Monitoring Thermoresponsive Morphological Changes in Individual Hydrogel Microspheres

Shusuke Matsui,† Yuichiro Nishizawa,‡ Takayuki Uchihashi,*§ and Daisuke Suzuki†,‡,§

†Graduate School of Textile Science & Technology and ‡Division of Smart Textile, Institute for Fiber Engineering, Interdisciplinary Cluster for Cutting Edge Research, Shinshu University, 3-15-1 Tokida, Ueda, Nagano 386-8567, Japan
§Department of Physics and Structural Biology Research Center, Graduate School of Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8602, Japan

Supporting Information

ABSTRACT: Real-time morphology/structure changes in individual hydrogel microspheres (microgels) were directly visualized at high spatiotemporal resolution using high-speed atomic force microscopy (HS-AFM) under temperature control ranging from room temperature to ~40 °C. The recorded HS-AFM movies demonstrate that the size and morphology of thermoresponsive poly(N-isopropyl acrylamide)-based microgels change with increasing temperature at the individual microgel level. Specifically, the height of the microgels gradually decreases and domain structures appeared even below the volume phase transition temperature. Moreover, the domain structure is retained, even after the microgels have fully collapsed. The present study thus demonstrates that temperature-controlled HS-AFM is a useful tool for monitoring stimulus-responsiveness of microgels. In the near future, it should furthermore be possible to extend this temperature-controlled HS-AFM to other stimulus-responsive materials, including autonomously oscillating microgels.

INTRODUCTION

As stimulus-responsive polymeric materials undergo dramatic changes in their structure/shape and physical/chemical properties in response to changes in the environment,1−7 they are also called “smart materials”. The key factors and requirements for stimulus-responsive materials to undergo changes are to be fast, reversible, complex, and robust in response to the external stimuli.5 One type of stimulus-responsive materials with the potential to fulfill the aforementioned requirements is hydrogel microspheres (microgels). Microgels are colloidal particles that can respond rapidly to external stimuli because of their microscopic dimensions.8−14 Owing to their rapid stimulus-sensitivity, microgels have been used for numerous applications in, for example, molecular separation, drug-delivery vehicles, sensors, and actuators.9−14 A recent significant advance in stimulus-responsive materials is autonomously oscillating microgels, which show periodic and reversible changes in size and the state of assembly in the absence of an external stimulus.15−18

So far, the stimulus-responsive properties of microgels have been mainly evaluated by scattering techniques,19−24 calorimetry,9,25,26 sensors,27,28 and so forth. However, these techniques provide ensemble-averaged signals for the entire system. Therefore, undesired artifacts such as unexpected morphology changes in microgels induced by external stimuli might be included in the obtained data, which would hamper the correct interpretation of the data. More reliable data and a deeper insight into the stimulus-responsive behavior of individual microgels should be obtained from real-time direct observations of microgels, which should provide accurate and relevant information on the morphological changes experienced by microgels. In this context, high-resolution microscopy techniques, such as atomic force microscopy (AFM),29−35 super-resolution microscopy,36−38 and liquid-cell in situ transmission electron microscopy (in situ TEM),39 have already been employed. Despite this considerable progress, there are still many issues that remain unresolved. For example, AFM and super-resolution microscopy are excellent techniques to visualize microgels on the nanoscale, albeit the temporal resolution remains problematic, that is, real-time observations cannot be achieved using these methods. In situ TEM allows monitoring microgels in aqueous solution; however, previous literature reports indicate that the spatial resolution is worse than that of cryo-TEM.35

In the present study, we have achieved a direct visualization of thermoresponsive morphological changes in individual microgels in real time in aqueous solution using high-speed atomic force microscopy (HS-AFM). HS-AFM enables
monitoring the structural dynamics of active biomolecules, such as proteins, under retention of their physiological functions.37–39 We have previously used HS-AFM to observe adsorption processes of microgels onto a solid substrate and found that soft colloidal particles adsorb on solid–liquid interfaces faster than hard ones.40 In the present study, we have investigated how highly water-swollen microgels respond to temperature changes using temperature-controlled HS-AFM (Figure 1a).

