Novel PEG-graft-PLA nanoparticles with the potential for encapsulation and controlled release of hydrophobic and hydrophilic medications in aqueous medium

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Abstract: This study concerns the encapsulation and controlled release of both hydrophobic and hydrophilic medications with one polymer, which are delivered together as a combined therapy to treat diseased tissue. To test our hypothesis that the novel PEG-graft-PLA (PEG, polyethylene glycol; PLA, polylactic acid) can deliver both the hydrophobic and hydrophilic medications on account of its amphiphility, charge, and graft structure, PEG-graft-PLA (molecular weight of PEG = 1900) with very low critical micelle concentration was synthesized. One hydrophilic (insulin) and one hydrophobic (naproxen) model medication were loaded in separately during its self-assembly in aqueous solution. The resulting nanoparticles (NPs) were narrowly distributed and spherical, with average particle size around 200 nm, zeta potential $\mu -10 \text{ mV}$, and encapsulation efficiency $\geq 50\%$. The NPs realized controlled release of insulin and naproxen for over 24 and 160 hours, respectively. Specifically, the bioactivity of the insulin released from the NPs was maintained. Owing to encapsulation, both for hydrophobic and hydrophilic medicines, and NPs obtained with similar size and zeta potential, as well as maintenance of bioactivity of loaded protein, we expect the applications of PEG-graft-PLA NPs in combination therapy.

Keywords: NPs, insulin, naproxen, controlled release, combination therapy

Introduction

Many studies have reported that, in many diseases, combination therapy is more effective than monotherapy.1–5 Although combination therapy is costlier than monotherapy in the short term, it offers significant savings in terms of lower treatment-failure rate, lower case-fatality ratios, slower development of resistance, and, consequently, is less expensive for the development of new drugs. In combination therapy, various medications, irrespective of their hydrophobic or hydrophilic nature, have been used.1–5 In particular, proteins, one of the most effective and fastest growing group of medicines for the treatment of nearly 150 diseases, including various severe chronic conditions such as cancer, diabetes, hepatitis, leukemia, and rheumatoid arthritis, have shown prominent effects.5 However, to date, very few studies have reported the delivery of both hydrophobic and hydrophilic medications with one biomaterial, even though many single-drug delivery studies have been conducted. Moreover, several problems still exist in protein-delivery vehicles, especially due to the denaturation of entrapped proteins.7–10 We aimed at developing a system with the capability of encapsulation and controlled release of both hydrophobic and hydrophilic medications, especially proteins,
and delivering them to the same diseased tissue to realize combination therapy. In this study, we focused on carrying the hydrophobic and hydrophilic drugs separately.

Polylactic acid (PLA), a known US Food and Drug Administration-approved biodegradable polymer for biomedical applications, has been studied as a drug carrier in particle form since 1970. However, various drawbacks have limited its further application. First, the emulsion method has to be used in particle preparation due to the hydrophobicity of PLA, where a relatively large amount of organic solvent (eg, chloroform, dichloromethane) and surfactant are added. Even though the organic solvent is removed afterwards, the residual solvent and surfactant may have harmful or cytotoxic effects. Second, the particle size prepared by emulsion is generally in the micrometer range, which limits the encapsulation efficiency and application of PLA particles compared with nanoparticles (NPs). Last, PLA particles are easily captured by the reticulo-endothelial system in vivo and eliminated from the body, which in turn limits the use ratio of loaded medicine.

