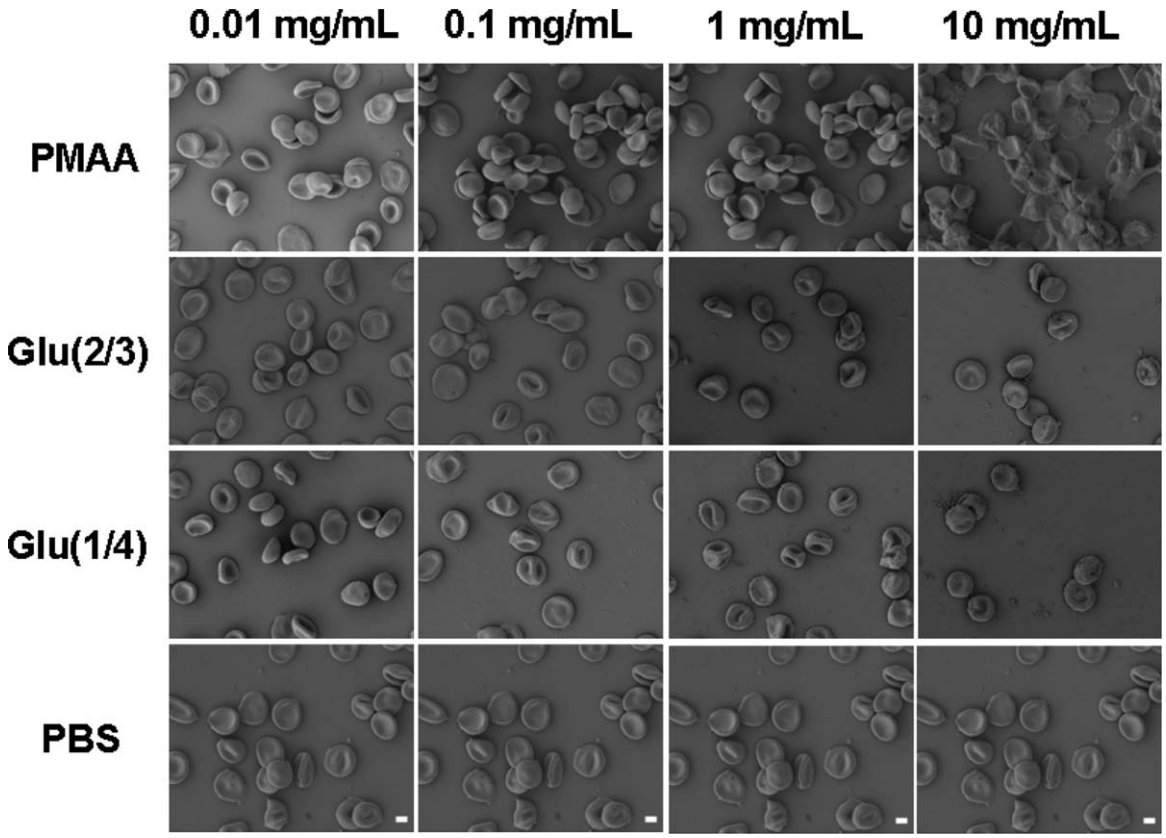

Figure 6. Effect of the different nanogels on the aggregation and morphology of RBCs.

blank nanogel itself had no hypoglycemic effect. When treated with free insulin, the blood glucose level of rats decreased sharply at once and the blood glucose concentration decreased about 90% after 2 h, which may result in serious side effects. However, after 8 h, the blood glucose level returned to basal level again.

Here, nanogel showed its advantage in drug delivery. For insulin-loaded nanogels, the insulin was released sustainably without resulting in a sharp decrease of blood glucose level compared with free insulin, and maintained a low blood glucose level (below the 70% of initial blood glucose level) for 12 h. These results indicated that nanogels could control the release of insulin and maintain a longer hypoglycemic effect compared with free insulin. Moreover, for insulin-loaded Glu(2/3) and PMAA samples, the glucose-sensitive Glu(2/3) showed a better controlled insulin release and the resultant hypoglycemic effect. For PMAA sample, due to its hydrophiphilic property, the blood glucose level reduced by 45% after 2 h, and returned to 75% at 12 h. It was worth noting that the blood glucose level reduced by nearly 80% after 6 h, which showed the too low level of the blood glucose. In fact, we hope to control the insulin released within a required concentration range. The excessive

