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ORIGINAL ARTICLE

Assembly of stimulus-sensitive gel particles with DNA–dye complexes

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In this study, an assembly of stimulus-sensitive gel particles with DNA–dye complexes was investigated for application in optical-sensing devices. This composite material of stimulus-sensitive gels and DNA detects external stimuli as a photo-signal. An assembly of the composite gel particles with DNA–dye complexes would be expected to achieve high responsiveness and to quickly detect environmental information as a photo-signal, because the porous structure produced by its interparticle spaces decreases with the relaxation time of the three-dimensional network structure. Poly(acrylic acid) (PAAc) gel particles with DNA were prepared by emulsion polymerization and the gel particles were assembled by a crosslinking reaction between the gel particles that utilized glutaraldehyde. It was possible to prepare the assembly of PAAc gel particles with DNA in sheet and block shapes by utilizing shaped forms for this synthesis process. The swelling ratio of the assembly increased with the pH, and the assembly reached equilibrium swelling in approximately 180 s. The response speed of the assembly was improved to more than 30 times faster than that of a typical PAAc block gel. Furthermore, the assembly of PAAc gel particles with DNA–ethidium bromide complexes detected pH information in an external solution as an optical signal.
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Keywords: assembly of stimulus-sensitive gel particles; DNA; optical property; pH-sensitivity

INTRODUCTION

Stimulus-sensitive gels change their volume in response to external environmental stimuli, such as pH, temperature, light, electric fields, and biomolecules.[1–7] By measuring the volume alteration quantitatively, the stimulus-sensitive gels can be used to detect a significant amount of environmental information and unique elements. These smart and soft materials are expected to be developed for use in applications such as sensor devices, actuators and drug carriers.[8–14] Previously, we reported on the application of the composite material consisting of stimulus-sensitive gels and DNA for optical sensor devices.[15] Stimulus-sensitive gels containing dye are expected to be useful as optical sensing devices to detect the fluorescence intensity, which changes with volume alteration in response to environmental stimuli. DNA was utilized as a dye-entrapped agent in stimulus-sensitive gels. The supplied DNA was obtained from salmon milt; most salmon milt is wasted in Japan. The utilization of DNA from salmon milt as a functional material is important and useful in terms of environmental programs and regional contributions. Furthermore, it has been reported that aromatic compounds such as dyes, harmful materials and carcinogens can be intercalated into double strands of DNA by molecular attraction because base-pairs of DNA exhibit aromaticity.[16–21] The double strand structure inhibits the dye aggregation in the gel. The concentration quenching of dye does not occur, and effective fluorescence emission can be expected. When the concentration of the dye is varied by altering the volume of the composite gel, the fluorescence intensity of the gel is increased or decreased in response to external stimuli because the amount of dye that is excited by laser illumination is changed.

In contrast, stimulus-sensitive gels are necessary to increase the response speed for applications in novel sensor devices. The relaxation time of a network of gels is proportional to the square of the diameter in equilibrium swelling. Recently, assemblies of polymer gel particles, which have excellent properties such as high stimulus responsiveness and colloidal photonic crystals, have been reported.[22–24] In nature, living things form in various shape of assembly in which the basic unit is optimally self-organized for the expression of various functions. The honeycomb structure has excellent physical properties and is often applied to artificial materials. The concept of organization and assembly on the microscale could provide various functions to smart materials.

Consequently, we also investigated the organization of the stimulus-sensitive gel particles with DNA by inducing a crosslinking reaction between the gel particles. An assembly of the stimulus-sensitive gel particles forms interparticle spaces. These microspaces are expected to reduce the relaxation time and diffuse solutions through

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the networks. Each gel particle composing the assembly responds to pH changes at nearly the same time as a result of the buffer solution diffusing through the interparticle spaces. That is, a microscopic change of the gel particles quickly induces a macroscopic change. The assembly of the stimulus-sensitive gel particles with DNA can achieve a high response speed similar to that of the gel particles. Additionally, the assembly of stimulus-sensitive gel particles with DNA could be used as an absorbent material for harmful substances in the external environment. DNA can immobilize harmful materials, such as carcinogens. The assembly has a highly porous structure, and its high-specific surface area is an advantage for absorbent material applications. The assembly of stimulus-sensitive gel particles with DNA would be expected to be used for sensing and absorbing materials.

In this study, an assembly of poly(acrylic acid) (PAAc) particles with DNA–ethidium bromide (EtBr) complexes was prepared, and the pH response characteristics were evaluated. The assembly of PAAc gel particles with DNA–EtBr complexes can be used to quickly detect pH information as a photo-signal.