To overcome these shortcomings, amphiphilic polymers of polyethylene glycol (PEG)—block-PLA, such as diblock polymer of PLA-PEG and triblock polymer of PEG-PLA-PEG, have been synthesized and applied in the encapsulation of medications of small molecular weight (Mw), including paclitaxel, papaverine, sulindac, and tetracaine. PEG-block-PLA is a surfactant polymer that can self-assemble into nanomicelles and entrap medicines simultaneously, which simplifies particle preparation and avoids or minimizes the use of the surfactant and organic solvent. Moreover, nanoscale vehicles improve the loading rate of medicine compared with microscale formulations. The introduction of PEG block also suppresses cellular and protein adsorption, thus increasing the circulation time of PEG-block-PLA micelles in vivo and the effectiveness of the medicine. However, although PEG-block-PLA improves PLA in many ways, only small hydrophobic medications have been encapsulated in them. Furthermore, to obtain stable nanomicelles, PEG with a molecular weight (Mw) >4000 Da is usually used in the PEG-block-PLA, which might not be degraded in the body. In our previous study, a PEG-graft-PLA with PEG of lower Mw (600 Da) self-assembled into NPs in water and showed good protein resistance and cellular compatibility in vitro.

In the current study, we hypothesized that PEG-graft-PLA could realize the delivery of both hydrophobic and hydrophilic medications via self-assembly in aqueous medium and form NPs with increased circulation time compared with particles of PLA alone, on account of its amiphiphility, charge, and graft structure. To test our hypothesis, PEG-graft-PLA with PEG of Mw 1900 Da was synthesized, followed by the critical micelle concentration (CMC) test. One protein and one small hydrophobic model medication, insulin and naproxen, respectively, were chosen to be loaded. Insulin, a peptide hormone composed of 51 amino acids with Mw of 5808 Da and isoelectric point of 5.3, was chosen to explore the possibility of PEG-graft-PLA to encapsulate protein medications and keep their bioactivity during entrapment and release. Naproxen, a nonsteroidal anti-inflammatory drug with a carboxyl group and Mw of 230 Da, was chosen to study the capability of PEG-graft-PLA to encapsulate small hydrophobic medications and compare the effect with PEG-block-PLA. The NPs encapsulated with or without (nude NPs) insulin or naproxen were prepared and characterized. The stability of the NPs under dilution or pH change as well as the controlled release of medications was studied in vitro.

**Methods**

**Synthesis of PEG-graft-PLA**

The PEG-graft-PLA was synthesized as described previously. Briefly, 20 and 5 mL of tetrahydrofuran (THF) were used to dissolve 6.12 g of O,O′-Bis-(2-aminopropyl) polypropylene glycol-block-polyethylene oxide-block-polypropylene glycol (H₃NPEO₄H₄, Mw 1900 Da, Sigma, St Louis, MO) and 10.00 g of 10% maleic anhydride graft PLA (MPLA), prepared in our lab, respectively. To MPLA, H₃NPEO₄H₄ was added dropwise at 50–55°C, followed by 1 more hour of stirring. The resulting product was purified by precipitation in 160 mL distilled water and dissolution in 20 mL THF three times before being vacuum-dried at room temperature to a stable weight. A white and nontransparent solid (that is, PEG-graft-PLA), soluble in acetone, chloroform, dimethylformamide (DMF), and dimethyl sulfoxide (DMSO) was yielded. A higher Mw PEG (Mw = 1900) was used in this study to realize the encapsulation of both the hydrophobic and hydrophilic materials.

**CMC tests**

To evaluate the surfacticity and the possibility of encapsulation, CMC of PEG-graft-PLA was determined using the established fluorescence method, with pyrene (Sigma, St Louis, MO) as extrinsic probe. Serial solutions with fixed pyrene concentration of 6.0 × 10⁻⁷ M and various concentrations of PEG-graft-PLA between 1.08 × 10⁻⁶ and 2.16 × 10⁻² mg/mL were prepared. The excitation intensity of pyrene at 329 and 327 nm was obtained (PerkinElmer LS-50B, Waltham, MA) according to the scanning excitation spectrum.
between 321 and 340 nm by fixing the emission spectrum at 394 nm and the excitation and emission bandwidth at 2.5 nm. The CMC was determined by taking the midpoint in the plot of the intensity ratio of 329/327 to the logarithm concentration of PEG-graft-PLA.

