Self-Assembly

DOI: 10.1002/anie.201001382

Regulation of a Real-Time Self-Healing Process in Organogel Tissues by Molecular Adhesives**

Pritam Mukhopadhyay, Norifumi Fujita,* Akihiko Takada, Takanori Kishida, Michihiro Shirakawa, and Seiji Shinkai**

Materials that change their physical properties on mechanical agitation are known as thixotropic materials. In the human body, for example, the functions of protoplasm, red blood cells, synovial fluid, and muscular activities are regulated by thixotropy.[1–3] Similarly, the properties of materials such as paints, bentonite clay, cosmetics, and foodstuffs arise from thixotropy. Despite the significant potential of this dynamic phenomenon, there is a lack of materials that can act as a model system to investigate vital natural processes such as muscle thixotropy and nerve fiber regeneration. In this respect, low-molecular-weight organogels[4] (LMOGs), which generally consists of 1D fibrous molecular assemblies, are envisioned to be attractive candidates when provided with thixotropic properties. However, not only are examples of LMOGs that exhibit thixotropy rare,[5–9] but there is also little information related to their aggregation and stability. These aspects circumvent the use of LMOGs for the study of this unique phenomenon.

Of the hundreds of functional LMOGs reported to date, only very few exhibit the unique property of thixotropy. In general, LMOGs are extremely sensitive to mechanical stress and these materials irreversibly expel solvent molecules from their network when subjected to flow. On removal of the external force, these materials behave as a solid suspension that loses its original elastic properties. On the other hand, thixotropic gels can disintegrate in solution under an external mechanical stress and can regain their elastic properties upon removal of the stress. This operation can normally be carried out for an infinite number of cycles.

Thixotropic LMOGs represent an intriguing and unique class of truly dynamic self-assembled supramolecular systems. Discernible visual insights into the process would be required in order to understand how such self-assembled entities evolve under mechanical stress followed by a resting time. To obtain such "snapshots", the thixotropic gel has to operate under a strict real concentration domain. However, the LMOGs reported to date show thixotopic properties only at concentrations of 10 wt% or more. At these high concentrations, the molecules are closely associated and therefore the reaggregation to the gel state from the disaggregated solution state occurs within seconds, thus rendering these systems unviable for real-time imaging. Hence, from the perspective of a supramolecular chemist, a successful thixotropic LMOG candidate has to fulfill three stringent requirements: firstly, the LMOG has to be an extremely efficient gelator (close to a super-gelator); secondly, it must exhibit the thixotropic property at such low concentrations; and thirdly, the gel has to maintain its original state without undergoing aging or forming crystalline domains during the cycles of breaking and regeneration processes. It is therefore difficult to obtain a supramolecular system that comprises all of these favorable functional features.

Herein we report the discovery of a naphthalenediimide (**1**)-based organogel[10] (Figure 1) that offers real-time imaging of thixotropic processes. TEM and AFM images confirm that the self-assembled 1D fibers tens of micrometers in length disintegrate under mechanical stress and undergo a self-healing process to reconstitute the 1D fibers during a resting time. However, the regenerated fibers are shorter, with a large number of active ends. Chemical stimulants that act as "molecular adhesives" significantly enhance the rate of recovery and promote further growth of these fibers. These observations show that self-assembly processes in artificial systems can potentially mimic some biological events such as self-healing of thixotropic muscular fibers and stimulant-induced regeneration of injured neural fibers.[11–13]

The naphthalenediimide-based organogel forms stable and transparent gels even at concentrations of 0.03 wt%. Organogel **1** transforms into the solution state under an external mechanical stress and, upon removal of the force, the gel state is reconstituted without forming precipitates or

---

[*] Dr. P. Mukhopadhyay,[+] Dr. N. Fujita,[++] Dr. T. Kishida,
Dr. M. Shirakawa, Prof. S. Shinkai[+++]
Department of Chemistry and Biochemsitry
Graduate School of Engineering, Kyushu University
744 Moto-oka, Nishi-ku, Fukuoka 812-8581 (Japan)

Dr. A. Takada
Division of Advanced Device Materials, Institute for Materials
Chemistry and Engineering, Kyushu University
Fukuoka 816-8581, (Japan)