EXPERIMENTAL PROCEDURE

Materials and reagents

Acrylic acid (AAc) and allylamine hydrochloride (AA) were purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). AAc was purified by reduced-pressure distillation. *N*,*N*′-methylene-bis-acrylamide, which was used as a crosslinking agent, was purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). The initiator, potassium persulfate, was purchased from Kanto Chemical Co. Inc. The *N*,*N*′-methylene-bis-acrylamide and potassium persulfate were used without further purification. DNA from salmon milt was supplied by Ogata Material Science Institute Corp. (Hokkaido, Japan). The molecular weight was approximately 6×10^7, the purity was 91.55% and the total amounts of N and P were 14.46% and 8.29%, respectively. Span 80, which was used as a surfactant, and cyclohexane were purchased from Kanto Chemical Co. Inc. EtBr, which was used as a fluorescent dye was purchased from Wako Pure Chemical Industries Ltd. Glutaraldehyde was purchased from Kanto Chemical Co. Inc.

Preparation of PAAc gel particles with DNA

PAAc gel particles with DNA were prepared by emulsion polymerization. First, 0.721 g of AAc (the molarity of AAc was adjusted to 1 mol l^{-1}), 0.096 g of AA (10 mol% relative to AAc) and 0.031 g of *N*,*N*′-methylene-bis-acrylamide (2 mol% relative to AAc) were dissolved in distilled water. DNA (the molarity of the DNA was adjusted to 1 mmol l^{-1}) was dissolved in distilled water, and each solution was mixed. This solution was dropped into 300 ml of cyclohexane, while simultaneously adding 2.1 g of Span 80, and the resulting mixture was stirred at 300 r.p.m. under a nitrogen atmosphere. Then, 0.005 g of potassium persulfate, which was deoxidized for 30 min, was added, and the polymerization was performed at 60 °C for 6 h. The obtained PAAc gel particles with DNA were microfiltered and washed repeatedly in methanol with centrifugation. The PAAc gel particles with DNA were finally immersed in distilled water for 24 h to remove the unreacted reagents.

Preparation of assembly of PAAc gel particles with DNA–EtBr complexes

The assembly of PAAc gel particles with DNA was prepared by a crosslinking reaction of the interparticles with glutaraldehyde. A suitable amount of the gel particles was put in a form which was composed of a silicone rubber sheet (1 mm in thickness) sandwiched between two glass plates (100 mm in height, 100 mm width, and 2 mm thickness). Glutaraldehyde was dissolved in a buffer solution at pH 9.0, and the solution was poured into the form. Subsequently, the form was set in a water bath and the crosslinking reaction was performed at 40 °C for 12 h. The obtained assembly of PAAc gel particles with DNA was repeatedly washed and immersed in distilled water to remove the unreacted reagents.

EtBr was used as a fluorescent dye. 10 ml of EtBr aqueous solution (1 mmol l^{-1}) was prepared by dissolved EtBr in distilled water. The assembly of PAAc gel particles with DNA–EtBr complexes was prepared by immersing 0.5 g of the assembly of PAAc gel particles containing DNA in the EtBr solution. The molar ratio of base pair vs EtBr was approximately 5.5:1. The obtained assembly of PAAc gel particles with DNA–EtBr complexes was then immersed in distilled water until equilibrium swelling was reached.

The assembly of PAAc gel particles with DNA–EtBr complexes was immersed for 24 h in distilled water and 0.01 N HCl aqueous solutions. The assembly of PAAc gel particles containing DNA–EtBr complexes in HCl aqueous solution was separated from the solution. The solution was measured by a UV–visible spectrophotometer (UV-1600, Shimadzu Corporation, Kyoto, Japan) to evaluate the elution of EtBr from the assembly containing DNA. The assembly of PAAc gel particles without DNA was similarly measured.