Preparation of nude and insulin or naproxen-loaded NPs

Procine insulin (Wanbang Biopharma, Jiangsu, China) was loaded into PEG-graft-PLA according to the following procedure: 10 mg PEG-graft-PLA dissolved in 1 mL organic solvent was dropped into 2 mg insulin solution, obtained by dissolution in 10 mL of 0.01 N HCl and pH adjusted to 5.3 with 2 N NaOH under constant stirring. Naproxen (Hengshuo Chemical Co Ltd, Hubei, China) was entrapped by co-dissolving 0.8 mg NaOH under constant stirring. Naproxen (Hengshuo Chemical Co Ltd, Hubei, China) was entrapped by co-dissolving 0.8 mg naproxen and 10 mg PEG-graft-PLA in 1 mL organic solvent and dropping into 10 mL distilled water under stirring (0.8 mg naproxen was supersaturated in 10 mL distilled water). A different organic solvent that can easily mix with water such as acetone, THF, DMF, or DMSO could be used in this preparation. After evaporation (for acetone or THF) or dialysis (for DMF or DMSO), to remove organic solvent, NPs encapsulated with insulin or naproxen were obtained. The main difference in using these solvents was the stability of the obtained NPs. The NPs prepared with THF were not as stable as those prepared with acetone, DMF, and DMSO. We applied the water solution of pH 5.3 (for insulin) and distilled water (for naproxen) during the entrapment to get lower solubility of the medicine in the solution and possible higher loading rate in the NPs. The nude PEG-graft-PLA NPs were prepared by dissolving 10 mg PEG-graft-PLA in 1 mL organic solvent and dropping into 10 mL phosphate buffered saline (PBS) under stirring before removal of the organic solvent.

NP characterization

The morphology of the nude NPs and the insulin- or naproxen-loaded NPs was observed by a Philips Tecnai 10 transmission electron microscope (Philips, Eindhoven, The Netherlands) under 80 kV after staining with 2% sodium phosphotungstate for 3 minutes. Average size and zeta potential of the NP solution were tested at room temperature by a dynamic light scattering (DLS) instrument (Zetasizer Nano S90; Malvern Instruments, Worcestershire, UK) using argon ion laser.

NP stability tests

Since NPs are diluted or encounter an acidic or basic environment besides the neutral conditions in the body, we studied the stability of various NPs under dilution (in aqueous solutions with PBS) or pH change (acidic and basic conditions). The stability of the NPs was also delineated as the average particle size and zeta potential change before and after dilution (10 or 100 times) or pH change (2.0 or 10.5, adjusted with 0.1 N HCl or NaOH, respectively).

Protein adsorption on the surface of NPs

The protein adsorption on the surface of the NPs was used to evaluate its possible circulation time in vivo. The fluorospectrophotometer was used with fluorescein 5-isothiocyanate conjugated bovine serum albumin (FITC-BSA) as model protein based on the fact that albumin is the dominant protein in human plasma when adsorbed on PLA. A 5-mL original NP solution was mixed with 5 mL FITC-BSA water solution (0.04 mg/mL) and incubated at 37°C for 40 minutes with stirring before NPs were collected by repetitive high-speed centrifuge (Ultra Centrifuge, Beckman Coulter, Inc, Brea, CA) at 18,000 rpm for 10 minutes and washed with Millipore water. The FITC-BSA-incubated NPs were vacuum-dried for 24 hours and dissolved with 3 mL DMF. The fluorescent emission of the DMF solution was tested with [θ] spectrofluorometer (PerkinElmer LS-50B, Maltham, MA) at 510 nm with 490 nm excitation. PLA particles prepared by dropping PLA solution in THF to 1% gelatin solution in water were used as control. The particle size was recorded before adsorption measurement. The ratio of FITC-BSA adsorbed on the particles of PEG-graft-PLA and PLA was calculated according to their particle size (D) and fluorescent emission (E) values as below:

$$\% \text{Ratio of FITC-BSA adsorption on the particles of PEG-graft-PLA and PLA} = \frac{D_{\text{PEG-graft-PLA}} \times E_{\text{PEG-graft-PLA}}}{D_{\text{PLA}} \times E_{\text{PLA}}} \times 100\%$$

