

AFM Observation of Immobilized Self-Oscillating Polymer

Yoshihiro Ito,^{*,†,‡} Yusuke Hara,[§] Hiroshi Uetsuka,^{||} Hirokazu Hasuda,[†] Hiroshi Onishi,^{||} Hideo Arakawa,[⊥] Atsushi Ikai,[⊥] and Ryo Yoshida[§]

Regenerative Medical Bioreactor Project, Kanagawa Academy of Science and Technology, KSP East 309, 3-2-1 Sakado, Takatsu-ku, Kawasaki, Kanagawa 213-0012, Japan, RIKEN (The Institute of Physical and Chemical Research), 2-1 Hirosawa, Wako, Saitama 314-0012, Japan, Department of Materials Engineering, Graduate School of Engineering, The University of Tokyo, Bunkyo-ku, Tokyo 113-8656 Japan, Surface Chemistry Project, Kanagawa Academy of Science and Technology, KSP East 404, 3-2-1 Sakado, Takatsu-ku, Kawasaki, Kanagawa 213-0012 Japan, and Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama, 226-8501, Japan

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Various types of nanocomponents have been developed to construct a nanodevice or nanomachine. Here, we add a new nanocomponent that has the function of self-oscillation. A thermoresponsive polymer carrying a Ru complex, a catalyst of the Belousov–Zhabotinsky reaction, was synthesized and immobilized on a glass plate. Periodic turbidity changes in the aqueous solution of the polymer were observed, and nanoscale self-oscillation of the immobilized polymer was observed by a scanning probe microscope.

Introduction

Many types of nanosize devices or molecular machines have been developed, including chemical-, protein-, and DNA-based devices.^{1–5} Recently, Hugel et al.⁶ realized a single-molecule optomechanical cycle using a photoresponsive polymer. Atomic force microscopy (AFM) observation of stimulus-responsive polymer brushes was performed by several researchers including us.^{7–10} However, observation of self-movement in nanodevices has not been reported. In the present study, for the first time we attempted to observe a nanoscale self-oscillation of a polymer brush using the Belousov–Zhabotinsky (BZ) reaction mechanism (chemical clock). It is known that the BZ reaction is a Ru(bpy)₃-catalyzed self-oscillating phenomenon.^{11,12} The overall process is the oxidation of an organic substrate such as malonic acid by an oxidizing agent (bromate) in the presence of a metal ion catalyst under acidic conditions. In the course of the reaction, a cyclic reaction network of intermediates is created, akin to metabolic reactions (e.g., the tricarboxylic acid cycle) in living organisms. In such a case, oscillations can occur, and the catalyst ion periodically changes between its reduced and oxidized states.

We have synthesized a new polymer combining this system into a thermal-responsive system.^{13–16} Here, the polymer was covalently immobilized on a surface, and the self-oscillation was directly observed at a molecular level by scanning probe microscopy (SPM). The oscillating chemical reaction is transformed into mechanical molecular movement. This self-oscillating device provides a new component for nanomachines.

Experimental Section

A self-oscillating polymer was synthesized using a Ru complex (Ru(bpy)₃, a BZ reaction catalyst), *N*-isopropylacrylamide (NIPAAm, a component of a thermal-responsive polymer), and an *N*-succinimidyl group (NAS, a component for linking to the substrate) as shown in Figure 1. 4-Vinyl-4'-methyl-2,2'-bipyridinebis(2,2'-bipyridine)bis(hexafluorophosphate) (Ru(bpy)₃) (0.2 g), *N*-isopropylacrylamide (3.6 g), and *N*-succinimidyl acrylic acid (0.2 g) were copolymerized in methanol (16 g) using 2,2'-azobis(isobutyronitrile) (0.16 g) as an initiator (total monomer concentration was 20 wt %). The polymerization was carried out at 60 °C for 24 h in vacuo. The resulting reaction mixture was dialyzed against water for 4 days followed by methanol for 3 days, and then freeze-dried. The weight-average molecular weight of the copolymer determined by static light scattering (Otsuka Electronics, DLS-7000) at 25 °C in the copolymer concentration range 4.0–8.0 mg/mL was 310 000.

For immobilization on the aminosilane-coupled glass plate, the aqueous solution of the polymer (5 wt %) was cast on the plate and then left standing for 18 h at room temperature. Subsequently, the plate was washed with pure water for further experiments.