[+] Current address: School of Physical Sciences
Jawaharlal Nehru University, New Delhi 110067, (India)
Fax: (+91) 11-2671-7537
E-mail: m_pritam@mail.jnu.ac.in

[++] Current address: Department of Chemistry and Biotechnology
School of Engineering, The University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656 (Japan)
Fax: (+81) 3-5841-7310
E-mail: m_pritam@mail.jnu.ac.in

[+++] Current address: C-205 4-22-1 Ikeda, Kumamoto 860-0082 (Japan)
Fax: (+81) 96-326-3494
E-mail: m_pritam@mail.jnu.ac.in

[**] We are grateful for the support provided by a Grant-in-Aid for Scientific Research (Nos. 20045014, 20685010) and for Innovative Areas "Coordination Programming" (area 2107), the 21st Century COE Project (Functional Innovation of Molecular Informatics), and the JSPS for a fellowship to P.M.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201001382.

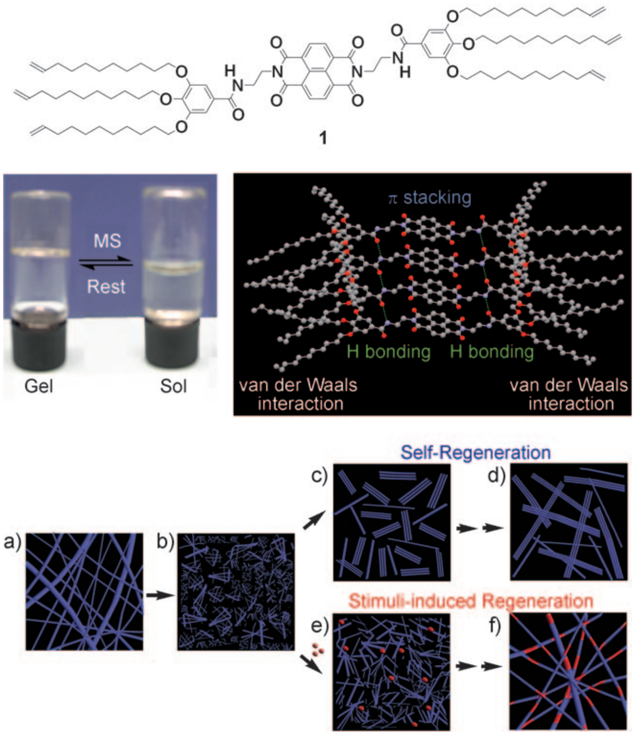

Figure 1. Top: Molecular structure of **1**. Center left: Thixotropic gel of **1** at 0.075 wt% that shows reversible gel–sol and sol–gel interconversion on mechanical shaking (MS) and rest, respectively. Center right: Proposed self-assembled suprastructure of **1**.[10] Bottom: Schematic representation of the thixotropic process and its regulation with donor molecules: a) Original gel with long fibers; b) short fibers after mixing by vortexing; c, d) self-regeneration of fibers and bundles; e, f) stimuli-induced regeneration of fibers in the presence of a molecular adhesive (red dots: 1,3-dihydroxynaphthalene).

crystalline domains for an infinite number of cycles. Intriguingly, compound **1** shows such a thixotropic phenomenon at concentrations as low as 0.075 wt%, hence providing the opportunity to monitor the dynamic formation process of the incipient fibers. Characterization of the organogel by UV/Vis and attenuated total reflectance IR (ATR-IR) spectroscopy confirmed that a combination of π–π stacking, hydrogen bonding, and van der Waals interactions is responsible for assembling this gel in a 1D fashion[10] (Figure 1). Rheological studies confirm that the organogel satisfies the conditions of a gel, that is, the storage modulus (G') is much greater than the loss modulus (G'') and G' is independent of the angular frequency ω. Data from creep and creep recovery measurements (details about the experiments are given in the Supporting Information) further support the characteristics of a true gel (Figure S1a and S1b in the Supporting Information). The thixotropy of **1** in a concentration range 150 times lower than the LMOGs reported to date allows us to study the phenomenon in real time. Because of the extraordinary ability of compound **1** to show thixotropy at such low concentrations, we found that the recovery time from the sol to the gel state can be easily tuned from a few seconds to hours and even days (Figure S5 in the Supporting Information). These properties provide us with the rare opportunity to track the consecutive states of the thixotropic process by TEM or AFM imaging.

