Heteromorphic Polymer Nanoparticles in Response to Rotational Magnetic Fields for Stirring inside Living Cells

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Abstract. Heteromorphic polymer nanoparticles containing iron oxide particles were prepared to obtain a nanometer-sized magnetic stir bar that remotely responds to external magnetic fields. The polymer nanoparticles were prepared by the mini-emulsion polymerization of styrene with small amounts of a cross-linker, and the spherical nanoparticles obtained were incorporated into a poly (vinyl alcohol) (PVA) film. The PVA film was stretched and deformed at 160°C to make the nanoparticles heterogeneous. The dimensions of the polymer nanoparticles with a heterogeneous morphology and rugby ball-like shape were about 210 nm \times 100 \text{nm} \text{ with an average aspect ratio greater than 2.0.} The dispersion ability of the nanoparticles was sufficient to use in aqueous circumstances. When an external magnetic field was applied to an aqueous suspension of the nanoparticles, the particles aggregated quickly. Also, on the application of a rotational magnetic field, the polymer nanoparticles began rotating with a velocity proportional to the rate of the external magnetic field rotation. Therefore, it was concluded that the heteromorphic polymer nanoparticles have the potential to stir a micrometer-scale reaction vessel, such as living cells.

1. Introduction
In the field of tissue regenerative medicine based on tissue engineering, it is necessary to quantitatively describe intracellular properties from an engineering viewpoint and to control cell function by active actions. It is a special molecular crowding environment in which high concentrations of biomolecules such as proteins are dissolved and distributed unevenly in cells, and it is difficult to reproduce artificially in vitro. Recently, intracellular environment factors such as temperature, viscosity, and pH have been directly analyzed using fluorescent probes such as polymer nanoparticles and quantum dots [1]. However, a reaction accelerating device for use inside cells has never been realized. Since fluorescent probes induce a nonspecific interaction with intracellular components, it causes analysis noise or deterioration of cell function. Therefore, we aim to prepare an intracellular magnetic stir bar having an excellent surface cytocompatibility and remote response to external magnetic fields (Figure 1). For this purpose, iron oxide nanoparticles encapsulated in heteromorphic polymer nanoparticles with surface cytocompatibility need to be designed. As the first step of this development, in this study, the heteromorphic polymer nanoparticles are prepared.

While incorporating super paramagnetic iron oxide particles into polymer nanoparticles, we considered the rotation characteristics provided by external magnetic fields applied to the nanoparticles. To achieve better stirring efficiency using physical rotation, the particles should have a heterogeneous morphology. The rotational motion of the polymer nanoparticles may quantitatively clarify the viscosity inside living cells. Furthermore, by controlling the dynamics of the molecules related to intracellular chemical reactions using this physical agitation, it is possible to dramatically change the cell function. Hence, we hope to contribute to the establishment of true cell engineering...
and the creation of an innovative medical technology by understanding the physical properties of intracellular environments and reaction control.

The heteromorphism of spherical particles was investigated by combining two methods: mini-emulsion polymerization of styrene (St) and divinylbenzene (DVB) using a small amount of anionic surfactant (sodium dodecyl sulfate: SDS) and physical stretching. In mini-emulsion polymerization, droplets of monomers emulsified and dispersed by ultrasonic irradiation are polymerized in situ. Therefore, it is relatively easy to encapsulate inorganic substances in the polymer particles. The effect of iron oxide content in the monomer droplets of the mini-emulsion polymerization on the magnetic responsiveness was examined. In addition, we aimed to achieve an aspect ratio of about 1.0–4.0, which is similar to that of commercially available stir bars. With regard to deformation by physical stretching, spherical polymer nanoparticles (poly(St-co-DVB) particles) were deformed in other polymer film by physical force. The aspect ratio of the polymer nanoparticles was controlled by changing the stretching ratio of the film.

Figure 1. Functionalities of polymer nanoparticle rotation inside cell

2. Experimental

2.1. Preparation of polymer nanoparticles containing iron oxide particles

An anionic surfactant SDS (0.25 mmol) as a dispersion stabilizer was dissolved in 50 mL of degassed water to prepare an aqueous phase. Desire amount of surface-hydrophobized iron oxide nanoparticles were dispersed in 29 mmol St and 1.2 mmol DVB was used as the crosslinking agent, and 0.15 moldodecyl peroxyde was dissolved to prepare a monomer solution. The monomer solution was added dropwise to the aqueous phase and emulsified and dispersed for 15 min using an ultrasonic homogenizer (Sonifier 250, Branson, and Danbury, CT, USA) with ice cooling and shaking. Thereafter, the emulsion was transferred to a 100 mL two-necked flask and argon gas was bubbled for 10 min while stirring at 250 rpm using a Teflon® stir blade. Thereafter, the two-necked flask was sealed, and the polymerization reaction continued for 24 h with stirring in an oil bath at 70 °C.