The encapsulation efficiency and in vitro release of insulin and naproxen

The encapsulation efficiency (EE) of insulin in the NPs was tested by centrifuging the loaded NPs at 16,000 rpm, 4°C for 20 minutes, collecting the supernatant, which contained the unencapsulated insulin, and measuring its concentration at 595 nm (PerkinElmer LS-50B) after reaction with Coomassie brilliant blue for 10 minutes. The EE was calculated according to the equation:

$$\text{EE} = \frac{\text{(the original insulin added in the NP preparation – the insulin in supernatant)}}{\text{the original insulin added in the NP preparation}} \times 100\%$$
The cumulative release of insulin was tested at 37°C and 150 rpm in 30 mL of NP solution, prepared with 5 mL original NP solution (without removing the unencapsulated insulin) plus 25 mL 0.01 M PBS. At various timepoints, 1 mL solution was taken out, centrifuged at 16,000 rpm to collect supernatant, and tested for insulin concentration (Coomassie brilliant blue coloration method) and bioactivity (porcine insulin enzyme-linked immunosorbent assay [ELISA] kit, R & D Systems, Inc, Minneapolis, MN). The cumulative release of insulin was expressed as the percentage change of insulin amount compared with total entrapped insulin (tested with Coomassie brilliant blue coloration and ELISA method) in bath solution over time. In parallel, the bioactivity of 0.08 mg/mL insulin water solution (same concentration as the unencapsulated insulin in the NP solution) was tested with ELISA as a control. The EE of naproxen was tested as the unencapsulated insulin in the NP solution) was tested of 0.08 mg/mL insulin water solution (same concentration method) in bath solution over time. Each test was replicated three times. The CMC of PEG-graft-PLA in water was 2.0 × 10⁻³ mg/mL, one of the most commonly used surfactants in particle preparation. The result showed its good surfactivity.

**Morphology and nanostructure of NPs**

The nude NPs as well as NPs loaded with insulin or naproxen were found to be spherical in structure, as shown in Figure 2. The representative average particle size was 114.7 nm (for nude NPs), 173 nm (for insulin), and 278.4 nm (for naproxen). The size distribution of NPs was very low (PDI [polydispersity index] was <0.3). The representative average zeta potential was −27.84 mV for nude NPs, −45.21 mV for insulin, and −14.42 mV for naproxen (Figure 3, original NPs).

**Stability of NPs under dilution and pH change**

The NPs loaded with or without insulin or naproxen were relatively stable after 10× and 100× dilution as well as pH change to 10.5 (Figure 3), while the NPs remained stable during the acidification, until the pH jumped to 2.0 when they aggregated immediately (Figure 3). The results suggested the buffering effect of PEG-graft-PLA in aqueous solution.

**BSA-resistant property of NPs**

There was 95.1% less FITC-BSA adsorbed on nude NPs of PEG-graft-PLA than on nude PLA particles. The results indicated the BSA-resistant property of PEG-graft-PLA NPs and possible longer circulation in the bloodstream.²¹

**Encapsulation and in vitro release of insulin and naproxen**

The encapsulation efficiency of insulin and naproxen in NPs of PEG-graft-PLA was >60% and 50%, respectively. About 80% of the loaded insulin and naproxen were released within 24 hours for insulin (tested with Coomassie brilliant blue coloration method) and 160 hours for naproxen (Figure 4). The burst release of insulin and naproxen within 2 hours was about 27% and 32%, respectively, which may relate to the surface adsorption of medicine on NPs (Figure 4). We found a slight drop at 4 hours compared with 2 hours in insulin release curve which may be due to the re-adsorption of burst-released insulin onto the NPs while there was less release, or no release at all, of inner insulin from the NPs.