In order to obtain "snapshots" of the complete process, that is, from disintegration of the original gel to the solution state and its reconstitution to the gel state, a concentration of 0.3 wt% of the organogel was first standardized. At this concentration, the recovery to the gel from the solution state takes approximately 24 hours. The organogel based on **1** was vortexed for 10 minutes with a vortex vibrator (operating at 60 Hz and 2600 rpm) and the solution or the recovering gel was immediately cast on a carbon-coated Cu grid. The casting of the recovered gel on the grid was done at regular intervals to get a large number of snapshots. The TEM observation unravels many features that were unreported to date and helps our understanding of this dynamic process. Figure 2 a, b shows that the original gel based on **1** consists of 1D fibers several tens of micrometers in length and 10-150 nm in diameter. Images obtained directly after vortexing (Figure 2 b, c) show the disintegration of the original long thin fibers and the thick bundles into numerous small fibers. Importantly, the original bundles undergo a drastic reduction of their length (maximum length of 4 μm) and their width (maximum width of 75 nm). With increasing resting time, the self-regeneration of the fibers takes place and after 24 hours the length of the bundles reaches a plateau value of 8–10 μm. It is apparent from Figure 2 h that the reconstituted gel consists of thicker bundles that are formed from loosely packed unimolecular fibers. Furthermore, the extraordinary ability of these fibers to self-regenerate even when after vortexing for 48 hours at a concentration as low as 0.07 wt% was confirmed by AFM and TEM images (Figure S3 and S4 in the Supporting Information). The AFM images clearly show small pieces of disintegrated fibers that self-regenerated in a 1D fashion with multiple joining points. This observation is the first direct visual evidence that self-assembled artificial tissues under mechanical agitation can disintegrate into small pieces of fibers, which can subsequently undergo self-regeneration in a self-healing process.

As the solution underwent reconstitution and reverted back to the gel state, it appeared that the recovered gel is slightly weaker than the original gel. We carried out rheological studies[14,15] in order to compare the recovered gel with the original gel. The thixotropic behavior of the gels was monitored by two independent experiments that utilized both the flow and the oscillation method. The reconstitution of the gel was studied by measuring the dynamic moduli as a function of the reconstitution time (oscillation method; Figure 3 a). At a very early stage, the storage modulus G' of the disintegrated gel has a low value that increases rapidly during the initial resting time, after which the rate of recovery is considerably slowed down and eventually reaches a plateau value of 6.6 Pa. A well-equilibrated sample of the original organogel based on **1** has a storage modulus G' of 9.7 Pa. This value indicates that the disintegrated gel underwent partial reconstitution (ca. 70%). Next, we compared the critical stress values (i.e., stress at which G' deviates from linearity; σ_c) and the fracture stress (σ_f) values (i.e., stress at $G' = G''$, which is one of the measures of sol–gel transition) of the original gel with those of the reconstituted gel by monitoring

