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Post-Self-Assembly Cross-Linking of Molecular Nanofibers for Oscillatory Hydrogels

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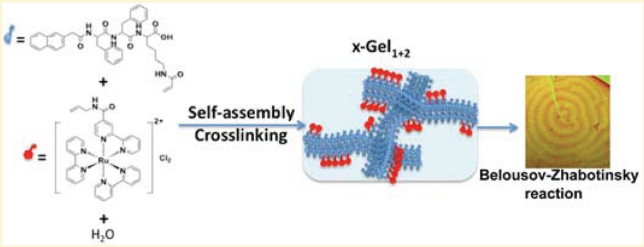
Supporting Information

ABSTRACT: After a polymerizable hydrogelator self-assembles in water to form molecular nanofibers, post-self-assembly cross-linking allows the catalyst of the Belousov−Zhabotinsky (BZ) reaction to be attached to the nanofibers, resulting in a hydrogel that exhibits concentration oscillations, spiral waves, and concentric waves. In addition to the first report of the observation of BZ spiral waves in a hydrogel that covalently integrates the catalyst, we suggest a new approach to developing active soft materials as chemical oscillators and for exploring the correlation between molecular structure and far-from-equilibrium dynamics.



This communication reports that cross-linking molecular nanofibers formed *via* self-assembly of hydrogelators can generate a new type of hydrogel suitable for exploring chemical oscillation and pattern formation in reaction-diffusion systems.[1,2] Among the few known oscillating chemical reactions that are able to sustain oscillations in a closed (batch) container for some time, the Belousov−Zhabotinsky (BZ) reaction,[3] the oxidation of an organic substrate (e.g., malonic acid) by acidic bromate catalyzed by metal ions or complexes (Figure 1A), has provided a useful chemical model of nonequilibrium biological phenomena such as pattern formation.[4,5] The BZ reaction was first explored in homogeneous aqueous solution,[6] in which it can oscillate, isolated from its environment, for hundreds of cycles in both stirred and pattern formation experiments. Gel materials, however, are excellent systems for studying BZ systems because they offer several advantages:[2] (i) In aqueous solution, convection can interfere with pattern formation. Gels, like several other heterogeneous or discontinuous systems (e.g., surfaces,[7] ion-exchange resins,[8] membranes,[9] and microemulsions[4,10]) eliminate such hydrodynamic effects, which makes them ideal for exploring spatiotemporal oscillation and pattern formation. (ii) The gel matrix facilitates immobilization and distribution of a variety of chemical species. That is, it is possible to have preselected concentration profiles and/or zero or very small effective diffusion coefficients for the catalyst, substrates, charged groups, and inhibitors or other substances of the BZ reaction, thus allowing control and modulation of pattern formation.[11] (iii) Gels can stabilize catalysts that otherwise may decompose in acidic solution. (iv) It is known that gels have a tendency to inhibit nucleation of heterogeneous processes by catching the nuclei on network chains, preventing their growth. Thus, gels suppress the formation of bubbles in the BZ reaction and help to create stable chemical waves.

The above promising features, in fact, have led to the development of self-oscillating polymeric hydrogels, mainly for generating chemomechanical effects.[12,13] For example, Yoshida et al. first demonstrated that replacement of a bipyridine ligand in the ruthenium(II) tris(bipyridine) complex (Ru(II)(bipy)$_3$Cl$_2$) by a 4-methyl-4′-vinyl-2,2′-bipyridine allows covalent attachment of the Ru(II)-center to poly(*N*-isopropylacrylamide), a well-known temperature responsive polymer,[14] via copolymerization to form a poly(NIPAAm-Ru) hydrogel[12,13] that exhibits self-sustained mechanical as well as chemical oscillations.[13] Despite this success, the poly(NIPAAm-Ru) hydrogel, consisting of random coils of copolymers, provides little insight into the correlation between the chemical oscillation and molecular organization, a fundamental issue that may be essential for a mechanistic understanding of the chemomechanical behavior of such gels. Therefore, we decided to use molecular self-assembly to introduce ordered nanostructures into hydrogels in order to construct new prototype systems that undergo chemical oscillation. Ultimately, we seek to explore pattern formation in reaction-diffusion systems with significant molecular order and to evaluate the correlation between macroscopic pattern formation and molecular arrangement.