Mechanical properties of assembly of PAAc gel particles with DNA–EtBr complexes

Young's modulus of the assembly of PAAc gel particles with DNA–EtBr complexes was measured by thermomechanical analysis (TMA-60, Shimadzu Corporation). The assembly of PAAc gel particles with the DNA–EtBr complex was fabricated with a 10 mm height, width and thickness and kept immersed in distilled water until equilibrium swelling. The assembly was then set on the sample stage and a load was applied (0.01 N min^{-1} of load rate and 0.1 N of object load) by a 25 mm^2 of a circle indenter at room temperature. Young's modulus was calculated from the stress-strain curves. Young's modulus of PAAc block gel was similarly measured and compared with the assembly. Furthermore, the relationship between Young's modulus and the swelling ratio of the assembly of PAAc gel particles with DNA was investigated.

pH-sensitivity of assembly of PAAc gel particles with DNA–EtBr complexes

The measurement of the swelling ratio in response to the pH of the assembly of PAAc gel particles with DNA–EtBr complexes was performed using the image analysis system of an optical microscope (STZ-168-TL, Shimadzu Corporation) with several types of buffer solutions with pH values ranging from 2.0 to 12.0. The assembly was kept immersed in distilled water until equilibrium swelling was reached. The distilled water was then removed, and the assembly was immersed in each buffer solution for 1 h. The volume of the assembly was measured using the image analysis system of the optical microscope. The swelling ratio of the assembly was calculated by evaluating the volumes of the assembly before and after immersion in each of the buffer solutions.

The evaluation of the pH response speed was performed by measuring the swelling ratio of the assembly with time. The assembly of PAAc gel particles with DNA–EtBr complexes was immersed in a buffer solution at pH 2.0 and then examined by the optical microscope. The time response of the swelling ratio was measured with the image analysis system of the optical microscope. Furthermore, the pH response speeds of the gel particle and the block-shaped gel, which had the same volume and chemical composition relative to the assembly, were similarly measured and compared with that of the assembly.

Optical properties of assembly of PAAc gel particles with DNA–EtBr complexes

The optical properties of the assembly of PAAc gel particles with DNA–EtBr complexes were evaluated to consider the application of the assembly in optical sensor devices. The absorbance of the assembly was measured with the UV–visible spectrometer. The assembly was kept immersed in each buffer solution for 1 h. The assembly was set in the center of a quartz cell, and the absorbance of the assembly was measured. The fluorescence intensity of the assembly was evaluated by using an image analysis system. The assembly of PAAc gel particles with DNA–EtBr complexes was kept immersed in each of the buffer solutions for 10 min. The assembly was irradiated with ultraviolet light at a wavelength of 265 nm and then observed with the optical microscope in the dark. The intensity of the assembly at each pH was calculated relatively by using the image analysis system.

RESULTS AND DISCUSSION

Preparation of PAAc gel particles with DNA–EtBr complexes

PAAc gel particles with DNA could be prepared by emulsion polymerization. The average diameter of the gel particles was approximately 330 μm, calculated from 100 measurements using the image analysis system of the optical microscope. For the PAAc gel particles with DNA, it was confirmed that DNA was included in the particles from the absorption peak at 260 nm, which was measured using the UV–visible spectrophotometer.

Preparation of assembly of PAAc gel particles with DNA–EtBr complexes

The assembly of PAAc gel particles with DNA was obtained by a crosslinking reaction of the interparticles with glutaraldehyde. The assembly was formed into a sheet shape according to the shape of the form, which was 100 mm in height, 100 mm width and 1 mm thickness (Figure 1, left side). Using a form that was 50 mm in height and 10 mm in width and thickness, a block-shaped assembly could be prepared (Figure 1, right side). The assembly of PAAc gel particles with DNA could be prepared in various shapes by utilizing various forms in this process.

The assembly of PAAc gel particles with DNA–EtBr complexes was prepared by immersing the assembly of PAAc gel particles containing DNA in an EtBr solution. After 1 h of immersion in the solution, the EtBr was incorporated in the assembly. The EtBr has an absorption peak at 490 nm. The assembly of PAAc gel particles with DNA–EtBr complexes was evaluated to measure the elution of EtBr from the assembly to the external solution by using a UV–visible spectrophotometer, as shown in Figure 2. In the case of the assembly without DNA, the absorption peak of EtBr in the external solution was observed at 490 nm. However, the external solution surrounding the assembly with DNA did not exhibit the absorption peak at 490 nm. These results indicate that EtBr was immobilized in the assembly with DNA and did not diffuse into the external solution because it was intercalated into the double strands of the DNA.