![Figure 1. (a) Schematic illustration of the HS-AFM apparatus equipped with the temperature-control device. (b) Time dependence of the temperature change in the sample holder during the HS-AFM measurements.](image)

**RESULTS AND DISCUSSION**

Thermoresponsive microgels were obtained from the aqueous free-radical precipitation polymerization of N-isopropyl acrylamide (NIPAm) and the cross-linker N,N′-methylenebis(acrylamide) (BIS; 1 or 5 mol %; hereafter denoted N1 and N5, respectively) (Table 1). Figure 2 shows the hydrodynamic diameter ($D_h$) of the polyNIPAm microgels in water as a function of the temperature determined by dynamic light scattering (DLS). The $D_h$ of the microgels decreases with increasing temperature, reaching an equilibrium at $\sim$40 °C. The volume phase transition temperatures (VPTT) for N1 and N5 were estimated to be $\sim$33 and $\sim$35 °C, respectively, on the basis of the maximum change ratio of the volume of the microgels.

| code | NIPAm (mol %) | BIS (mol %) | $D_h$ at 25 °C (nm) | $D_h$ at 40 °C (nm) |
|------|---------------|-------------|---------------------|---------------------|
| N1   | 99            | 1           | 396 ± 15            | 157 ± 1             |
| N5   | 95            | 5           | 450 ± 28            | 242 ± 1             |

Table 1. Chemical Composition and DLS-Derived $D_h$ Values for the Microgels in Aqueous Solution

Subsequently, the substrate was thoroughly rinsed with pure water to remove any excess microgels. The adsorbed microgels on the HOPG substrate were imaged by HS-AFM in pure water at room temperature ($\sim$25 °C), and then, the temperature-control device was employed to heat the solution ($\sim$1.5 °C/min) during the imaging (Figure 1b). Figure 3a shows clipped HS-AFM images of the N1 microgels during the heating (Movie S1). The N1 microgels exhibited an inhomogeneously collapsed morphology surrounded by a flattened corona of a loosely cross-linked polymer shell at 25 °C (Figure 3a), which is probably due to their highly soft nature. Here, a maximum height of the adsorbed microgels was approximately 50 nm (Figure 3b at 25.0 °C and Figure 3e), which was $\sim$8 times smaller than $D_h$ of the N1 microgels at 25 °C (396 nm), indicating highly deformed N1 microgels on the substrate because of their high softness. Upon heating the solution, the height of the N1 microgels gradually decreased (Figure 3e). During the whole process, the N1 microgels exhibited an inhomogeneous and nonhemispherical morphology (Figure 3a,b, as well as Movie S1). In contrast, the N5 microgels with a higher cross-linking density exhibited a relatively hemispherical morphology at 25 °C compared to that of the N1 microgels on the same substrate. Here, a maximum height of the adsorbed N5 microgel was 146 nm (Figure 3d at 25.6 °C and Figure 3f), which was $\sim$3 times smaller than the $D_h$ of the N5 microgels at 25 °C (450 nm). Upon heating the solution, the N5 microgels gradually contracted, and then, polymer-rich domains clearly appeared on the surface (Figure 3c and Movie S2).

![Figure 2. Temperature dependence of the DLS-derived $D_h$ values of N1 and N5 microgels in aqueous solution.](image)

Figure 4 shows the temperature dependencies of the normalized height of the microgels determined by the HS-AFM images and the normalized $D_h$ of the microgels determined by DLS. Here, the heights and $D_h$ of the microgels correspond to nonequilibrium and equilibrium states of the microgels at each temperature, respectively. Both the height and $D_h$ of the N1 microgels gradually decreased up to $\sim$31 °C and then rapidly decreased up to the VPTT ($\sim$33 °C), above which both the height and $D_h$ approached the equilibrium state (Figure 4a,b). In the case of the N5 microgels with a higher cross-linking density, both the height and $D_h$ gradually decreased up to the VPTT ($\sim$35 °C), above which both the height and $D_h$ reached a near-equilibrium state (Figure 4c,d). Since the tendencies of the temperature dependence for the normalized height and $D_h$ of these microgels are similar, it can be concluded that the size change of the microgels detected by HS-AFM must follow the temperature change on the
individual microgel level. This behavior seems to be reflected in the rapid stimulus-responsiveness of microgels.

