Notes

Stimuli-Responsive Hydrogel Based on Poly(propylene phosphate)

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Introduction

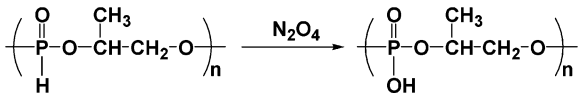
Materials capable of in situ transformation from liquid precursors to solids are attractive for drug delivery, gene delivery, and tissue engineering applications. More specifically, these include cell delivery, barriers for tissue regeneration guidance, injectable temporary scaffolds for tissue regeneration, and tissue adhesives for healing and injectable controlled release devices.[1,2] In situ gelation is usually achieved by photopolymerization or phase transition. The latter is generally accomplished by amphiphilic block or graft copolymers that can self-assemble in water to form organized structures or gels in the presence of stimuli such as a pH or temperature change. Many block copolymers of poly(ethylene glycol) and propylene glycol, for example, display a lower critical solution temperature (LCST) around room temperature, rendering them injectable biomaterials that can gel in situ at body temperature.[3−5] There are few systems where the thermosensitivity of the gelation is also dependent on ion concentration.

Polyphosphates have been synthesized since the 1970s by Penczek and colleagues as analogues of nucleic and teichoic acids.[6,7] The biodegradability and biocompatibility of this class of polymers render them attractive biomaterials for drug delivery, gene delivery, and tissue engineering.[8−15] We reported here poly(propylene phosphate), first synthesized by Penczek and colleagues in 1982,[16] as a thermosensitive injectable biomaterial whose gelation is induced by calcium ions. The sol−gel transition temperature of this system can be adjusted by the polymer and calcium ion concentrations. This system shows promising characteristics as a minimally invasive injectable material for controlled drug and gene delivery.

Experimental Section

Polymer Synthesis. Poly(propylene phosphate) was synthesized according to a modified method reported by Biela et al.[16] as shown in Scheme 1. Poly(propylene H-phosphonate) (10 g) in CH_2Cl_2 was concentrated to 20 mL and precipitated into 500 mL of anhydrous benzene, dried under vacuum, and redissolved in 200 mL of anhydrous DMF. This solution was cooled at −15 °C and passed through anhydrous N_2O_4 until a persistent green color appeared. The mixture was reacted at

Scheme 1



4 °C for 24 h and room temperature for 48 h in turn. The excess N_2O_4 was removed, and the solution was concentrated. The polymer was obtained by precipitation in acetone followed by drying under vacuum. It was further purified by dialysis against water in cellulous dialysis tubing with a molecular weight cutoff of 3500 (Pierce, Rockford, IL). The polymer structure and purity were confirmed by ^{1}H NMR, and the weight molecular weights (M_w) of the polymers were determined by gel permeation chromatography as to be 12 900 with a polydipersity of 2.6.

Sol−Gel Transition. Poly(propylene phosphate) was dissolved in 0−700 mM $CaCl_2$ solutions at concentrations ranging from 5 to 25 wt % and incubated in a temperature-controlled water bath for 5 min. The temperature of the bath was increased in steps of 2 °C. Conditions of gel formation (no flow) and solution (flow) were determined by inverting the vial vertically in the bath.

Rheological Measurements. Polymer solutions were injected into the test chamber of the RFS3 rheometer (Rheometric Scientific Inc.). The upper plate (25 mm in diameter, rigid and nonpermeable) was lowered to a 0.25 mm gap. The temperature of the chamber was maintained for 10 min at the gelation temperature for a particular gel composition and calcium concentration, followed immediately by a dynamic shear strain sweep at 25 °C. The dynamic shear strain sweep, using the parallel-plate configuration, was performed in a strain range from 1000% to 0.3% at a frequency of 1.57 rad/s. The dynamic modulus is reported as G' and G'', where G' is the elastic modulus, and G'' is the viscous modulus, and the phase angle δ ($=\tan^{-1}(G''/G')$) provides a measure of the relative magnitude of internal dissipation (i.e., the viscosity) over the energy storage (i.e., the elasticity).