As a powerful strategy for making ordered nanostructures, molecular self-assembly[15] offers ample opportunities to develop novel materials for various applications.[16] For example, self-

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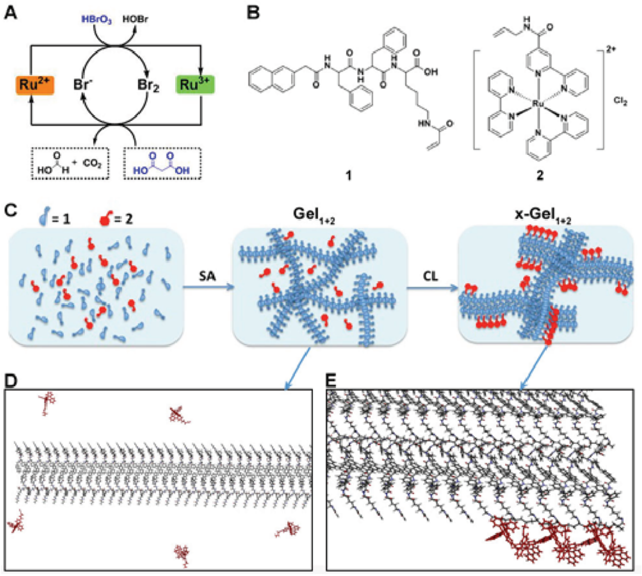

Figure 1. (A) Core process of chemical oscillation, typically occurring after addition of $NaBrO_3$ and malonic acid to an acidic solution containing a Ru complex as catalyst. (B) Molecular structures of the hydrogelator bearing a polymerizable group and a catalyst for BZ reaction. (C) Schematic illustration of the postself-assembly cross-linking process to make the gel for chemical oscillation (SA = self-assembly; CL = cross-linking). (D) Molecular model of nanofibers of **1** (in black color) and free catalyst **2** (in brown color). (E) Molecular model of cross-linked nanofibers of **1** integrated with catalyst **2**.

organization of supramolecular helical dendrimers has led to a new class of supramolecular materials suitable for electronic and optoelectronic applications;[17] self-assembly of planar π-conjugated molecules has resulted in organic nanodevices;[18] and self-assembly of peptide amphiphiles has created bioactive nanofibers as biomaterials for regenerative medicine.[19] Encouraged by the above works, advances in supramolecular gels,[20] and other supramolecular materials based on small peptides,[21] we chose to utilize molecular self-assembly to make new soft materials for exploring chemical oscillations, an approach that has yet to be explored. Specifically, we have designed and synthesized a small peptide hydrogelator that self-assembles in aqueous phase to form supramolecular nanofibers. Unlike most previously reported peptidic hydrogelators, our hydrogelator bears a polymerizable group, acrylamide, that allows polymerization after formation of the nanofibers. To integrate the BZ catalyst into the nanofibers, we cross-linked the molecular nanofibers of the hydrogelator in the presence of the BZ catalyst after self-assembly of the hydrogelator resulted in the hydrogel (Figure 1C, D, and E).

Based on the process illustrated in Figure 1C, we investigated the chemical oscillation of our supramolecular hydrogels before and after post-self-assembly cross-linking. Before cross-linking and under the conditions for a BZ reaction, Gel$_{1+2}$, the hydrogel consisting of **1** and **2**, displayed chemical oscillation, which represents the first example of a supramolecular hydrogel that tolerates the demanding conditions of the BZ reaction (strongly acidic medium and the presence of strongly oxidizing species) for chemical oscillation. After the postself-assembly cross-linking of Gel$_{1+2}$, the resulting cross-linked hydrogel, x-Gel$_{1+2}$, successfully generated chemical oscillation and spiral waves with a frequency five times higher than that of the poly(NIPAAm-Ru) hydrogel.[13] In addition, we found that cross-linking immobilizes the catalyst into the nanofibers at little expense to the molecular order, and the cross-linked hydrogel (x-Gel$_{1+2}$) has relatively large pores that likely contribute to its relatively fast chemical oscillation.