2.2. Characterization of polymer nanoparticles containing iron oxide particles

Measurements of the size and size distribution of the polymer nanoparticles were performed by dynamic light scattering (DLS) (Malvern Instruments Co., Ltd., Worcestershire, UK) using a 592 nm He-Ne laser.

The morphology of the polymer nanoparticles was examined using scanning electron microscopy (SEM) (SM-200, TOPCON, Tokyo, Japan). The aqueous dispersion of the prepared polymer nanoparticles was diluted with water, and it was added dropwise to a poly (ethylene terephthalate)(PET) substrate and dried overnight. Thereafter, a 20-nm-thick gold film was vapor-deposited on the PET substrate by vacuum evaporation to obtain an observation sample. The SEM observation was performed at an acceleration voltage of 20 kV.
The aqueous dispersion of the polymer nanoparticles was diluted 100-fold with an aqueous solution of 1.0 mM NaCl. The diluted dispersion was placed in a dedicated disposable cell using a syringe, and the surface zeta potential of the particle was measured by DLS.

The iron oxide content in the polymer nanoparticles was determined by the following procedure. Approximately 1.0 mg of lyophilized polymer nanoparticles was taken in a platinum sample pan. Using a thermo gravimetric analyzer (TG/DTA 6200, Seiko Instruments Inc., Tokyo, Japan), the temperature was raised from room temperature to 800 °C under nitrogen atmosphere at a rate of 20 °C/min to thermally decompose the polymer part. From the weight of the remaining iron oxide, the encapsulation ratio of the iron oxide particles in the polymer nanoparticles was calculated.

2.3. Evaluation of magnetic responsiveness of polymer nanoparticles containing iron oxide particles

Magnetic response of the polymer nanoparticles containing iron oxide particles was evaluated. A neodymium magnet with a surface magnetic flux density of 520 mT was applied to 1 mg particles dispersed in 1 mL NaCl aqueous solution. Time changes in the polymer nanoparticle recovery rate were measured from the changes in absorbance at 600 nm using an ultraviolet-visible spectrophotometer (V-560, JASCO, Tokyo, Japan).

2.4. Preparation of heteromorphic polymer nanoparticles containing magnetic particles

In 5.0 wt% aqueous solution of PVA, 2.0 mL aqueous dispersion of the polymer nanoparticles containing magnetic particles was mixed and casted in a poly (St) dish, and the solvent was evaporated overnight on a shaker to avoid precipitation of the polymer particles. The PVA film containing polymer nanoparticles was cut into a sample with a width of 1.0 cm and a length of 3.0 cm and uniaxial stretched to 5.0 cm in the width direction in an oil bath heated to 160 °C. Thereafter, the oil attached on the PVA film was washed twice with 2-propanol (IPA), and 30% IPA aqueous solution was added and left overnight. After heating to 160°C to dissolve PVA, the PVA solution was centrifuged at 13,000 rpm for 30 min and polymer nanoparticles precipitated were dispersed again with the same mixed solvent. This operation was repeated three times. Finally, the precipitated heteromorphic polymer nanoparticles were dispersed in water.

2.5. Evaluation of rotation characteristics of heteromorphic polymer nanoparticles

Magnetic tweezers were used to evaluate the rotational characteristics of the heteromorphic polymer nanoparticles containing magnetic particles. Magnetic tweezers are devices that generate rotating magnetic fields using electromagnets. Thin paper was glued to one glass plate using silicone grease, and another glass plate was placed on it. An aqueous dispersion of the heteromorphic polymer nanoparticles (20 μL) was injected into the gap between the glass plates to prepare an observation specimen. The rotation rates of the applied magnetic fields were controlled to be 2 Hz and 5 Hz. The rotation was observed by a video camera, and the video images were analyzed using Image J Software (Wayne Rasband National Institute of Health). The center of gravity position of the particles was calculated using a plug-in (ADACHI), and the center of rotation was set. Thereafter, the angle of the particles with respect to the rotation center and the cumulative rotation number was calculated.

3. Results and discussion

3.1. Magnetic responsiveness of polymer nanoparticles

The polymer nanoparticles obtained had a spherical morphology with a diameter of approximately 130 nm. The surface zeta potential of the nanoparticles was -50 mV due to the existence of an anionic surfactant during the emulsion polymerization. The negative value might facilitate good dispersion ability in an aqueous medium. The encapsulation rate of the iron oxide particles in the polymer nanoparticles measured by thermo gravimetric analysis increased with increasing iron oxide content in the feed. The iron oxide content was varied from 5.0wt% to 25 wt% in the polymer nanoparticles.