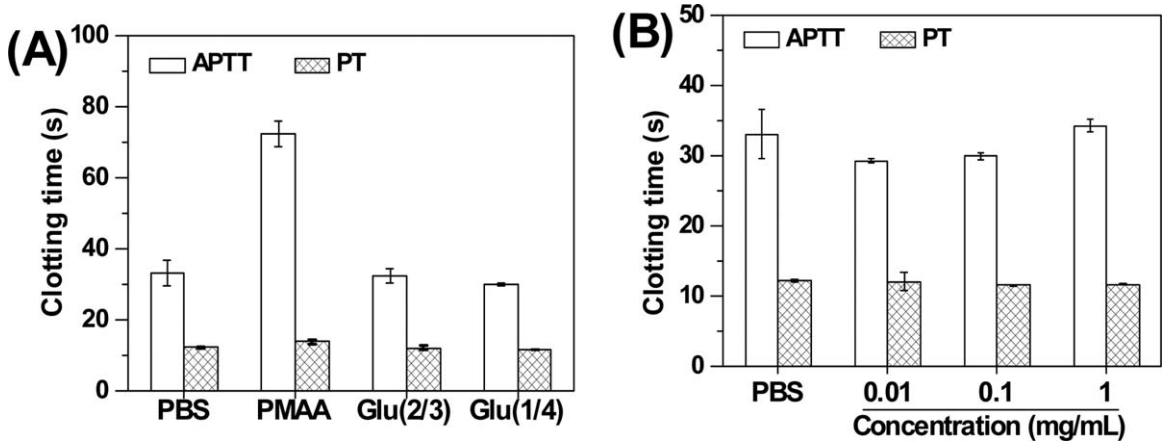

Figure 7. (A) Effect of 0.1 mg/mL nanogels on APTT and PT; (B) Effect of different Glu(2/3) concentrations on APTT and PT.

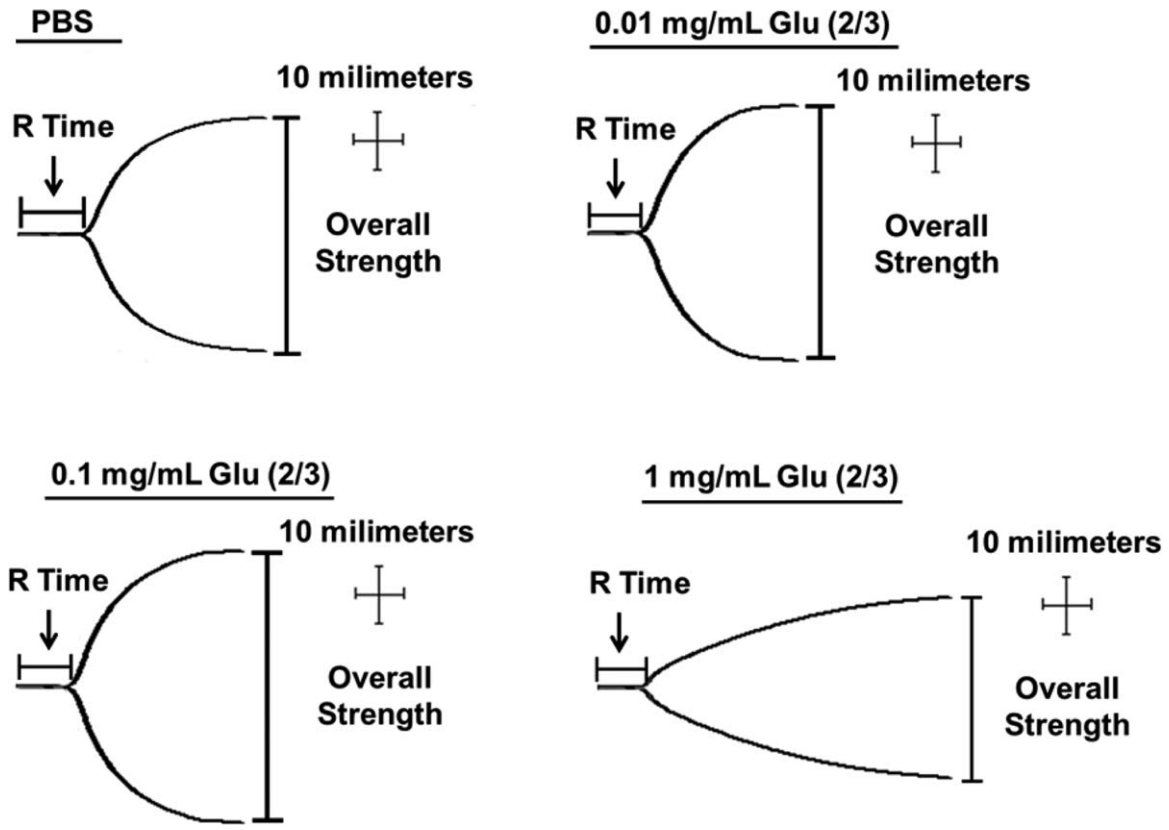

Figure 8. Representative TEG traces of the whole blood coagulation in the presence of different concentrations of Glu(2/3).