Mechanical properties of assembly of PAAc gel particles with DNA–EtBr complexes

The Young's modulus of the assemblies of the PAAc gel particles with DNA that were prepared at different AA molar ratios was evaluated by calculations based on the stress-strain curve. The Young's modulus of the assembly exponentially increased with the AA molar ratio of the assembly, and the Young's modulus of the assembly that was prepared at 20 mol% of the AA molar ratio was approximately 10 kPa. It was found that the Young's modulus of the assembly was almost the same as that of the typical PAAc block gel. Additionally, the swelling ratio of the assembly of PAAc particles with DNA was measured. The swelling ratio of the assembly decreased exponentially with the AA molar ratio of the assembly. It was assumed that the assembly formed a highly porous structure that was composed of the gel particles, and the stimulus responsiveness of the assembly was controllable by the amount of crosslinking points between the gel particles. The formation of the interparticle spacing increased the swelling speed and made it possible to include and release micromaterials, such as drugs, biopolymers and other functional particles. Based on the relationship between Young's modulus and the swelling ratio, the assembly that was prepared with a 10 mol% AA molar ratio was used (Figure 3).

pH-sensitivity of PAAc gel particles with DNA–EtBr complexes

Figure 4 shows the optical micrographs of the assembly of the PAAc gel particles with DNA–EtBr complexes in various pH buffer solutions. The assembly changed its volume in response to pH. Furthermore, a dye density change of the assembly caused by the volume alteration was also observed. The dye density in the assembly increased with the shrinkage of the assembly. Additionally, the dye density of the assembly changed repeatedly with the volume alteration. These results indicate that pH-sensing could be accomplished by optical analysis of the assembly. Figure 5 shows the swelling ratio of the assembly in the pH range from 2.0 to 12.0. The swelling ratio of the assembly of PAAc gel particles with DNA–EtBr complexes regularly increased with the pH in constant ionic strength buffers.

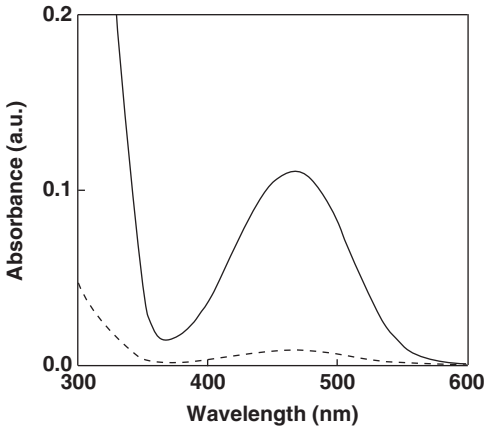

Figure 2 UV–visible spectra of the solution in which the assembly of PAAc gel particles with or without DNA containing EtBr was immersed. The solid line indicates the UV–visible spectrum of the solution of the assembly without DNA. The broken line indicates the assembly with DNA.

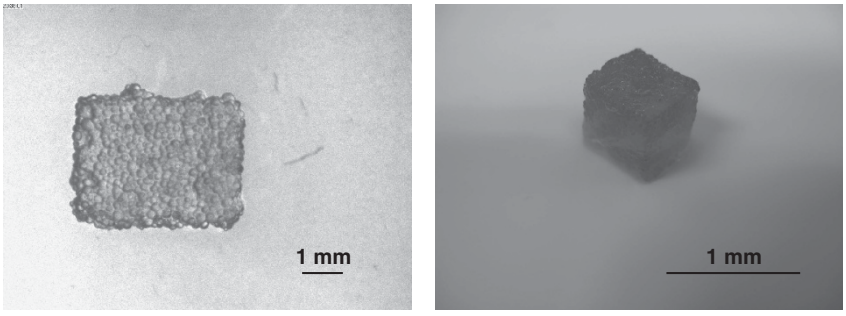

Figure 1 Optical micrograph and photograph of the assembly of PAAc gel particles with DNA–EtBr complexes. The left side indicates the sheet-shaped assembly and the right side indicates the block-shaped assembly.

Figure 6 shows the time course of the swelling ratio of the assembly of PAAc gel particles with DNA–EtBr complexes, the gel particles and the block gel at a pH of 2.0. The assembly shrunk rapidly, and the volume was constant after 180 s, which implies that equilibrium swelling was reached in approximately 180 s. Furthermore, this value of the assembly was approximately the same as the value of the gel particle, although their volumes were quite different. In contrast, the block gel, which had the same volume and chemical composition as the assembly, was also synthesized, and the pH response speeds of the block gel and the assembly were compared. The block gel with DNA shrunk slowly, and the volume was constant at approximately 6000 s. Therefore, the pH response speed of the assembly was approximately 30 times faster than that of the block gel, in spite of the same volume. The increase in the pH response speed of the assembly was assumed to be caused by the assembly forming a porous structure. The porous structure of the assembly decreased with the relaxation time of the three-dimensional network structure and external solution easily diffused into the network of the assembly. Therefore, the particles making up the assembly responded to external stimuli at approximately the same time.