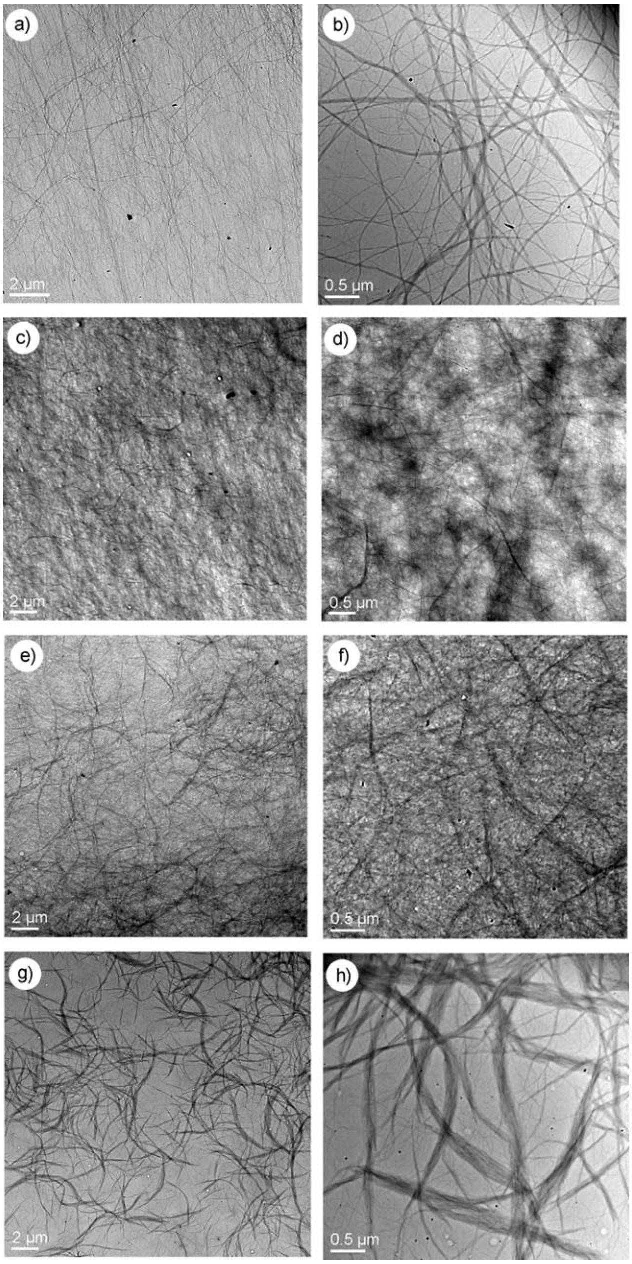

Figure 2. TEM images (at low and high magnifications) of a, b) original organogel **1** with extremely long (several tens of μm) fibers and large bundles with 10-100 nm in diameter; c, d) images taken just after the disintegration of the gel (within 60 s) show small fibers and clusters of many small fibers (black regions); e, f) images taken after 6 h show the self-regeneration of the fibers and bundles have grown up to 5 μm in length; g, h) images after 40 h show the regeneration of the fibers and bundles that have grown up to 10 μm in length. Conditions: 0.3 wt % **1** in cyclohexane.

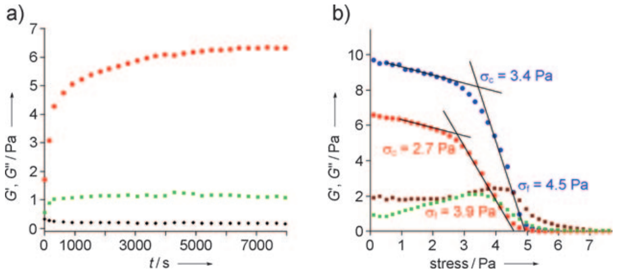

Figure 3. a) Plots of G' (red circle) and G'' (green square) of organogel **1** as a function of time, showing the reconstitution of the disintegrated gel (application of stress, $\sigma = 1$ Pa and angular frequency, $\omega = 0.1$ rad sec^{-1}), black diamond: tanδ; b) Plots of G' and G'' as a function of applied stress to compare σ_c and σ_f of the original organogel based on **1** (blue circle: G', brown square: G'') and of the reconstituted organogel (red circle: G', green square: G''). Conditions: 0.6 wt % **1** in cyclohexane.

G' and G'' as a function of the applied stress (Figure 3 b). The reconstituted gel regained 79 % of the σ_c value of the original gel. In a different set of experiments in which the stress was measured versus the shear rate (flow method), a 0.6 wt % gel maintained a σ_c value that was 74 % of the value of the original gel (Figure S5 in the Supporting Information). To confirm that reconstitution of the organogel is independent of the concentration, we carried out the same experiment at 0.3 wt %. At this concentration the organogel maintained a σ_c value that is 72 % of the value of the original gel. Therefore, the rheological studies are consistent with the TEM and AFM observations and support the hypothesis that the organogel based on **1** undergoes partial reconstitution after a disintegration process. Hence, it can be presumed that the regeneration of the fibers and bundles that results in smaller lengths of the objects (with "active ends") is predominated by kinetic factors.[16]