release of insulin could also result in the serious side effects, such as hypoglycemia, which also threatened the human life. Because of a glucose-sensitive, Glu(2/3) showed a better controlled release of insulin. Glu(2/3) released insulin faster than PMAA and the blood glucose level reduced about 55% after 2 h, showing a rapid response to a high blood glucose level. The Glu(2/3) also showed a milder and broader interval of low blood glucose, which could avoid side effects resulting from sharp fluctuation of blood glucose and reduce the frequency of drug use.

Blood Compatibility

Although all reported glucose-sensitive materials are designed to response to the glucose in the blood, the blood safety of this kind of materials has not been reported. The instability and nonspecific interactions of biomaterials in the blood could seriously decrease the half-life and target ability of biomaterials, and will to be one of the threaten for human being's life.[28] The blood compatibility of nanogels was first assessed by spectrophotometric measurement of hemoglobin release from erythrocytes after contacting with nanogels with different concentrations. As evident from the data given in Figure 5, PMAA nanogels caused serious hemolysis when their concentration was higher than 1 mg mL^{-1}, because of the erythrocyte membrane disruption. However, Glu(1/4) and Glu(2/3) showed a much better blood compatibility. Even if the concentration was up to 10 mg mL^{-1}, glucose-sensitive nanogels showed nonhemolytic with the extent of hemolysis lower than the permissible level of 5%.[29]

RBCs are abundance in blood cells and have a high volume fraction in whole blood (normally about 40–50%), so the safety of interaction between RBCs and biopolymer is need to be evaluated.

Table I. Clotting Kinetics Values of the Whole Blood Mixed with PBS or Glu(2/3)

Polymer solutions	*R* (min)	*K* (min)	α (deg)	MA (mm)
Normal range	5–10	1–3	53–72	50–70
PBS control	9.5	2.6	57	54.1
0.01 mg mL^{-1} Glu (2/3)	6.6	2.3	58.1	50
0.1 mg mL^{-1} Glu (2/3)	6.5	2.2	59.3	53.2
1 mg mL^{-1} Glu (2/3)	5.6	10.3↑	37.4↓	35.3↓

The sign ↓ indicates a lower value and ↑ indicates a higher value compared with the normal range provided by the TEG analyzer.

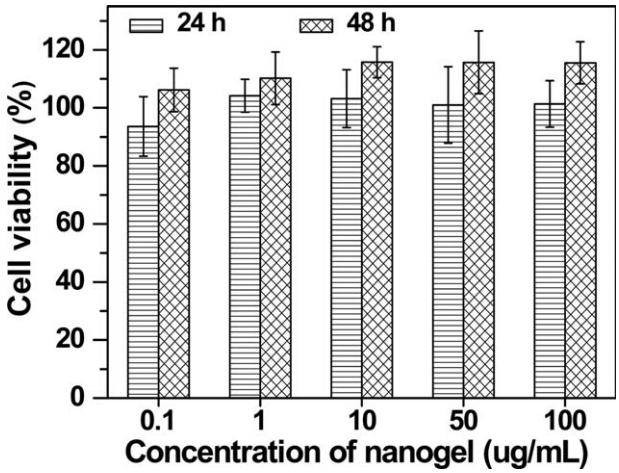

Figure 9. The cytotoxicity of Glu(2/3) with different concentrations on 3T3 cells.

In this study, the aggregation and morphological changes of the RBCs were examined in the presence of various nanogels at different concentrations by SEM observation. As the Figure 6 shown, compared with the PBS control, both Glu(2/3) and Glu(1/4) did not cause RBC aggregation and morphological change obviously from 0.01 to 10 mg mL^{-1}. However, PMAA nanogels have more obvious effect on the RBCs morphology. When PMAA concentration was up to 10 mg mL^{-1}, the aggregated RBCs fully changed their shape to gather together and eventually formed big lumps like concrete. This result indicated that glucose-sensitive nanogels have less interaction with RBCs, showing good blood compatibility. According to some previous studies,[30,31] the interaction of foreign materials with RBCs is being driven mainly by the electrostatic interactions between the positive polycations and the negative RBCs surface, and/or by the hydrophobic interaction between the hydrophobic groups of amphiphilic polymers and the lipid bilayer of RBCs membrane. Hence, the negative surface and hydrophilic property of glucose-sensitive nanogels resulted in the less effect on RBC aggregation and morphology.