Turbidity was measured by monitoring the transmittance at 574 nm. X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (TOF-SIMS) measurements were performed by S-probe (model 2703, Surface Science Instrument Co.) and TFS-2000 (iPhysical Electronics Co.), respectively.

Scanning probe microscopy (SPM) measurement was carried out using a JSPM-4200 (JEOL) equipped with a cantilever (OMCL-AC160TS, Olympus) at 20 °C. The frequency and spring constant were 150 kHz and 42 N/m, respectively. First, a topological image was obtained and the cantilever put on the

[†] Regenerative Medical Bioreactor Project, Kanagawa Academy of Science and Technology.

[‡] RIKEN.

[§] The University of Tokyo.

^{||} Surface Chemistry Project, Kanagawa Academy of Science and Technology.

[⊥] Tokyo Institute of Technology.

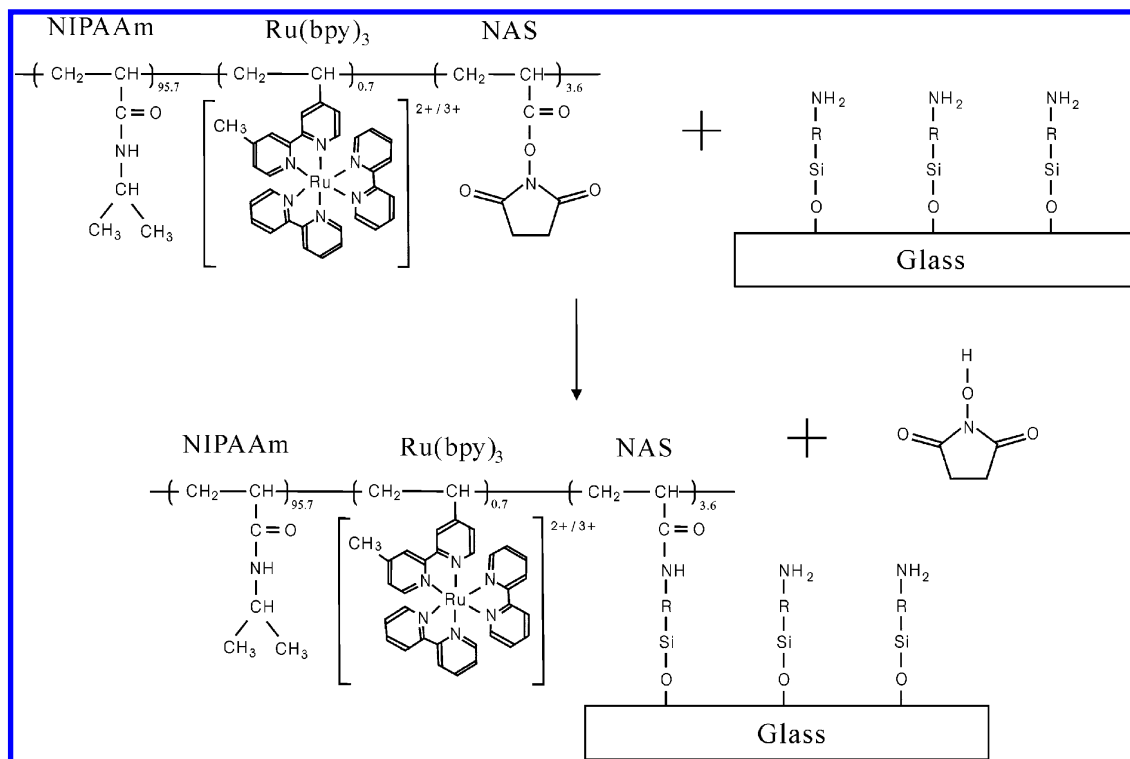


Figure 1. Preparation of self-oscillating polymer and the immobilization on a aminosilane-coupled glass surface.

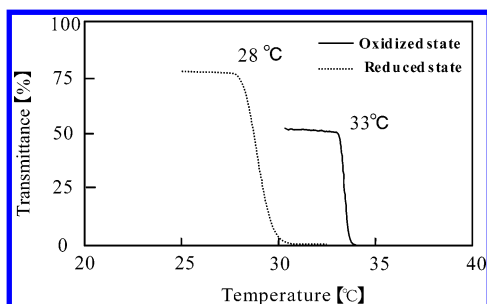


Figure 2. The thermal property of the prepared polymer. The concentrations of polymer, HNO_3 , and $\text{Ce}_2(\text{SO}_4)_3$ or $\text{Ce}_2(\text{SO}_4)_2$ were 0.5 wt %, 0.3 M, and 1 mM, respectively. The temperature was raised at a rate of 0.5 °C/min. The solution was stirred with a magnetic stirrer.

immobilized plate. After the measurement, the fact that there was no lateral movement was confirmed by the coincidence with the topological image.