At this point, we investigated whether the active ends generated by the disintegration and reconstitution of the fibers would respond to chemical stimuli (apart from heat stimuli) that in turn would further promote the regeneration of fibers when exposed to the bulk solution. It is well known that nerve fiber regeneration in patients that suffer from spinal cord injury (SCI) remains one of the most difficult challenges for spinal cord research. Recent findings highlight that injured nerve fibers can indeed regenerate in the presence of specific stimuli such as cAMP and neurotrophins.[17,18] Replication of such stimuli-induced regeneration processes in artificial organogel fibers is an interesting challenge that can lead to potential medicinal applications. To achieve this target, we have employed donor molecules as chemical stimuli that would presumably interact with the acceptor naphthalenediimide scaffold of the organogel. The donor molecule functions as a molecular adhesive to bring the active ends together in order to complete the fiber regeneration. Nature has quite efficiently evolved such molecular adhesives, for example, cadherins[19] (one of the major classes of cell adhesion molecules) that play pivotal roles in tissue organization and function. For a successful implementation of our strategy, it is mandatory that such donor–acceptor interactions operate strictly under ambient conditions (i.e., without heating). After screening a large number of donor molecules, 1,3- and 1,6-dihydroxynaphthalenes were selected as they were found to interact with the gel fibers under ambient conditions.

The active ends of the fibers were generated by mixing the gel by vortexing for 10 minutes in order to disintegrate the gel, and subsequently the molecular adhesive 1,3-dihydroxynaphthalene was mixed by vortexing to have a homogeneous distribution of the donor molecules. Over time, a completely transparent violet gel appeared, which was characterized by UV/Vis spectroscopy. A charge-transfer band with $\lambda_{max} = 570$ nm signifies the donor–acceptor interaction between the electron-rich 1,3-dihydroxynaphthalene and the electron-deficient naphthalenediimide scaffold of the organogel based on **1** (Figure S6a and S6b in the Supporting Information).

The reconstitution of the organogel based on **1** was monitored by taking "snapshots" at regular time intervals in the presence of 1,3-dihydroxynaphthalene. TEM images clearly show that the disintegrated small fibers of the organogel based on **1** underwent stimuli-induced regeneration and grew in lengths of several tens of micrometers, which are comparable to the dimensions of the original gel (Figure 4a–d). Moreover, the bundles formed through this process are closely packed with individual fibers and have maximum diameters of 1 μm. Therefore, the molecular adhesive 1,3-dihydroxynaphthalene can bring the active ends of the disintegrated fibers closer together which eventually promotes regeneration of the fibers.

