Biomaterials

Light-Sensitive Polypeptide Hydrogel and Nanorod Composites**

*Manoj B. Charati, Ian Lee, Kolin C. Hribar, and Jason A. Burdick**

Stimuli-responsive hydrogels exhibit structural changes based on changes in local temperature or pH and analytes and are highly valued in fields ranging from controlled drug release to tissue repair and also in microdevices. Hydrogels that respond to external stimuli, such as light or magnetic fields, offer additional advantages with respect to on-demand and triggered response. With this in mind, we formulated a composite of thermoreversible polypeptide-based hydrogels, formed from a genetically engineered multiblock polypeptide that exhibits a temperature-dependent transition from a solid to liquid state, and gold nanorods. Near-infrared-light (NIR) exposure of the nanorods induces local heating and, consequently, melting of the gels. These networks were explored for the controlled release of a macromolecule and the release profiles were controlled by the extent and timing of light exposure, including stepwise release with intermittent light. The infrared-light-controlled dissociation of these hydrogels offers unique opportunities, such as for the delivery of drugs and growth factors with transdermal light exposure or for incorporation of hydrogel actuators in microdevices.

Smart biopolymeric hydrogels are a new generation of biomaterials that may exhibit reversible physicochemical changes in response to their environment.[1] These stimuli-responsive gels are finding applications in many fields, such as for the delivery of therapeutic molecules, biomedical devices, such as actuators and biosensors/diagnostics, and as scaffolds for tissue engineering and regenerative medicine.[1,2] A unique feature of such materials is that they undergo significant conformational changes upon variation in one or more physicochemical stimuli such as pH, temperature, analytes, or light. Although many variables have been explored, hydrogels that reversibly respond to temperature changes have been the subject of major investigation over the past two decades.

These thermoresponsive systems are typically centered around synthetic polymers such as poly(*N*-isopropylacrylamide) (PNIPAm) and its derivatives or biomimetic elastin-based polypeptides and undergo a reversible volume/phase transition upon change in temperature of the surrounding environment. PNIPAm materials exhibit a lower critical solution temperature (LCST), where polymer phase separation occurs and networks tend to shrink or collapse as the temperature is increased past the LCST. This feature has led to significant investigation of PNIPAm and its copolymers for drug-delivery applications, where triggered release may be advantageous,[1] thermosensitive surface coatings to manipulate cells,[3] actuators to control cell development and morphology,[4] and as valves[5] or as a chromatography matrix[6] in microfluidic devices. Genetically engineered polypeptides, particularly those based on elastin, show similar thermoresponsive behavior to PNIPAm gels and have been used previously as drug-delivery vehicles,[7] tissue-engineering scaffolds,[8] and biosensors.[9] These systems offer advantages for the production of well defined macromolecules that assemble into unique structures and allow for precise control over the conditions at which the transition in material properties occur via elegant design of the amino acid sequence.

Building on this work, genetically engineered triblock polymers comprising random-coil midblocks flanked by two associative leucine-zipper end blocks have been designed that self-assemble to form physically cross-linked hydrogels (where the leucine-zipper domains serve as junction points) and also exhibit reversible gelation in response to pH and temperature.[10] These coiled-coil polypeptide hydrogels are solution–gel (sol–gel) materials that self-assemble to form a network that can be switched on and off by controlling pH and temperature, which opens up opportunities for a variety of applications where stimuli-responsive materials are useful. Specifically, Tirrell and coworkers have designed a library of triblock polypeptides, consisting of leucine-zipper end blocks, forming hydrogels with varying mechanical properties and erosion rates.[11–14] One such polypeptide hydrogel, PC10P, was designed to suppress the dissociation rate of the physically crosslinked hydrogel via selective molecular recognition in order to increase gel stability. The triblock polypeptide, PC10P, consists of relatively short ''leucine zipper'' end blocks derived from the amino acid sequence of rat cartilage oligomeric matrix protein (COMP) domain and a water-soluble polyelectrolyte domain in the center.