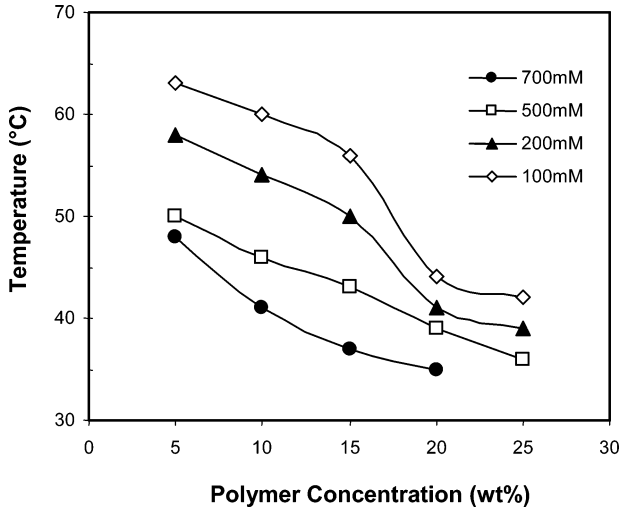


Figure 1. Phase transition temperatures as a function of poly(propylene phosphate) concentration at different Ca^{2+} concentrations.

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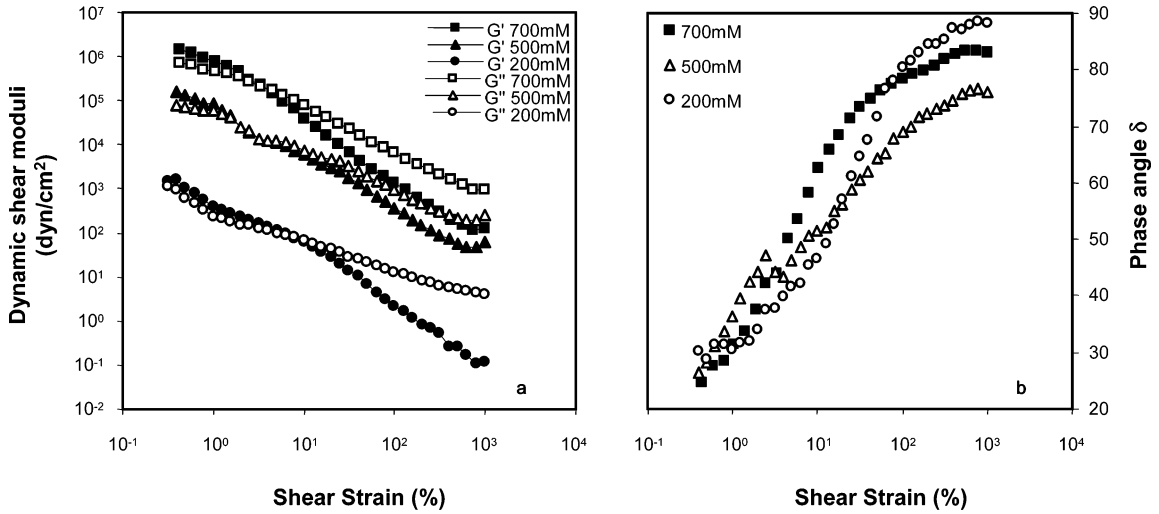

Figure 2. Rheological properties of poly(propylene phosphate) hydrogels cross-linked at different Ca^{2+} concentrations.

In Vitro Drug Release. 200 μL of poly(propylene phosphate) solution containing 30 mg of polymer and 1.5 mg of lysozyme (or 150 μg of plasmid DNA) were prepared in 700 mM $CaCl_2$ and injected into a 1.5 mL cuvette. This was incubated in a 37 °C water bath for 10 min to form a gel, followed by addition of 1 mL of PBS (0.01 M, pH 7.4). The area of the gel exposed to the PBS was 0.4 cm^2. At designated time points, 0.5 mL of sample was taken and the equivalent quantity of fresh PBS added. The amount of plasmid DNA released was determined by UV−vis spectrophotometric measurements at 260 nm. The amount of lysozyme released was determined using a BCA microassay kit (Promega, Madison, WI).

Results and Discussion

Solutions of poly(propylene phosphate) in distilled water did not exhibit phase transition temperature at any concentration. Addition of Ca^{2+} to the aqueous solution, however, significantly changed the phase transition properties. At room temperature, the aqueous polymer solutions (up to 20 wt %) remained a liquid in the presence of up to 700 mM $CaCl_2$, only becoming partially insoluble at 1 M $CaCl_2$. The solutions maintained their fluidity even at higher polymer concentrations if the $CaCl_2$ concentration was reduced. For example, a 25 wt % polymer solution can be obtained at room temperature in 500 mM $CaCl_2$. This solution exhibited a rapid phase transition to a nonflowing gel, within 5 min, when the temperature was raised to 36 °C. Hydrogel formation was observed at polymer concentrations 15 wt % or above, in the presence of Ca^{2+} and above the LCST. At concentrations below 10 wt %, only precipitates would be observed even at high temperatures. The phase transition temperature is a function of polymer concentration as well as Ca^{2+} concentration. Increasing the polymer or the Ca^{2+} concentration led to lower phase transition temperatures. As shown in Figure 1, the sol−gel transition temperature can easily be manipulated by varying these two parameters.