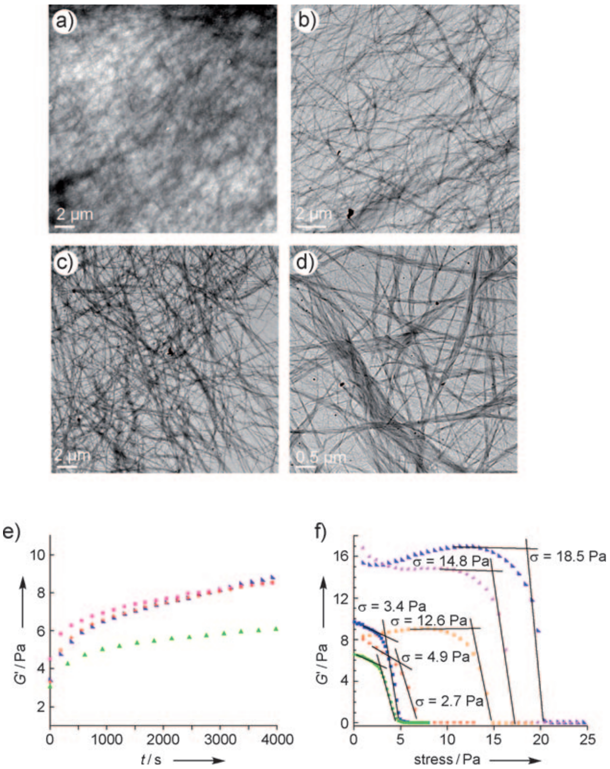

Figure 4. TEM images of organogel **1** in the presence of 2 equiv of the molecular adhesive 1,3-dihydroxynaphthalene a) just after mixing by vortexing (within 60 s); b) after 2 h; c, d) after 20 h. Conditions: 0.3 wt% in cyclohexane. e) Left: determination of G' versus time of the recovery process of the original gel and in the presence of varying amounts of 1,3-dihydroxynaphthalene (purple triangle: 0.3 equiv, magenta square: 0.75 equiv, red diamond: 2 equiv, green triangle: original gel); right: determination of G' versus stress, to elucidate σ_c with varying amounts of 1,3-dihydroxynaphthalene at 0.6 wt% in cyclohexane (purple triangle: 0.3 equiv, magenta triangle: 1 equiv, yellow triangle: 1.5 equiv, red diamond: 2 equiv, blue square: original gel, green circle: recovered gel).

To confirm that the disintegrated fibers with active ends can indeed undergo morphological modulations after binding to donor molecules, a control experiment with 1,6-dihydroxynaphthalene was carried out. This donor–acceptor-complexed gel had an opaque appearance, thus suggesting the presence of 2D assembled structures;[13,20] therefore gel formation from the disintegrated small fibers would be easier to follow and visualize. In fact, TEM observation clearly showed that 2D sheetlike structures develop gradually from disintegrated fibers (Figure S7 in the Supporting Information). Dynamic rheological experiments were carried out in order to determine how the rate of recovery of the gel is affected by the addition of 1,3-dihydroxynaphthalene (Figure 4e, left). Interestingly, a twofold enhancement in the rate of recovery of the disintegrated gel was observed in the presence of 1,3-dihydroxynaphthalene. Rheological experiments were performed in order to substantiate that the regeneration of fibers observed from TEM images is correlated with the mechanical strength of the gel. Interestingly, the recovered gel sustained higher critical stress (σ_c) values in the presence of fewer equivalents of 1,3-dihydroxynaphthalene. The σ_c value that the gel can sustain was found to be 18.5, 14.8, 12.6, and 5 Pa in the presence of 0.3, 1.0, 1.5, and 2.0 equivalents of 1,3-dihydroxynaphthalene, respectively (Figure 4e, right). As the concentration of the donor molecule increases, the σ_c value that the gel can sustain decreases. The ability of very small amounts of the donor molecule to enhance the gel stability could be due to the lengthening of the fibers, the tight packing during lateral aggregation, and toughness of the individual fibers.

In summary, we have found an extraordinarily efficient self-assembly process in an organogel system that undergoes stimuli-responsive disintegration and self-regeneration processes in real time. This process allows us for the first time to take snapshots of these dynamic processes, hence unraveling many interesting features, for example kinetically-induced partial self-regeneration and self-healing of the fibers. We have been able to regulate the regeneration process of the disintegrated fibers in a supramolecular fashion by utilizing simple donor–acceptor interactions. The donor molecules act as molecular adhesives that bring the disintegrated active ends of the fibers together closely, thus completing the stimuli-induced regeneration and self-healing of the artificial tissues. We believe, therefore, that the conclusions obtained in the present study are significant and helpful to understand the basic thixotropic phenomenon that is present in the complex natural system and the simplified synthetic system.

Experimental Section

TEM measurements: These measurements were performed at 120 kV using a JEOL 2010 TEM. The gel of **1** in cyclohexane was cast on a carbon-coated copper grid, and dried at room temperature. Measurements were performed without staining the sample.

Rheological measurements: Dynamic viscoelastic, shear creep, shear flow, and time evolution measurements were carried out with a CLS-100 stress-controlled rheometer (Carri-MED, ITS Japan), by using a parallel-plate geometry (the plate diameter was 40 mm and the gap width was 500 μm). All viscoelastic measurements were performed at (25 ± 0.1) °C. Dynamic viscoelastic measurements were performed with the range of the oscillatory angular frequency, $\omega = 10^{-1}$–100 rad s^{-1} and with the strain, $\gamma = 0.05$ to ensure the linear viscoelasticity behavior, except for the stress dependence measurements.