Figure 5 shows the magnified HS-AFM images of the N5 microgels upon heating the solution. While the microgels gradually contracted, domains (several tens of nanometer in size) continuously and simultaneously formed on the microgels even at $\sim 27.0^\circ C$, that is, well below the VPTT of the microgels ($\sim 35^\circ C$). These domains persisted near the VPTT and did not disappear, not even at near $40^\circ C$, where the microgels had almost entirely collapsed. According to a previous study using small-angle neutron scattering (SANS), swollen pNIPAm-based microgels prepared by precipitation polymerization in aqueous solution present a core−shell structure with uniform segment distribution within the core and thin shell ($\sim 20$ nm), where the shell thickness is not affected by the temperature.21 Considering the present study, the surface and/or shell of the microgels may not always exhibit a uniform structure that may transform into an inhomogeneous raspberry structure upon heating. On the other hand, Höfl et al. have observed the appearance of a surface pattern on pNIPAm-based microgels adsorbed on a substrate above the VPTT by conventional AFM.30 They speculated that such surface patterns are probably due to the presence of collapsed dangling polymer chains that lead to rigid globules on the microgel surface.30 Additionally, Ikkai et al. have observed a microphase separation (size scale: 20−30 nm) in a weakly charged microgel near the VPTT by SANS,41 which is comparable to the domain size found in the present study. On the basis of these reports, the formation of domains observed in the present study upon increasing the temperature should presumably be attributed to polymer collapsing and/or polymer−polymer association. We have clarified that the domain formation occurs gradually at temperatures below the VPTT and the domain formation as well as the contraction of the microgels occurs simultaneously upon increasing the temperature.

It should be noted that the density fluctuation near the volume phase transition is largely influenced by the adsorption of the microgels on the solid substrate.35 This means that the thermoresponsive behavior of microgels in the dispersed state or when adsorbed on a solid substrate might be different, although reports describing such differences in detail remain elusive. Nevertheless, we believe that the findings in the present study are significant and help clarify the relationship between the morphology/structure and physical properties of microgels adsorbed on solid substrates, which may lead to the development of advanced functional materials, such as switchable cell culture substrates for tissue engineering,36 where the surface roughness as well as hydrophilicity/hydrophobicity are important parameters.

**CONCLUSIONS**

We have successfully observed temperature-induced morphological changes in individual microgels at the nanoscale in real
time by temperature-controlled HS-AFM. The morphology of substrate-adsorbed microgels varies with cross-linking density. The change of the shape of the microgels follows the change in temperature, whereby the normalized height and $D_h$ of the microgels showed similar behavior because of the rapid stimulus-responsiveness of the microgels. Furthermore, the formation of domains was observed during the collapse of the microgel structures, even below the VPTT; these domains persist, even in the collapsed state, which indicates that the contracted microgel state may not always be a homogeneous sphere, but often an inhomogeneous raspberry-shaped one. The direct visualization of the behavior of individual microgels on the nanoscale is important to gain further insight into thermoresponsive materials. In the near future, it should be possible to apply temperature-controlled HS-AFM to a wider range of temperatures and stimulus-responsive materials, which may further accelerate the development of novel smart materials.

### EXPERIMENTAL SECTION

**Materials.** N-Isopropyl acrylamide (NIPAm, 98%), $N,N'$-methylenebis(acrylamide) (BIS, 97%), sodium dodecyl sulfate (SDS, 95%), and potassium persulfate (KPS, 95%) were purchased from Wako Pure Chemical Industries (Japan) and used as received. The water used in all reactions, in the preparation of solutions, and in the purification of polymers was distilled and ion-exchanged (EYELA, SA-2100E1).