Optical properties of assembly of PAAc gel particles with DNA–EtBr complexes

The dye density of the assembly could be changed by volume alteration in response to pH. The evaluation of the optical properties of the assembly is important for optical sensor device applications. The absorbance of the assembly at a wavelength of 500 nm decreased exponentially with the pH value. Figure 7 shows the relationship between fluorescence intensity and the pH of the assembly. The fluorescence intensity of the assembly was measured relatively with the image analysis system by irradiating UV light at a wavelength of 265 nm in buffer solution at pH 2.0, 4.0, 6.0, 7.0, 9.5, 11.0 and 12.0. The intensity of the assembly exponentially decreased with pH. In particular, the intensity of the pH values from 2.0 to the neutral region was clearly detected. In contrast, the fluorescence intensity at pH values over 11.0 was slight. Therefore, the color of the assembly of PAAc gel particles with DNA–EtBr complexes changed several times in response to pH, and it was confirmed that the EtBr was hardly eluted resulting from the absorbance spectra of DNA–EtBr in HCl and NaOH aqueous solutions. These results assume that the

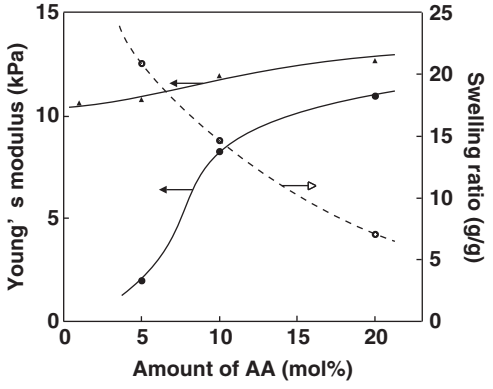

Figure 3 Relationship between Young's modulus (filled circle) and the swelling ratio (open circle) of the assembly of PAAc gel particles with DNA–EtBr complexes. Young's modulus of the PAAc block gel, which had the same volume and chemical composition as the assembly (filled triangle), is shown in comparison with the assembly.

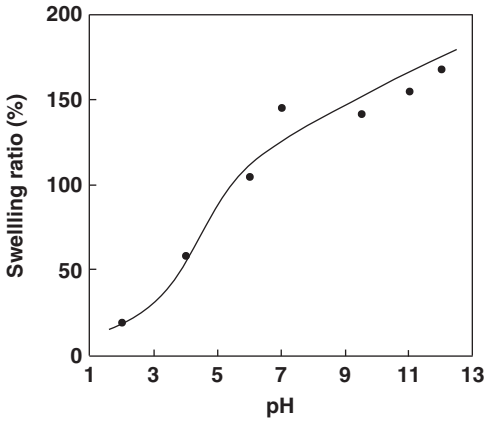

Figure 5 Swelling ratio of the assembly of PAAc gel particles with DNA–EtBr complexes in various buffer solutions at the pH values of 2.0, 4.0, 6.0, 7.0, 9.5, 11.0 and 12.0.

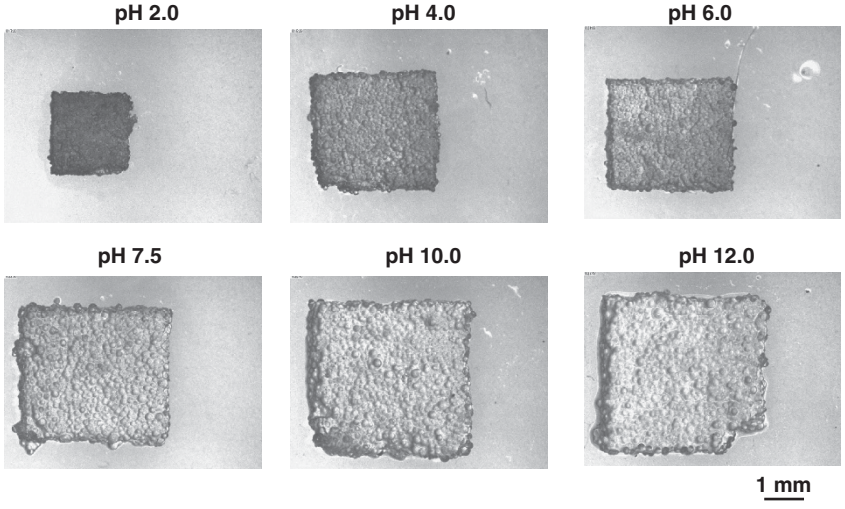

Figure 4 Optical micrographs of the assembly of PAAc gel particles with DNA–EtBr complexes in various buffer solutions at the pH values of 2.0, 4.0, 6.0, 7.5, 10.0 and 12.0.