In this study, we synthesized, purified, and characterized the PC10P polypeptide using conventional protein-biosynthesis techniques.[13] The thermoreversible characteristics of PC10P hydrogels were monitored under dynamic oscillatory shear mode at 25 °C using a cone-and-plate configuration (Figure 1). Specifically, a 7 wt% PC10P solution was observed to gel rapidly between the plates with a plateau storage modulus (G')

[*] M. B. Charati, I. Lee, K. C. Hribar, Prof. J. A. Burdick
Department of Bioengineering
University of Pennsylvania
210 S 33rd Street, Philadelphia, PA 19104 (USA)
E-mail: burdick2@seas.upenn.edu

[**] This work was supported by a Fellowship in Science and Engineering from the David and Lucile Packard Foundation and MRSEC DMR05-20020 from the National Science Foundation. The authors acknowledge the generous gift of the PC10P plasmid from the Tirrell Laboratory.

DOI: 10.1002/smll.201000162

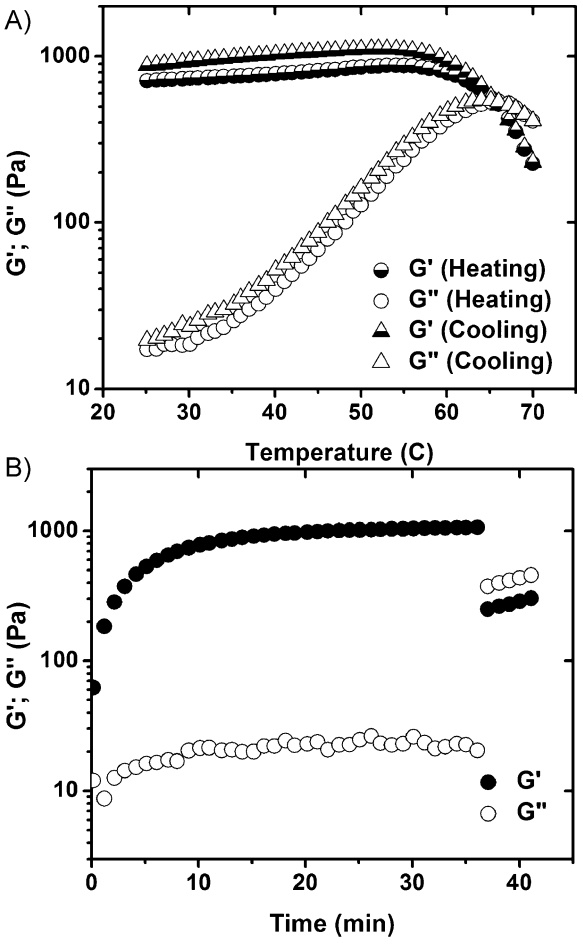

Figure 1. A) Dynamic oscillatory temperature sweep of PC10P gels (7 wt%, 10 mM phosphate buffer, pH 7.0) at a heating rate of 2 °C min^{-1}. B) Dynamic oscillatory time sweep of PC10P gels at 25 °C and an instantaneous temperature change to 65 °C at 35 min.

of 800 Pa in less than 15 min (see Figure 1B). The equilibrium shear modulus of the PC10P hydrogel is comparable to other physically crosslinked coiled-coil hydrogels of similar molecular weight.[11,14] A gradual increase in the temperature resulted in thermal unfolding of the leucine-zipper aggregates and thus melting of the PC10P gel, as indicated by a decrease in the storage modulus (G') and an increase in the loss modulus (G'') (see Figure 1A); complete transition from an elastic solid to a viscous liquid was observed at ≈60 °C. Similar gel–sol behavior has been observed for coiled-coil polypeptides via circular dichroism spectroscopy in dilute solutions.[10]

The dissociated PC10P solution was observed to recover into a stable and elastic gel state upon cooling the solution to room temperature, indicating thermoreversible behavior (see Figure 1A). The minimal hysteresis observed after a complete heating/cooling cycle could imply time dependence correlated with the refolding of the coiled-coil network junctions and/or evaporation of solvent under the experimental setup. To investigate the instantaneous melting of the PC10P gel, the temperature was rapidly increased to 65 °C at a rate of 10 °C s^{-1}. As shown in Figure 1B, there is a rapid transition from a solid gel to a viscous liquid with heating, as indicated by crossing of the G' and G'' values. The instantaneous gel–sol transition of PC10P and similar genetically engineered coiled-coil hydrogels therefore offers unique opportunities for applications in biomedical devices as on-demand drug-delivery vehicles or biosensors. The temperature and the rate at which the gel–sol transition occurs can be easily tuned by rational choice of amino acid sequences of the associative leucine-zipper domains.