In contrast, the gelation kinetics was found to be independent of polymer or Ca^{2+} concentrations. Gelation characteristics of a polymer solution (15 wt % in 700 mM Ca^{2+}) were determined by rheological measurements at 37 °C using a time sweep in the dynamic shear configuration (w = 6.28 rad/s, shear strain = 20%). When the temperature was raised to 37 °C, both G' and G'' increased with time. The gel time point (defined as $G' = G''$, a phase angle of 45°) was determined to be 220 s.

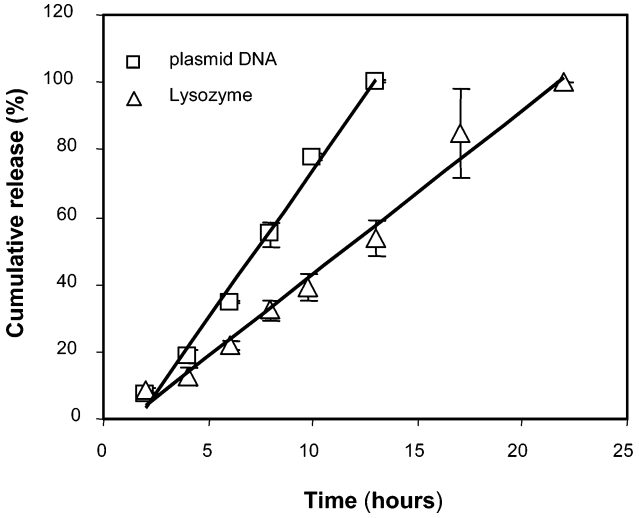

Figure 3. In vitro release profile of plasmid DNA and lysozyme from poly(propylene phosphate) hydrogel.

The dynamic shear modulus of the gels formed with different Ca^{2+} concentrations exhibited a nonlinear dependence on the amplitude of the shear strain (Figure 2a). Both G' and G'' increased proportionally with Ca^{2+} concentration, reflecting a higher degree of cross-linking by Ca^{2+}. However, at higher shear strains, both G' and G'' decreased monotonically. The phase angle δ increased with increasing shear strain for Ca^{2+} concentrations ranging from 200 to 700 mM (Figure 2b). The elasticity was higher than the viscosity below an approximate 10% shear strain (δ < 45°). Above this, the material exhibited a dominance of viscosity over elasticity (δ > 45°). This behavior is unexpected of other covalently cross-linking hydrogels. The results suggest that the Ca^{2+} cross-linking in the network is continuously broken at high shear deformation.

One of the most obvious applications of these thermosensitive hydrogels would be for drug delivery. In vitro release studies were conducted with plasmid DNA or lysozyme as model drugs. The initial loading was 0.5% and 5%, respectively. The polymer and plasmid (or lysozyme) were dissolved in a 700 mM Ca^{2+} solution and gelled at 37 °C. The cumulative release from the gel is shown as a function of time in Figure 3. Interestingly, the release followed zero-order kinetics for both model drugs after an onset of 1 h, with no burst release.

Plasmid DNA was released at a near constant rate of 3.3 μg h^{-1} cm^2, achieving 100% release over 12 h. Release of lysozyme was slower, reaching complete release in 22 h. The slower release of the lysozyme is hypothesized to be a consequence of the charge interaction between the polymer and the lysozyme. In addition, the release was accompanied by reversal of the Ca^{2+} cross-linking as evidenced by the dissociation of the gel, presumably due to the diffusion of Ca^{2+} into the PBS buffer. The poly(propylene phosphate), with a $pK_a \approx 2.3$ (determined by titration), showed no toxicity to COS-7 and MRC-5 cells up to a concentration of 5.4 mg/mL, indicating its relatively noncytotoxic characteristics.

In summary, poly(propylene phosphate) solutions display a sol−gel transition temperature that can be controlled by adjusting the concentrations of polymer and Ca^{2+} so that the therapeutic delivery cargo is liquid at room temperature but solidifies at the in vivo temperature of 37 °C. The gels show rapid gelation kinetics but instability at high shear strains. Ion exchange between buffer and gel induces a dissociation of the network with time, leading to a zero-order controlled release of plasmid DNA or lysozyme. These hydrogels, produced by a mechanism of gelation induced by both calcium cross-linking and temperature change in physiological solvent, is an interesting novel injectable gel system for minimally invasive therapeutic delivery applications.

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Supporting Information Available: ^1H and ^{31}P NMR of poly(propylene phosphate), cytotoxicity assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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