Figure 1B shows the structures of the hydrogelator (**1**) and the catalyst (**2**). We used solid-phase synthesis to make the peptide hydrogelator (NapFFK)[22] before coupling it with acrylic acid via an amide bond in a one-step liquid-phase reaction assisted by *N*-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), which formed the hydrogelator **1** in 62% yield. Despite its apparently simple structure, the synthesis of the polymerizable catalyst **2** of the BZ reaction required multiple steps:[23] First, 2-trimethylstannyl-pyridine (**3**) was reacted with methyl 2-bromoisonicotinate (**5**) via a Stille cross-coupling reaction[24] to form 2,2′-bipyridine-4-carboxylic acid (**6**). Second, assisted by 1-hydroxybenzotriazole (HOBt) and *N,N*′-dicyclohexylcarbodiimide (DCC), **6** was coupled with an allylamine to form *N*-allyl-(2,2′-bipyridine)-4-carboxamide (**7**) in 85% yield. Third, **7** was reacted with dichloro-bis(dipyridyl)-ruthenium(II) dihydrate at high temperature in a water−ethanol mixture to give Ru-catalyst **2** in 90% yield after purification by column chromatography (column: Sephadex; eluent: acetonitrile and methanol).

To obtain Gel$_{1+2}$ (Figure 2A), we mixed **1** (1.1% (w/v)) and **2** (0.2% (w/v)) in water and adjusted the pH to 10−12 to produce Gel$_{1+2}$. After degassing Gel$_{1+2}$ using N_2, we added V_{50}, the initiator for photopolymerization, at 0.2 wt % of **1**+**2**, to Gel$_{1+2}$ and mixed them well. After being poured into a glass mold and sealed, the mixture was subjected to UV irradiation (mercury lamp) for 20 min to form the cross-linked hydrogel, x-Gel$_{1+2}$ (Figure 2A). Then, x-Gel$_{1+2}$ underwent dialysis for 3 days to remove the initiators. Finally, we immersed x-Gel$_{1+2}$ in a saturated KPF_6 solution for 3 days to exchange the anions from

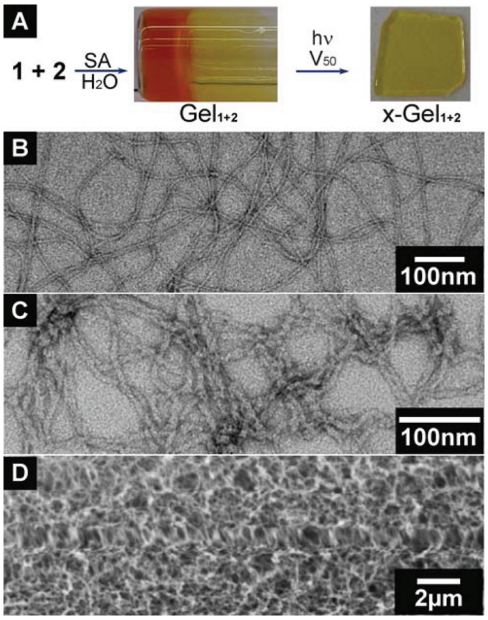

Figure 2. (A) Process for making the molecular hydrogel without and with cross-linking to form Gel$_{1+2}$ and x-Gel$_{1+2}$, respectively (SA = self-assembly). V50 = 2,2′-azobis(2-methylpropionamidine)-dihydrochloride, a water-soluble photoinitiator. (B,C) TEM images of (B) Gel$_{1+2}$ and (C) x-Gel$_{1+2}$. (D) SEM image of x-Gel$_{1+2}$.