In combination with stimuli-responsive materials, the application of external triggers of the response, such as magnetic/electric fields, ultrasound waves, and/or light, may be desirable for many applications. For example, magnetic nanoparticles have been employed with thermosensitive gels for modulating drug delivery, where molecule release is instigated either via application of oscillatory magnetic motion to mechanically force pore opening to enhance diffusion or magnetic heating of the nanoparticles to induce the thermo-response of the material.[15] An alternate approach is to induce transitions with light-sensitive particles, such as gold nanorods or nanospheres, where absorbed photons in the NIR spectrum are efficiently converted into heat. NIR light is particularly advantageous since it penetrates tissue very well and has been used extensively for biomedical applications, such as photo-dynamic therapy, thermal ablation of tumors, and imaging.[16,17] As an example of light-triggered composites, gold nanoshells have been applied for photothermally modulated drug delivery from PNIPAm gels.[18] However, one limitation to this approach is that the thermal response of PNIPAm gels in the above-mentioned system is slow and on the order of hours. Recently, our lab demonstrated that heating and subsequent shape transitions can be triggered in a composite of gold nanorods (Au-NRs) and a biodegradable polymer upon exposure to infrared light and passing through the material glass-transition temperature.[19] However, this used a highly crosslinked polymer system that would not be applicable for delivery of large molecules.

To date, there are no examples where light exposure has been used to trigger dissociation in polypeptide materials, despite the speed at which dissociation occurs and the tunability of properties that is possible with an engineered polypeptide. In this work, we used Au-NRs to induce instantaneous thermal transitions in genetically engineered PC10P hydrogels. The Au-NRs were synthesized via a seed-mediated growth process with a well defined and uniform size distribution.[19–21] To assess the effect of Au-NR incorporation on the gelation of PC10P, a 7 wt% PC10P and 10^{-10} M PEGylated Au-NR solution was mixed and allowed to gel on a rheometer (PEG = polyethylene glycol). Although the PC10P/Au-NR solution was observed to gel more quickly than PC10P without Au-NR (Figure 2), the incorporation of Au-NRs, in the experimental concentration regime, did not have any effect on the equilibrium shear modulus of the gel.

The ability of a PC10P/Au-NR gel to undergo thermal melting was evaluated via exposure to NIR light (diode pumped solid state (DPSS) laser, 808 nm, 1W) and was visually captured via high-definition (HD) video. Images of the gels (with and without Au-NRs) before, during, and after light exposure are shown in Figure 3. The PC10P gels with and without Au-NRs were placed on a glass slide with the light source at a distance of ≈10 cm. Significant heating of the gels was observed in PC10P/Au-NR within 2 min, leading to the complete dissociation of the physically crosslinked network and

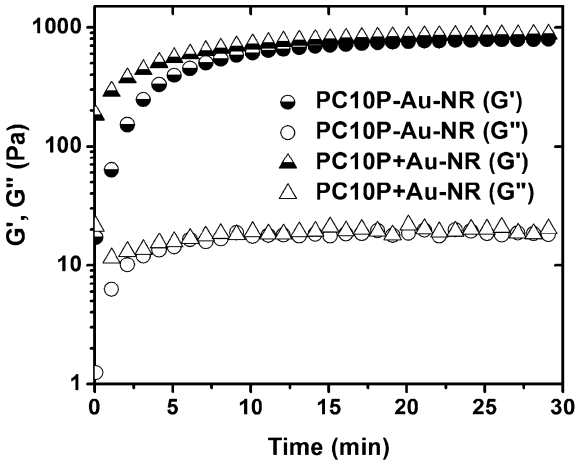

Figure 2. Oscillatory time-sweep measurements of PC10P gels with and without Au-NRs.

subsequent melting of the gel. Additionally, the heating was noticed to be highly localized in the area where NIR light was exposed. On the contrary, no heating or melting was observed in PC10P gels without Au-NRs and the gel shape appeared as it did prior to light exposure. As shown previously in an alternate polymer system,[19] the light-induced temperature increases can be controlled effectively by the concentration of Au-NR in the gel and the intensity and time of exposure to NIR light. In this gel system, the Au-NRs are expected to have the same effect, adding additional tunability to the speed of heating and gel dissociation. Although the actual temperature within the gel was not measured, it clearly exceeded the dissociation temperature, since gel melting was observed.