Figure 2 shows the evaluation of the magnetic responsiveness of the nanoparticles using a neodymium magnet. When applying a magnetic field, the polymer nanoparticles aggregated and finally completely gathered (Figure 2, left). After removing the magnetic field and shaking the
suspension, the polymer particles showed good dispersion ability. As the encapsulation rate of the iron oxide nanoparticles increased, the particles were recovered more quickly, and for particles with an encapsulation rate of 25 wt%, about 80% of the polymer nanoparticles could be recovered in about 60 min. By increasing the iron oxide content in the polymer nanoparticles, the magnetic responsiveness could improve.

Figure 2. Aggregation of polymer nanoparticles containing iron oxide particles by applying a magnetic field. In the right panel, the open circles, closed circles, and open triangles represent iron oxide contents of 25 wt%, 10 wt%, and 5 wt% in the polymer nanoparticles, respectively.

3.2. Heterogeneous morphology of the polymer nanoparticles by physical stretching

The procedure for preparing heteromorphic polymer nanoparticles is shown in Figure 3. Generally, the particles assume a spherical morphology because of the minimal surface free energy in the medium. Therefore, it is difficult to produce particles with a heterogeneous morphology directly in one step. Various methods for preparing heteromorphic particles have been reported. The production of deformed particles using physical stretching was first reported by Ho et al. [2]. First, a PVA film containing polymer nanoparticles was prepared by blending spherical poly (St) particles and an aqueous PVA solution and casting the suspension on a substrate heated to a temperature higher than the glass transition temperatures of poly (St) and PVA. Then, uniaxial stretching was performed to deform the particles. Thereafter, by dissolution of PVA, rugby ball-shaped irregular particles were obtained. By changing the stretching ratio of the film during uniaxial stretching, it was possible to produce irregular-shaped particles of various aspect ratios.

Figure 3. Physical stretching for preparation of heteromorphic polymer nanoparticles

Figure 4 shows the SEM image of the polymer nanoparticles with a heterogeneous morphology and the aspect-ratio distribution chart. It can be seen that every polymer nanoparticle attained a heterogeneous morphology due to physical stretching. Although the original spherical particle size
was 130 nm, the particles assumed morphology with a long axis of about 210 nm and a minor axis of about 100 nm. Thus, the aspect ratio of the heterogeneous polymer nanoparticles was 2.1 and the distribution was not so broad, between 1.5 and 2.5.

Figure 4. SEM image of polymer nanoparticles with heterogeneous morphology and aspect-ratio distribution chart

3.3. Rotational movement of heteromorphic polymer nanoparticles in response to external magnetic field

Figure 5 shows a representative example of the rotational movement of the heteromorphic polymer nanoparticles. The heteromorphic polymer nanoparticles demonstrated a rotational motion when a rotating magnetic field was applied to the suspension of the polymer particles on the magnetic tweezers. The rotational motion continued throughout the magnetic field application period and stopped after the magnetic field was removed. That is, the rotational movement of the polymer nanoparticles was due to the response to the magnetic force generated by the iron oxide particles present in the nanoparticles, and not a Brownian motion of the colloidal particles. The rotational rate of the polymer particles almost agreed with that of the external magnetic field, confirming the good magnetic responsiveness of the polymer particles. As shown in Figure 6, the rotational motion of the heteromorphic polymer nanoparticles corresponded well to the rotation rate of the external magnetic field. That is, when the rotation rate was 5 Hz, the rotation of the heteromorphic polymer nanoparticles was almost 5 Hz and the correlation factor was 1.0. A low rotation rate of 2 Hz induced slow rotation of the heteromorphic polymer nanoparticles. The polymer nanoparticles also followed the rotation very well.

We believe that the heteromorphic polymer nanoparticles can be used like a magnetic stir bar in living cells after much surface modification for application in cells.

Figure 5. Rotational motion of heteromorphic polymer nanoparticles under 5 Hz rotational magnetic field. Scale bar: 500 nm
Figure 6. Accumulated rotation number of heteromorphic polymer nanoparticles with application time of rotational magnetic field having various rotation rates

4. Conclusion
We successfully prepared polymer nanoparticles containing iron particles with good responsiveness to external rotational magnetic fields. Also, the morphology of the nanoparticles was altered to be heterogeneous, that is, a rugby ball-shaped morphology. The responsiveness of the heteromorphic polymer nanoparticles to an external magnetic field was sufficient to allow rotational motion.

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6. References
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