Results and Discussion

When the turbidity of the polymer solution was measured, the lower critical solution temperature (LCST) of the polymer was observed (Figure 2). Since the maximum absorbencies of the reduced and oxidized polymer were observed at 453 and 421 nm, respectively, the turbidity was measured at 574 nm to avoid the effect of color change. Through addition of Ce^{3+} and Ce^{4+} , the polymer was reduced and oxidized, respectively. The LCST in the reduced state was 28 °C, which is lower than that of the homopolymer of NIPAAm. Copolymerization with the hydrophobic monomer $\text{Ru}(\text{bpy})_3$ resulted in a decrease of the LCST. We previously observed a decrease of the LCST by incorporation of phenyl groups into the NIPAAm polymer.^{13,14} On the other hand, the LCST in the oxidized state was 33 °C, which is only slightly higher than that of the NIPAAm homopolymer. This result indicates that an increase in the cationic charge due to oxidation of the $\text{Ru}(\text{bpy})_3$ moiety causes

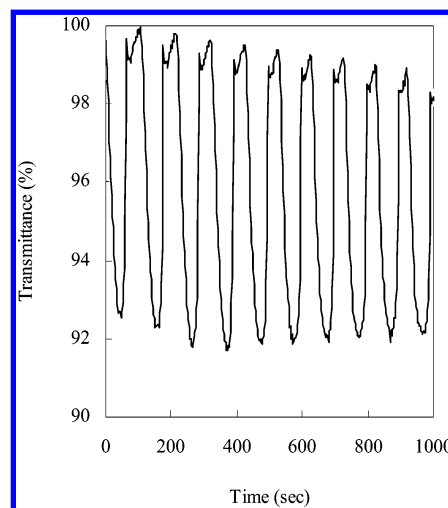


Figure 3. Self-oscillating behavior in solution. The concentrations of polymer, malonic acid, NaBrO_3 , and HNO_3 were 0.25 wt %, 0.1 M, 0.2 M, and 0.3 M, respectively. The solution was stirred with a magnetic stirrer at 20 °C.

an increase of water absorption onto the charge site of the copolymers.

Figure 3 shows oscillation of the polymer solution. During the BZ reaction, the color oscillated, and the turbidity also changed between the reduced state ($[\text{Ru}(\text{bpy})_3]^{2+}$) and the oxidized state ($[\text{Ru}(\text{bpy})_3]^{3+}$). As mentioned above, because a periodic color change of the Ru complex was observed during the BZ reaction, the oscillation was monitored at 574 nm as the turbidity changed. The amplitude and frequency of the oscillation were controlled by the concentration of reactants and the temperature, as previously reported for the polymer carrying no *N*-succinimide groups (NAS).^{14,15}

When dynamic light scattering (DLS) experiments were performed using a dilute solution of the polymer in aqueous 0.3 M HNO_3 solutions containing 1 mM $\text{Ce}_2(\text{SO}_4)_3$ and 1 mM $\text{Ce}(\text{SO}_4)_2$, the cumulant diameters were 23.9 and 59.6 nm,