This use of NIR light to trigger thermal transitions and melting in coiled-coil hydrogels offers unique opportunities to photothermally modulate the release of encapsulated molecules from gels. To illustrate this, the release of 10 kDa fluorescein isothiocyanate (FITC)–dextran from composite hydrogels was observed and measured via fluorescence spectroscopy. The release of FITC–dextran from PC10P gels without exposure to NIR light observes a typical diffusion-mediated release profile with ≈50% release of the encapsulated dextran at 36 h (Figure 4A). However, 100% release of the

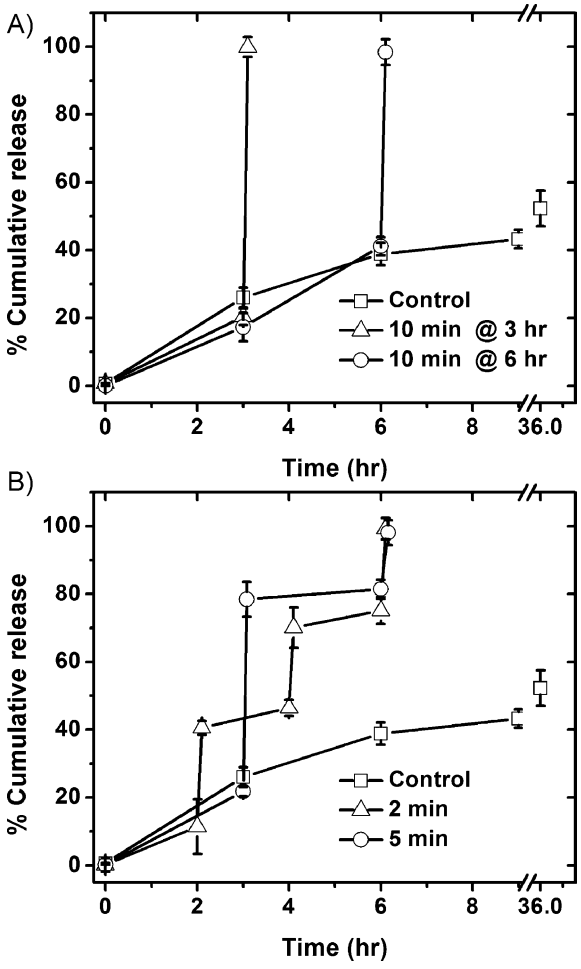

Figure 4. A) Release of FITC–dextran after 10-min exposure of NIR light. B) Release of FITC–dextran after 2- and 5-min exposure of NIR light.

encapsulated dextran was achieved via gel exposure to NIR light for approximately 10 min at any given point. In addition to diffusion-mediated release, exposure to NIR light results in melting of the gel, as described earlier, and thus gel dissociation is likely the primary driving force for the release. Beyond release of all of the dextran at once, the composite gels can also