To determine G' and G'' versus the reconstitution time, the organogel based on **1** was initially disintegrated for 10 min by using a mechanized vibrator (vortex) operating at 60 Hz and 2600 rpm. The disintegrated gel was immediately put on the rheometer and the time evolution experiment was performed.

Received: March 8, 2010

Keywords: charge transfer · gels · molecular assemblies · self-assembly · self-healing

---

[1] U. Proske, D. L. Morgan J. E. Gregory, *Prog. Neurobiol.* **1993**, *41*, 705–721.

[2] R. W. Briehl, *Nature* **1980**, *288*, 622–624.

[3] K. E. Hagbarth, J. V. Hugglund, M. Nordin, E. U. Wallin, *J. Physiol.* **1985**, *368*, 323–342.

[4] P. Terech, R. G. Weiss, *Chem. Rev.* **1997**, *97*, 3133–3159.

[5] M. Shirakawa, N. Fujita, S. Shinkai, *J. Am. Chem. Soc.* **2005**, *127*, 4164–4165.

[6] M. Lescanne, P. Grondin, A. d'Aleo, F. Fages, J.-L. Pozzo, O. M. Monval, P. Reinheimer, A. Colin, *Langmuir* **2004**, *20*, 3032–3041.

[7] J. Brinksma, B. L. Feringa, R. M. Kellog, R. Vreeker, J. van Esch, *Langmuir* **2000**, *16*, 9249–9255.

[8] T. Shirosaki, S. Chowdhury, M. Takafuji, D. Alekperov, G. Popova, H. Hachisako, H. Ihara, *J. Mater. Res.* **2006**, *21*, 1274–1278.

[9] D. M. Blow, A. Rich, *J. Am. Chem. Soc.* **1960**, *82*, 3566–3571.

[10] P. Mukhopadhyay, Y. Iwashita, M. Shirakawa, S.-i. Kawano, N. Fujita, S. Shinkai, *Angew. Chem.* **2006**, *118*, 1622–1625; *Angew. Chem. Int. Ed.* **2006**, *45*, 1592–1595.

[11] L. Schnell, M. E. Schwab, *Nature* **1990**, *343*, 269–272.

[12] M. E. Schwab, D. Bartholdi, *Physiol. Rev.* **1996**, *76*, 319–370.

[13] H. Song, G. Ming, Z. He, M. Lehmann, L. McKerracher, M. Tessier-Lavigne, M. Poo, *Science* **1998**, *281*, 1515–1518.

[14] *Rheology: Principles, Measurements and Applications* (Ed.: C. W. Macosko), VCH, Weinheim, **1994**.

[15] *Rheology for Chemists: An Introduction* (Ed.: J. W. Goodwin), Royal Society of Chemistry, Cambridge, **2000**.

[16] The growth of the disintegrated fibers under ambient conditions is regulated by kinetic factors. This regulation was confirmed by heating the reconstituted gel, which shows the formation of fibers comparable in length to those of the original gel in the presence of heat as stimulus.

[17] P. Lu, Y. Hong, L. L. Jones, M. T. Filbin, M. H. Tuszynski, *J. Neurosci.* **2004**, *24*, 6402–6409.

[18] P. Lu, L. L. Jones, E. Y. Snyder, M. H. Tuszynski, *Exp. Neurol.* **2003**, *181*, 115–129.

[19] E. Perret, A. Leung, H. Feracci, E. Evans, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 16472–16477.

[20] For an example of an opaque gel that forms 2D assemblies, see M. Shirakawa, N. Fujita, S. Shinkai, *J. Am. Chem. Soc.* **2003**, *125*, 9902–9903.

[21] Studies to help understand the different binding mechanisms of 1,3-dihydroxynaphthalene and 1,6-dihydroxynaphthalene with organogel **1** are underway.

[22] In this work, we have explored self-healing as well as its real-time visualization and regulation in self-assembled fibrous nanomaterials. Self-healing materials have also been realized in polymer composites by utilizing covalent bonding as a tool, see S. R. White, N. R. Sottos, P. H. Geubelle, J. S. Moore, M. R. Kessler, S. R. Sriram, E. N. Brown, S. Viswanathan, *Nature* **2001**, *409*, 794–797.