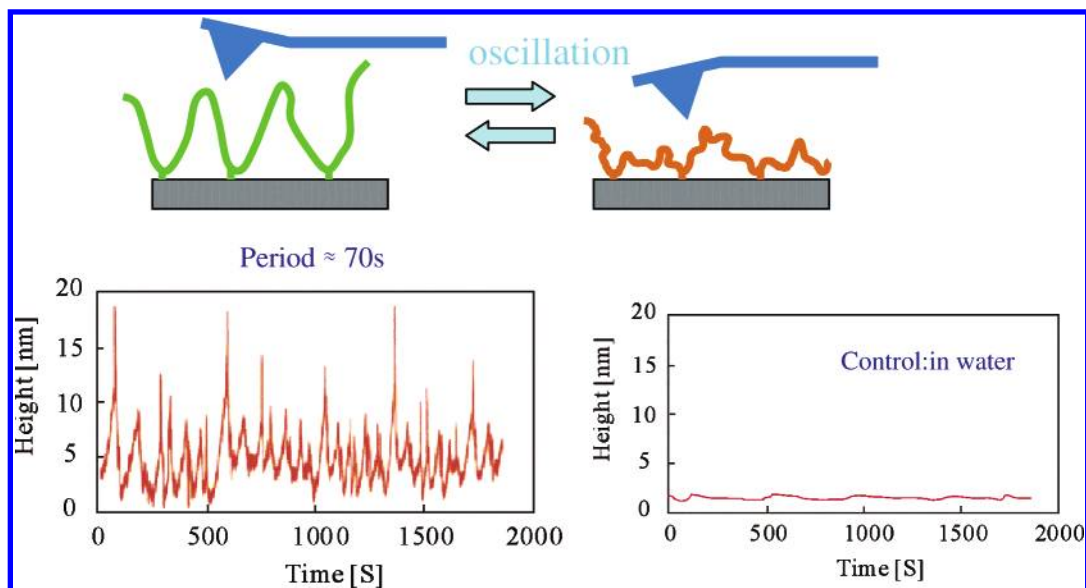


Figure 4. Self-oscillating behavior of the immobilized polymer in BZ reaction solution. The concentrations of malonic acid, NaBrO_3 , and HNO_3 were 0.1, 0.3, and 0.3 M, respectively.

respectively. This result shows that the reduction and oxidation induced conformational change of one polymer.

The synthesized copolymer was immobilized on an aminosilane-coupled glass plate. The surface was analyzed by XPS and TOF-SIMS. XPS revealed a weak peak of $\text{Ru}3d_{5/2}$ at 280.5 eV. On the other hand, in the positive secondary ionic spectrum of TOF-SIMS, $^{96,99,100,101,102,104}\text{Ru}^+$ were observed. These results demonstrated that Ru-containing polymer was immobilized on the glass plate.

The immobilized copolymer was measured by SPM operating in tapping mode. The plate was incubated in an aqueous solution containing malonic acid. After the addition of sodium bromate and nitric acid, the SPM cantilever was put on the plate. The z -axis movement of the cantilever was monitored; this is plotted in Figure 4.

While no oscillation was observed in pure water, nanoscale oscillation was observed in an aqueous solution of the BZ reaction, which consisted of malonic acid, NaBrO_3 , and HNO_3 . The amplitude was about 10–15 nm, and the period was about 70 s, although some irregular behavior was observed. In the case of the aqueous solution of polymer, stirring was very important in order to observe the regular oscillation shown in Figure 3. When it was not stirred, no regular oscillation was observed. However, in the case of the SPM experiment, it was impossible to stir the solution because of disturbance to the cantilever. In thin layers of unstirred BZ solutions, which would show otherwise homogeneous oscillations in well-stirred solutions, the usual phenomenon is considered to be spiral. For a local observer, like the cantilever, this was a local oscillation; nevertheless, the global phenomenon was more complex. Although no stirring could lead to the observed irregularity, the oscillation was reproducibly observed. The period of about 70 s was almost the same as observed in Figure 3 under the same concentration of BZ reaction.

The amplitude of the oscillation of the immobilized polymer (about 10–15 nm) was lower than that in the solution, as observed by DLS (23.9 and 59.6 nm). This smaller amplitude may be because the structure of the immobilized polymer was a loop-train-tail: the moving regions were shorter than that of the soluble polymer, as illustrated in Figure 4. In addition, the oscillation amplitude may have been suppressed because of the force applied by the weight of the cantilever, although the effect

was not quantitatively evaluated. The amplitude and the frequency were controlled by the concentration of reactant, as observed in the solution.

Finally, the oscillations in the concentrated solution (more than 2.0 wt %) did not continue for a long time (more than 2000 s). In contrast, the oscillations of the immobilized polymer on the glass surface continued for 2000 s without significant reduction. This reduced time dependence of the surface polymer is most likely due to less aggregation resulting from the interaction between polymers after they were immobilized.

The BZ reaction has previously been observed in solution, and it was applied to gel oscillation.¹⁶ On the other hand, local nanomechanical motion of the living cell wall was observed by SPM.¹⁷ Here, we observed nanoscale molecular self-oscillation for the first time. Some ideas for nano-oscillators were reported for the construction of a nanoclock or nanosensor.^{18–22} In the future, the oscillation polymer chain may be used as a component of a nanoclock or nanomachine.

References and Notes

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