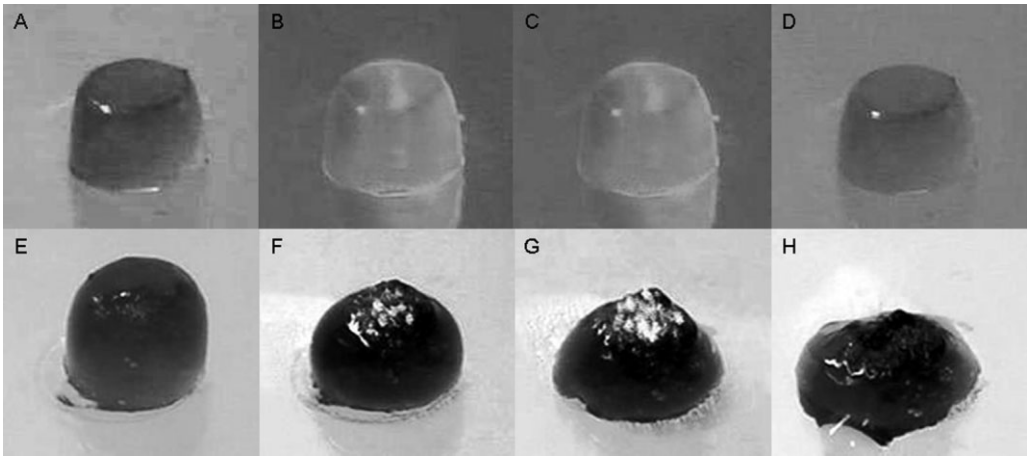

Figure 3. Snapshots of PC10P gels A–D) without and E–H) with Au-NR demonstrating melting of the gel only with Au-NRs: before NIR exposure (A, E), during NIR exposure (B, C, F, G), and after NIR exposure for 10 min (D, H).

be utilized to precisely modulate the drug-release profile by tuning the duration of NIR-light exposure (e.g., removing light before complete dissociation occurs). Specifically, the stepwise release of FITC–dextran from PC10P/Au-NR gels via periodic exposure of NIR light is possible (Figure 4B). Instantaneous release was observed once the laser was ON and the amount of release was dependent on the time of exposure. When the laser was turned OFF, the passive release profile observed in the control samples was merely resumed due to the reversibility of the sol–gel transition. This process could be repeated until all of the encapsulated molecules were released.

In conclusion, the composite polypeptide hydrogel with Au-NRs described here exhibits unique features suitable for on-demand changes in gels, potentially applicable as drug-release devices. The gels undergo instantaneous thermal transitions, gel–sol, by application of external NIR light, providing a technique to regulate drug release, a technique that could be performed with transdermal exposure. The introduction of polypeptide gels allows for tunability in material design and properties and rapid network transitions that may increase the utility of such composites for a range of applications.

Experimental Section

Polypeptide synthesis and gelation: The plasmid-bearing PC10P gene (pQE9-PC10P) in *E. coli* strain SG13009 was a gift from Prof. David Tirrell at the California Institute of Technology, Pasadena, CA. The protocol for PC10P expression and purification was followed as described previously.[10,12] 7 wt% PC10P gels were prepared by dissolving the lyophilized polypeptide in phosphate buffer (10 mM, pH 7.0). To prepare gels with Au-NRs, PEGylated Au-NRs were suspended in the buffer at the desired concentration and mixed with the polypeptide.

Nanorod fabrication, characterization, and encapsulation: Nanorods were fabricated via a seed-mediated growth process.[20] Briefly, a solution of cetyltrimethylammonium bromide (CTAB), $HAuCl_4 \cdot 3H_2O$, silver nitrate, L-ascorbic acid, and a seed solution containing CTAB, sodium borohydride, and $HAuCl_4 \cdot H_2O$ were mixed for 2 h and then PEGylated by reaction with PEG–thiol ($M_W = 5$ kDa).[21] The Au-NRs were imaged on a transmission electron microscopy (TEM) system (JEOL 2010F) at an accelerating voltage of 200 kV by placing a drop of the nanorod/dichloromethane solution on a TEM carbon-coated grid (SPI Supplies) to assess their dimensions. The absorption spectrum of the Au-NRs was also confirmed (TECAN infinite 200 UV/Vis spectrophotometer).

Mechanical characterization: Dynamic oscillatory time and temperature sweeps were performed using an AR2000ex rheometer (TA Instruments) with a 20-mm anodized-aluminum cone-and-plate geometry and a 27-μm gap. 7 wt% PC10P solution was prepared in 10 mM phosphate buffer, pH 7.0, and loaded between the plates on the rheometer; care was taken to avoid bubble formation during loading. Dynamic oscillatory time sweeps were recorded at 25 °C, angular frequencies of 6 rad s^{-1}, and 0.5% strain and the temperature sweeps were recorded with a heating rate of either 2 or 10 °C min^{-1}. A solvent trap was used to prevent sample evaporation.