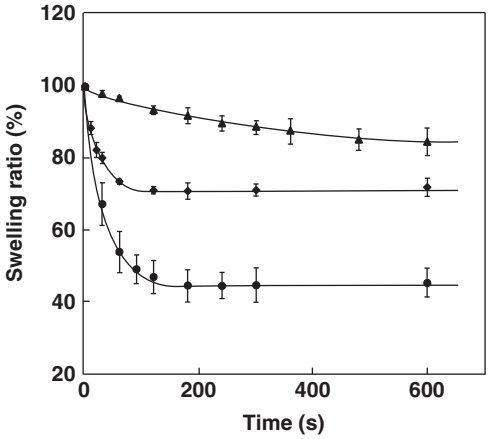

Figure 6 Time course of the swelling ratio of the assembly of PAAc gel particles (circle), the PAAc gel particle (diamond) and the PAAc block gel (triangle) in pH 2.0 buffer solution.

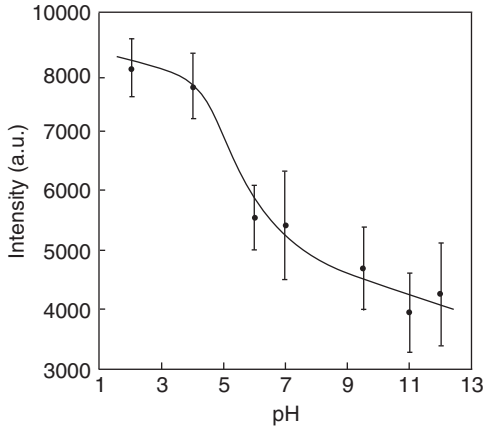

Figure 7 The fluorescence intensity of the assembly of PAAc gel particles with DNA–EtBr complexes in various pH buffer solutions at the pH values of 2.0, 4.0, 6.0, 7.0, 9.5, 11.0 and 12.0.

fluorescence intensity of the assembly of PAAc gel particles with DNA–EtBr complexes corresponded with the swelling behavior of the assembly. It is clear that the assembly of PAAc gel particles with DNA–EtBr complexes can be used to detect and quickly measure the pH in the surrounding environment via the fluorescence intensity and that this approach could be used to create optical sensor devices.

CONCLUSIONS

In this paper, we demonstrated the preparation of an assembly of PAAc gel particles with DNA–EtBr complexes and evaluated its pH sensitivity for application in optical detection devices. PAAc gel particles with DNA–EtBr complexes were prepared by emulsion polymerization, and the assembly was prepared by a crosslinking reaction between the gel particles that utilized glutaraldehyde. DNA was trapped in the PAAc gel particles and it was not eluted from the gel particles. EtBr could be immobilized into the assembly by intercalation with DNA, and EtBr did not diffuse in the external solution. The assembly of PAAc gel particles with DNA–EtBr complexes could be fabricated in various shapes by utilizing various forms. The Young's modulus of the assembly was almost the same as that of the typical block gel, and it was controlled by the AA molar ratio. The swelling ratio of the assembly of PAAc gel particles with DNA increased regularly with pH, and the response speed of the assembly was approximately 180 s. This speed was approximately 30 times faster than that of the block gel, which had the same volume and chemical composition as the assembly. The fluorescence intensity of the assembly of PAAc gel particles with DNA–EtBr complexes was measured with an image analysis system, and it exponentially decreased with pH. The assembly of PAAc gel particles with DNA–EtBr complexes can quickly detect the pH in the surrounding environment as an optical signal. The high stimulus-sensitivity of the assembly of stimulus-sensitive gel particles with DNA is due to its highly porous structure, and the gel particles that formed the assembly responded to external stimuli at the same time. The porous structure is an advantage for absorbent materials. DNA can immobilize not only dyes but also harmful materials, such as carcinogens. It has previously been reported that gas sensor devices using DNA could detect harmful materials. The assembly might be utilized as an absorbent filter for harmful materials because the assembly has a high-specific surface area and can be fabricated in various shapes based on the mold used. The assembly would also be able to detect the surrounding environmental conditions as an optical signal at the same time. Consequently, the utilization of DNA is a novel and useful approach for functional materials, and the assembly of gel particles with DNA is expected to be useful as a novel optical sensing material.

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