We found that the bioactivity of insulin in PBS solution decreased with time (half-life was about 8 hours), while the bioactivity in NP solution appeared to increase a little and then drop (half-life was about 24 hours) (Figure 5A).

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The_cumulative_release_of_insulin_was_tested_at_37°C_and_150_rpm_in_30_mL_of_NP_solution,_prepared_with_5_mL_original_NP_solution_(without_removing_the_unencapsulated_insulin)_plus_25_mL_0.01_M_PBS. At various timepoints, 1_mL_solution_was_taken_out, centrifuged_at_16,000_rpm_to_collect_supernatant, and tested_for_insulin_concentration_(Coomassie_brilliant_blue_coloration_method) and_bioactivity_(porcine_insulin_enzyme-linked_immunosorbent_assay_ELISA_kit, R & D_Systems, Inc, Minneapolis, MN). The_cumulative_release_of_insulin_was_expressed_as_the_percentage_change_of_insulin_amount_compared_with_total_encapsulated_insulin_(tested_with_Coomassie_brilliant_blue_coloration_and_ELISA_method) in_bath_solution_over_time. In_parallel, the_bioactivity_of_0.08_mg/mL_insulin_water_solution_(same_concentration_method) in_bath_solution_over_time. Each_test_was_replicated_three_times. The_CMC_of_PEG-graft-PLA_in_water_was_2.0 × 10⁻³ mg/mL, one_of_the_most_commonly_used_surfactants_in_particle_preparation. The_result_showed_its_good_surfactivity.

**Morphology_and_nanostructure_of_NPs**

The_nude_NPs_as_well_as_NPs_loaded_with_insulin_or_naproxen_were_found_to_be_spherical_in_structure, as_shown_in_Figure_2. The représentative_average_particle_size_was_114.7 nm (for_nude_NPs), 173 nm (for_insulin), and 278.4 nm (for_naproxen). The_size_distribution_of_NPs_was_very_low (PDI [polydispersity_index] was <0.3). The représentative_average_zeta_potential_was −27.84 mV for_nude_NPs, −45.21 mV for_insulin, and_−14.42 mV for_naproxen (Figure_3, original_NPs).

**Stability_of_NPs_under_dilution_and_pH_change**

The_NPs_loaded_with_or_without_insulin_or_naproxen_were relatively_stable_after_10× and_100×_dilution_as_well_as_pH_change_to_10.5 (Figure_3), while the_NPs_remained_stable_during_the_acidification, until_the_pH_jumped_to_2.0 when they_aggregated_immediately (Figure_3). The_results_suggested_the_buffering_effect_of_PEG-graft-PLA_in_aqueous_solution.

**BSA-resistant_property_of_NPs**

There_was_95.1% less FITC-BSA_adsorbed_on_nude_NPs_of_PEG-graft-PLA_than_on_nude_PLA_particles. The_results_indicated_the_BSA-resistant_property_of_PEG-graft-PLA_NPs and_possible_longer_circulation_in_the_bloodstream.²¹

**Encapsulation_and_in_vitro_release_of_insulin_and_naproxen**

The_encapsulation_efficiency_of_insulin_and_naproxen_in_NPs_of_PEG-graft-PLA_was >60% and 50%, respectively. About_80% of_the_loaded_insulin_and_naproxen_wereReleased_within_24 hours_for_insulin (tested_with_Coomassie_brilliant_blue_coloration_method) and_160 hours_for_naproxen (Figure_4). The_burst_release_of_insulin_and_naproxen_within_2 hours_was about 27% and 32%, respectively, which_may_relate_to_the_surface_adsorption_of_medicine_on_NPs (Figure_4). We_found_a_slight_drop_at_4 hours_compared_with_2 hours in insulin_release_curve which may_be_due_to_the_re-adsorption of_burst-released_insulin_onto_the_NPs while there was less release, or no release at all, of inner_insulin_from_the_NPs.²¹