**Microgel Synthesis.** PolyNIPAm microgels were synthesized by aqueous free-radical precipitation polymerization. A mixture of NIPAm (8.402 g/99 mol % or 8.063 g/95 mol %), BIS (0.116 g, 1 mol % or 0.578 g/5 mol %), and SDS (0.072 g/0.5 mM for N1) in water (495 mL) was placed in a three-necked round-bottomed flask (1000 mL) equipped with a mechanical stirrer, a condenser, and a nitrogen gas inlet. The monomer solution was heated in an oil bath to 70 °C under nitrogen sparging (30 min) and constant stirring (250 rpm). After stabilization for 30 min, the KPS initiator (0.270 g/2 mM) dissolved in water (5 mL) was added to the flask to initiate the polymerization reaction. Thereafter, stirring was continued for 4 h before the dispersion was cooled to room temperature. The obtained microgels were purified by two cycles of centrifugation (70 000 g or 415 000 g; 15 °C), decantation of the supernatant, and redispersion of the precipitate in water. The dispersion was then dialyzed for a week with daily water changes.

**Dynamic Light Scattering (DLS) Measurements.** The hydrodynamic diameter ($D_h$) of the microgels was determined by DLS (Malvern Instruments Ltd., Zetasizer NanoS) measurements in aqueous solution. The DLS data represent averages of three individual measurements of 15 consecutive runs (30 s acquisition time of the intensity autocorrelation). The microgel concentration was fixed at 0.001 wt %. The samples were allowed to thermally equilibrate at each temperature for 10 min before the measurements. The time-dependent scattering intensity was detected at a total scattering angle of 173°. The $D_h$ of the microgels was calculated from the measured diffusion coefficients using the Stokes–Einstein equation (Zetasizer software v6.12).

**High-Speed Atomic Force Microscopy (HS-AFM).** We used the laboratory-built HS-AFM in this study, the details of which are described elsewhere. For the HS-AFM imaging, small cantilevers (length: 6–7 μm; width: 2 μm; thickness: 90 μm were used.

![Figure 4](image-url)
nm) developed by Olympus were employed. Typical spring constant, resonant frequency, and quality factor values for the aqueous solution of the cantilever are \(\sim 0.1 \text{ N/m} \), \(\sim 600 \text{ kHz} \), and \(\sim 2 \), respectively. Because the small cantilever has only a blunt bird-beak structure at the end, a sharp amorphous carbon tip was grown on the original tip by electron beam deposition. Then, the carbon tip was etched to \(\sim 4 \text{ nm} \) in radius by a RF plasma etcher under an argon atmosphere. For the HS-AFM imaging of the microgels, the cantilever free-oscillation amplitude was set to \(5^{-30} \text{ nm} \) and the set-point amplitude to \(70^{-90} \% \) of the free-oscillation amplitude (depending on the size of the microgels).

Droplets (3 \(\mu\text{L} \)) including the microgel dispersion ([microgel] = 0.001 wt \%) were loaded on highly oriented pyrolytic graphite (HOPG) substrates. After incubation (5 min), the substrate was thoroughly rinsed with pure water to remove residual microgels, and HS-AFM imaging was performed at room temperature \((T \approx 25 \text{ °C} \); scanning area: \(1000 \times 1000 \text{ nm}^2\); 120 \(\times\) 120 pixels\(^2\); frame rate = 1 fps\).

To monitor the real-time thermoresponsive morphological changes in the microgels, a temperature-control device was equipped in HS-AFM. The solution was heated by flowing a direct current through indium-tin-oxide glass at the bottom of the cantilever holder. The thermocouple was attached to the cantilever holder and immersed in the solution. The solution temperature can be controlled by a feedback control on the software of HS-AFM. Using this system, the temperature of the aqueous solution within the HS-AFM fluid cell was increased at a rate of \(\sim 1.5 \text{ °C/min} \). The temperature was measured every 10 s at different values of input voltage (1.7 to +0.2 V every 30 s) and plotted as a function of time (Figure 1b).

### ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b01770.

- Information on the HS-AFM movies of the thermoresponsive behavior of the N1 and N5 microgels in aqueous solution (PDF)
- HS-AFM movies of the thermoresponsive behavior of the N1 and N5 microgels in aqueous solution (MPG)

### AUTHOR INFORMATION

**Corresponding Authors**

*E-mail: d_suzuki@shinshu-u.ac.jp* (D.S.).

*E-mail: uchihast@d.phys.nagoya-u.ac.jp* (T.U.).

**ORCID**

Shusuke Matsui: 0000-0003-3129-1755

Daisuke Suzuki: 0000-0003-0444-156X

**Notes**

The authors declare no competing financial interest.
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