Release studies: FITC-conjugated dextran (10 kDa, Molecular Probes, Invitrogen) was used as a model drug. 100 μL PC10P gels were prepared by mixing together the polypeptide, Au-NRs at a nanorod concentration of 1.3×10^{-10} M and 1 wt% 10 kDa FITC–dextran. The solution was vortexed to dissolve the components, spun down in a microcentrifuge tube, and allowed to gel for 30 min. 1 mL of ddH_2O (doubly distilled H_2O) was added on top of the gel and the supernatant was sampled to monitor the release of FITC–dextran using a fluorescence spectrophotometer ($\lambda_{ex} = 485$ nm, $\lambda_{em} = 530$ nm). A DPSS laser (808 nm, 1W) was used to trigger release of encapsulated dextran from a distance of 10 cm. The gel was exposed to the NIR laser from the top either for 10 min to trigger complete release of encapsulated dextran or for 2–5 min for partial release. The data for each study was recorded in triplicate and reported as an average.

Keywords:
biomaterials · nanorods · polypeptides · thermosensitive

[1] J. F. Mano, *Adv. Eng. Mater.* **2008**, *10*, 515–527.
[2] S. K. Ahn, R. M. Kasi, S. C. Kim, N. Sharma, Y. X. Zhou, *Soft Mater.* **2008**, *4*, 1151–1157.
[3] R. M. P. Da Silva, J. F. Mano, R. L. Reis, *Trends Biotechnol.* **2007**, *25*, 577–583.
[4] A. Pelah, R. Seemann, T. M. Jovin, *J. Am. Chem. Soc.* **2007**, *129*, 468–469.
[5] Q. Z. Luo, S. Mutlu, Y. B. Gianchandani, F. Svec, J. M. J. Frechet, *Electrophoresis* **2003**, *24*, 3694–3702.
[6] N. Malmstadt, P. Yager, A. S. Hoffman, P. S. Stayton, *Anal. Chem.* **2003**, *75*, 2943–2949.
[7] M. F. Shamji, L. Whitlatch, A. H. Friedman, W. J. Richardson, A. Chilkoti, L. A. Setton, *Spine* **2008**, *33*, 748–754.
[8] D. L. Nettles, K. Kitaoka, N. A. Hanson, C. M. Flahiff, B. A. Mata, E. W. Hsu, A. Chilkoti, L. A. Setton, *Tissue Eng.* **2008**, *14*, 1133–1140.
[9] J. Y. Kim, S. O'Malley, A. Mulchandani, W. Chen, *Anal. Chem.* **2005**, *77*, 2318–2322.
[10] W. A. Petka, J. L. Harden, K. P. McGrath, D. Wirtz, D. A. Tirrell, *Science* **1998**, *281*, 389–392.
[11] W. Shen, J. A. Kornfield, D. A. Tirrell, *Macromolecules* **2007**, *40*, 689–692.
[12] W. Shen, R. G. H. Lammertink, J. K. Sakata, J. A. Kornfield, D. A. Tirrell, *Macromolecules* **2005**, *38*, 3909–3916.
[13] W. Shen, K. C. Zhang, J. A. Kornfield, D. A. Tirrell, *Nat. Mater.* **2006**, *5*, 153–158.
[14] W. Shen, J. A. Kornfield, D. A. Tirrell, *Soft Mater.* **2007**, *3*, 99–107.
[15] T. Hoare, J. Santamaria, G. F. Goya, S. Irusta, D. Lin, S. Lau, R. Padera, R. Langer, D. S. Kohane, *Nano Lett.* **2009**, *9*, 3651–3657.
[16] A. M. R. Fisher, A. L. Murphree, C. J. Gomer, *Lasers. Surg. Med.* **1995**, *17*, 2–31.
[17] R. Weissleder, C. H. Tung, U. Mahmood, A. Bogdanov, *Nat. Biotechnol.* **1999**, *17*, 375–378.
[18] S. R. Sershen, S. L. Westcott, N. J. Halas, J. L. West, *J. Biomed. Mater. Res.* **2000**, *51*, 293–298.
[19] K. C. Hribar, R. B. Metter, J. L. Ifkovits, T. Troxler, J. A. Burdick, *Small* **2009**, *5*, 1830–1834.
[20] T. K. Sau, C. J. Murphy, *Langmuir* **2004**, *20*, 6414–6420.
[21] H. W. Liao, J. H. Hafner, *Chem. Mater.* **2005**, *17*, 4636–4641.

Received: February 1, 2010
Revised: May 17, 2010
Published online: July 5, 2010