We_found_that_the_bioactivity_of_insulin_in_PBS_solution decreased_with_time (half-life_was_about 8 hours), while the_bioactivity_in_NP_solution_appeared_to_increase_a_little and then_drop (half-life_was_about 24 hours) (Figure_5A).
The increase within 2 hours was from the burst release, which showed that the burst-released insulin was bioactive. The bioactivity of insulin released from the NPs, calculated by the bioactivity in NPs solution minus the bioactivity in PBS, increased with time (Figure 5B), from which we inferred that the bioactive insulin was released from the NPs for over 24 hours (Figure 5B).

**Discussion**

Although a number of studies have reported the delivery of single medicines, very few studies have shown the development of self-assembled NPs with one polymer in aqueous solution which could carry both the hydrophobic and hydrophilic medications, particularly the large proteins with sustained bioactivity. PEG-graft-PLA is a kind of amphiphilic polymer with graft PEG structure that is flexible in solution. It contains free amino and carboxyl groups that ionize and interact in water. These features enable it to self-assemble and entrap chemicals via hydrophilic/hydrophobic and electrostatic interaction. Our results demonstrated the good surfactivity of PEG-graft-PLA and its capability to load insulin and naproxen during self-assembly in aqueous medium to receive NPs and realize controlled release. In fact, we also found that the PEG-graft-PLA can encapsulate during the self-assembly and controlled release of many other hydrophobic and hydrophilic chemicals, including sodium fluorescein and fluorescein isothiocyanate in aqueous solution (data not shown). The potential encapsulation of PEG-graft-PLA for the hydrophobic and hydrophilic materials implied that PEG-graft-PLA formed a hydrophobic and hydrophilic inner-layer with a hydrophilic out-layer during self-assembly in aqueous solution, as shown in Figure 6. Thus, we expected these NPs loaded with different drugs would arrive at the same diseased tissue and realize the combination therapy according to their similar chemical character, particle size, and zeta potential. Of course, it needs further study.

We obtained the spherical, narrowly distributed, and negatively charged NPs loaded with insulin or naproxen by way of self-assembly, with no surfactant and much less of the organic solvent compared with when PLA particles
were used. So we would expect the PEG-graft-PLA NPs to be more biocompatible than the PLA particles. The NPs have particle size around 200 nm, zeta potential $>-10$ mV, and encapsulation efficiency $>50\%$. The first order kinetics with $>80\%$ release of insulin and naproxen was realized within 24 hours and 160 hours, respectively, in vitro (Figures 4A and 4B). In addition, the NPs of PEG-graft-PLA showed BSA-resistant properties compared with PLA particles. In fact, we have previously demonstrated the BSA resistance of PEG-graft-PLA film compared with PLA film. The good protein-resistant property of PEG-graft-PLA NPs may ensure its long circulation in the bloodstream. The stability study disclosed that the NPs could be stable after administration in the body and diluted by blood or encountered with neutral (ie, pH 7.0) and basic tissue fluid, eg, intestinal fluid, but may aggregate in acidic fluid, especially in the gastric fluid. Therefore, it is reasonable to expect that the NPs of PEG-graft-PLA could be acidic-environment targeting and used for targeted and controlled drug delivery for stomach diseases and many tumors that have lower pH than normal tissue. We also found the original NP solution loaded with insulin or naproxen was stable at room temperature for at least 1 week.

Figure 3 Particle size (top figures), its polydispersity (the value above the column on the top figures) and zeta potential (bottom figures) of nude NPs (A), NPs encapsulated with insulin (B) or naproxen (C), with or without dilution and pH changes, tested by dynamic light scattering.
Abbreviation: NP, nanoparticle.