APTT and PT are used extensively in clinical for detecting the abnormality of the blood plasma.[32] The blood coagulation cascade contains three types of pathways: intrinsic, extrinsic, and common pathway. APPT is used to evaluate the intrinsic and common coagulation pathways, and refers to the time required to form a fibrin clot after adding a partial thromboplastin reagent and calcium chloride ($CaCl_2$) to plasma. PT is used to evaluate the performance of the extrinsic and common coagulation pathways, and refers to the time to form a fibrin clot after adding tissue thromboplastin to plasma.[33] Results are shown in Figure 7. As shown in Figure 7(A), 0.1 mg mL^{-1} PMAA nanogels made APTT increase obviously, which may be resulted from the interactions between PMAA and the coagulation factors in the partial thromboplastin reagent and/or the plasma. Conversely, the glucose-sensitive nanogels showed no distinct difference in APTT from PBS control, indicating no interactions with the coagulation factors and/or partial thromboplastin reagent. For Glu(2/3) samples with the concentrations of 0.1–1 mg mL^{-1}, there was also no significant difference in APTT, shown in Figure 7(B). Moreover, compared to PBS control, all nanogel samples did not significantly change PT. These results indicated that the nanogels have little effect on the extrinsic pathway of blood coagulation. For PMAA nanogels, it has more effect on the intrinsic than extrinsic coagulation route, while the obtained glucose-sensitive nanogels showed no effect on blood plasma.

As previously reported, APTT and PT are not enough to accurately reflect the biomaterial-induced anticoagulant activity.[34] In comparison, TEG could provide the overall, dynamic process of whole blood coagulation by detecting the blood clot strength. TEG mainly contains four principle parameters: (1) reaction time (R), the time from adding the initiator to the initial fibrin formation; (2) coagulation time (K), represents the dynamics of clot formation; (3) α angle, the rate of clot aggregating or fibrin crosslinking; (4) maximum amplitude (MA) of the tracing, represents the maximum blood clot strength.[35] Here, Table I shows the principle TEG data of the whole blood coagulation process in the presence of the Glu(2/3), with the representative TEG traces are given in Figure 8, indicating the clot formation in PBS and Glu(2/3) at a concentration of 0.01–1 mg mL^{-1}. From these results, representative TEG trace and main parameters were obtained when clotting was carrying out in PBS or nanogels systems. Compared with the PBS control, it was seen that Glu(2/3) at the concentration of 0.01 and 0.1 mg mL^{-1} showed no difference in TEG trace and the four parameters were in normal range. Although ATPP and PT assay indicated that there was no effect of 1 mg mL^{-1} Glu(2/3) on coagulation course, the TEG result gave a different viewpoint. When Glu(2/3) concentration rised up to 1 mg mL^{-1}, three abnormal TEG values were recorded except *R* value (Table I). The coagulation course was affected obviously, expressing that the coagulation time increased and clot strength decreased. The anticoagulant effects may be attributed to the removal of thrombin from the blood by the nanogels via electrostatic interactions.[36]

Toxicity

The cytotoxicity of nanogels on 3T3 cells was studied by a CCK-8 assay. As is shown in Figure 9, the cell viability results of the 3T3 cells cultured in the medium treated with different Glu(2/3) concentrations for 24 or 48 h. As seen, Glu(2/3) had no toxicity compared with PBS control even if the concentration reached 100 μg mL^{-1}. Moreover, for 48-h cultivation, 3T3 cells showed a slight proliferation compared to that at 24 h. As is reported,[37,38] the toxicity of biomaterials is influenced by the size, chemical structures, biodistribution, exposure duration, as well as the nature of the surface and terminal groups. The nonobserved cytotoxicity of nanogels could be attributed to its smaller size and the characteristic of molecular structure, such as the negative charges on the surface.

CONCLUSIONS

A glucose-sensitive nanogel with uniform and spheroidal morphology was prepared through one-step thermally initiated precipitation polymerization. The obtained nanogels showed the glucose-dependent insulin release *in vitro* and prolonged effect

of lowing blood glucose level *in vivo*. The blood compatibility of nanogels has been studied particularly and confirmed that the obtained glucose-sensitive nanogels had better blood compatibility than PMAA nanogels. Although TEG results showed that 1 mg mL^{-1} Glu(2/3) could interact with thrombin in the blood to affect the coagulation time, the safe concentration of 0.1 mg mL^{-1} could be enough to meet the clinical demand. Moreover, the nontoxicity of the obtained nanogels suggested a potential application in diabetes therapy.

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