Cl^- to PF_6^-. Conventional free radical initiated polymerization of copolymer takes about 20 h,[12] while the cross-linking of self-assembled nanofibers and the attachment of the BZ catalyst requires only about 20 min. Moreover, post-self-assembly cross-linking allows more catalyst to be integrated into the nanofibers, up to 20.0 wt % compared to about 5.0 wt % in poly(NIPAAm-Ru).[13]

Before carrying out the BZ reaction with the gel, we used transmission microscopy to examine Gel_{1+2} and x-Gel_{1+2}. As shown in Figure 2B and C, the nanofibers of Gel_{1+2} were quite uniform in width (about 6 ± 2 nm). Although the widths of the nanofibers in x-Gel_{1+2} increased to about 11 ± 2 nm after cross-linking, the diameters of the nanofibers remained uniform. The increase in the width of the nanofibers and the observation of the parallel nanofibers in Gel_{1+2} (SI Figure s1) indicate interfiber cross-linking. In addition, scanning electron microscopy (SEM) revealed that the pores of the network in x-Gel_{1+2} are about 200−300 nm in diameter. These results confirm that hydrogelator **1** self-assembles in water to form ordered secondary structures, which tolerate the cross-linking in spite of the increase in width of the nanofibers. Moreover, rheological tests showed that the storage modulus of x-Gel_{1+2} is 3 orders of magnitude higher than that of Gel_{1+2} (SI Figure s2), suggesting that the cross-linking successfully builds covalent links for the hydrogel network and significantly improves the physicochemical stability of the gel. For example, before cross-linking, addition of ethanol dissolved or collapsed Gel_{1+2}, but x-Gel_{1+2} remained as a hydrogel upon the same treatment.

After EM study, we tested Gel_{1+2}, x-Gel_{1+2}, and a control (the poly(NIPAAm-Ru) gel[13]) for chemical oscillation under the initial conditions of the BZ reaction: ($[HNO_3] = 0.8$ M; $[NaBrO_3] = 0.2$ M; $[CH_2(COOH)_2] = 0.08$ M). We included the third gel because there has been little study of pattern formation in it. Unlike the poly(NIPAAm-Ru) gel, which showed small volume changes with chemical oscillation,[12,13] both Gel_{1+2} and x-Gel_{1+2} maintained their volume in the BZ solution, which is quite acidic (pH < 1). Under the same conditions, the three gels had different oscillation frequencies. Gel_{1+2} and x-Gel_{1+2} oscillated at 39 and 52 s, respectively (Figure 3), significantly faster than the poly(NIPAAm-Ru) gel (212 s, SI Figure s3).[12]

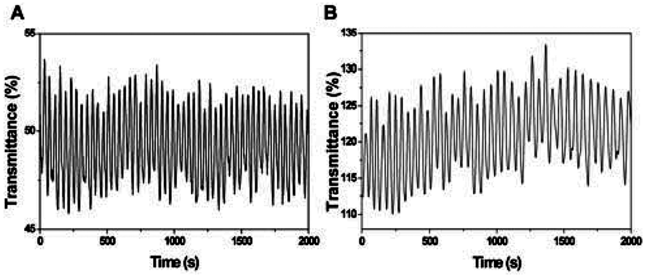

Figure 3. Oscillatory profile of optical transmittance for Gel_{1+2} (A) and x-Gel_{1+2} (B) at 20 °C in aqueous solution with initial composition $[HNO_3] = 0.8$ M; $[NaBrO_3] = 0.2$ M; $[CH_2(COOH)_2] = 0.08$ M. The average oscillation periods of Gel_{1+2} and x-Gel_{1+2} are 39 and 52 s, respectively.