Figure 4 Typical in vitro insulin (A) (tested with Coomassie brilliant blue coloration method) and naproxen (B) release profile from nanoparticles of PEG-graft-PLA.
Abbreviations: PEG, polyethylene glycol; PLA, polylactic acid.
Many studies have used chitosan with or without other polymers, such as Eudragit L 100-55,\textsuperscript{25} arabic gum,\textsuperscript{26} dextran sulfate,\textsuperscript{27} and alginate\textsuperscript{28} to prepare NPs with encapsulated insulin owing to its positive charge, and potential mucoadhesive and membrane permeable properties. They achieved particle size of 200–750 nm, zeta potential of $-5$ to $-15$ mV, encapsulation efficiency of 40%–85%, entrapment of 3.38%,\textsuperscript{25} and controlled release of <30 minutes\textsuperscript{25} and 6 hours.\textsuperscript{27} A crosslinked dextran,\textsuperscript{29} insulin-sodium oleate complex-loaded poly(lactide-co-glycolide),\textsuperscript{30} and polyelectrolyte\textsuperscript{31} NPs got an encapsulation efficiency of 45%–91.2%, particle size of 100–250 nm, and controlled release with 75%–95% entrapped insulin within 48 hours\textsuperscript{29} and 6 hours.\textsuperscript{31} We have achieved particle size and encapsulation efficiency comparable to the NPs obtained in these studies, but prominent higher zeta potential value even after dilution or pH change to basic conditions (Figure 3). The high zeta potential hints at the stability of NPs under storage.\textsuperscript{32} Moreover, the high negative charge of insulin-loaded PEG-graft-PLA NPs favors their long stay in the bloodstream because the surface of blood cells and endothelia are negatively charged.\textsuperscript{33} Sarmento et al also tested the bioactivity and concentration of insulin released from polysaccharide NPs with ELISA and the Bradford method and found that the relative bioactivity, a comparison of bioactivity with the concentration, remained for up to 24 hours.\textsuperscript{34} We found the relative concentration of both the pure insulin in PBS and insulin released from NPs decreased with time in 24 hours, even though the latter decreased slower than the former (data not shown). In addition, the storage of raw insulin influenced the loading and release results due to its aggregation. The results we report here were from fresh insulin. Our study demonstrated that insulin released from NPs retained its bioactivity, which makes PEG-graft-PLA NPs more attractive for other therapeutic protein delivery, although more in vivo studies are needed.

Poly(D,L-lactide-co-glycolide),\textsuperscript{35} poly-caprolactone,\textsuperscript{36} liposomes,\textsuperscript{37} and aspartate-based dendrimers\textsuperscript{38} have been

**Figure 5** The bioactivity of insulin versus time in PBS and NPs/PBS solution (A), as well as released from the NPs calculated by the bioactivity in NPs/PBS solution minus the bioactivity in PBS (B).

**Abbreviations:** NP, nanoparticle; PBS, phosphate buffered saline.

**Figure 6** The formation of Peg-graft-PLA NPs when loaded with insulin (A) and naproxen (B).

**Abbreviations:** PEG, polyethylene glycol; PLA, polyactic acid.
used for naproxen delivery, with particle size ranging from 100 to 723 nm, a loading ratio of up to 98%, and a sustained 1-month in vivo release, and an increased solubility of up to two-fold at pH 6 and 7. PEG-block-PLA for small hydrophobic-medicine delivery gave particle sizes as small as 45 nm, loading rate up to 25%, and controlled release of up to 450 hours, and 5000-fold increased solubility. We obtained effects comparable with the published naproxen delivery systems and the PEG-block-PLA for small hydrophobic medications.

In conclusion, we have developed PEG-graft-PLA NPs with the potential for encapsulation and controlled release of both hydrophobic and hydrophilic medications. Specifically, they retain the bioactivity of the entrapped protein.

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Disclosure

The authors report no conflicts of interest in this work.

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