More importantly, because of the differences in their molecular structures, the three gels exhibited different spatial behaviors, displaying distinctive patterns of chemical waves and wave transitions (Figure 4, also see video in Supporting Information). After being immersed in the BZ solution for about 6 min, x-Gel_{1+2} displayed trigger wave patterns at two or three random sites. The wave propagated, and at 10 min, a spiral wave began to form (Figure 4A), propagating out from the center to the edge of the gel (Figure 4B,C). This pattern, with a wavelength of 1.0 mm, remained in the gel for more than an hour. As soon as it was covered by BZ solution, Gel_{1+2} started to oscillate. Several spiral waves formed initially (Figure 4D). About 20 min later, the pattern changed to several traveling waves terminating at the same spot (Figure 4E). The wavelength of the chemical wave in Gel_{1+2} was 1.1 mm, almost the same as that seen in x-Gel_{1+2}. The comparable wavelengths of the chemical waves in Gel_{1+2} and x-Gel_{1+2} are probably due to their similar networks. The poly(NIPAAm-Ru) gel started oscillating after being immersed in BZ solution for about 5 min. Traveling waves started moving at similar speeds from the four edges toward the center of the gel. After another 5 min, the traveling waves from two edges with a joint corner overcame the other waves and formed the pattern in Figure 4A. After another 10 min, only the traveling waves from one edge remained, with all of them traveling across the gel in the same direction. The stable average wavelength was around 2.0 mm. These results clearly confirm that the molecular arrangement in the gel affects the dynamical behavior.

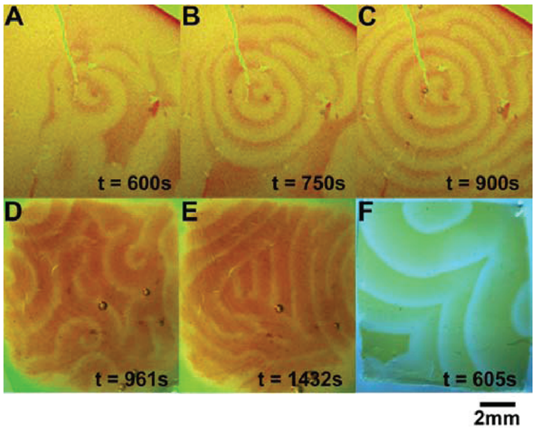

Figure 4. Optical images of patterns formed by chemical waves on x-Gel_{1+2} (A,B,C), Gel_{1+2} (D and E), and the poly(NIPAAm-Ru) gel (F) at 24 °C in aqueous solution with the same initial conditions as Figure 3. Except for F, the contrast of the images was enhanced.

In conclusion, this work illustrates several important advantages for future development of chemical oscillatory hydrogels. First, it confirms the stability of the peptide bond under the rather severe conditions of the BZ reaction. Thus, it is feasible to employ sophisticated peptide motifs[25] in hydrogels for generating chemical or chemomechanical oscillation. Second, the synthesis of **2** is much easier than that of the catalyst for poly(NIPAAM-Ru) gel,[22] and each component for the post-self-assembly cross-linking (e.g., **1** and **2**) can be stored over long times (e.g., >12 months). Third, the formation of x-Gel is complete in a relatively short time and eliminates the need for solvents other than water, thus opening up new experimental possibilities for the study of chemical waves in more elaborate microstructures. Fourth, it establishes the correlation between molecular structure, chemical oscillation, and pattern formation in heterogeneous systems such as gels. That is, the x-gel and the poly(NIPAAm-Ru) gel have different molecular structures, which lead to differences in the pore sizes in the matrices of the hydrogels and the distribution of the catalysts, thus resulting in chemical waves with different spatial properties. Fifth, it verifies that post-self-assembly cross-linking preserves ordered molecular nanostructures, which implies that this approach can help integrate other catalysts into supramolecular hydrogels. Therefore, this work opens many exciting opportunities for exploring self-oscillatory materials via self-assembly.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis of hydrogelator **1**, rheological data, and a video showing chemical waves. This material is available free of charge via the Internet at http://pubs.acs.org.

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