**Nano-sized Magnetic Stirring Device For Intracellular Control of Cell Functions**

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**Statement of Purpose:** Cells have been frequently used as a material for medical supplies with the development of regenerative medicine. Thus, it is important to provide uniform cells as a product. Therefore, the quantitative description of properties of the intracellular environment and active control of its functions are essential. Due to the uniqueness and complexity of the intracellular environment, it is difficult to construct the similar environment *in vitro*. For these reasons, the direct analysis of the intracellular environment is required. Cells are starting to be considered and evaluated as a field of micro chemical reactions upon the quantitative description of the cell properties. There are few tools or methods to conduct such direct analysis. Active control of cell functions based on the behavior of molecules involved in intracellular reactions has not been reported yet. Therefore, it is necessary to design and develop suitable tools in terms of the active control. The purpose of this study is to develop an intracellular magnetic stirrer. It is consisted of the $Fe_3O_4$-encapsulated anisotropic polymer nanoparticles covered by phospholipid polymer. This is a new intracellular device, which rotates in the cell and enables the intracellular direct analysis and control cell functions.

**Methods:** $Fe_3O_4$-encapsulated polystyrene (PSt) spherical nanoparticles having the rhodamine units inside them were synthesized by soap-free polymerization. The physical anisotropy was given to the nanoparticles by stretching the nanoparticles-embedded poly(vinyl alcohol) (PVA) film at 160°C which is above the glass transition temperature of PSt (102°C) and PVA (85°C). The initiator groups for atom transfer radical polymerization (ATRP) were introduced at the surface of the nanoparticles by seed emulsion polymerization of the methacrylate with the initiator group in the side chain. Poly(methacryoyloxylethyl trimethyl ammonium chloride (TMAEMA)-*block*- 2-methacryloyloxyethyl phosphorylcholine (MPC)) (PT*b*M-90-10) chains were grafted at the surface of the nanoparticles by surface-initiated ATRP. The numbers after PT*b*M represent the polymerization degree of TMAEMA and MPC. First, poly(TMAEMA) chains were grafted from the surface of the nanoparticles, then poly(MPC) chains were extended from the edge of the poly(TMAEMA) chains. The nanoparticles with the poly(MPC) chains (PT*b*M-0-100) were also synthesized. Human epithelial carcinoma (HeLa) cells were cultured in the medium containing 20 μg/mL nanoparticles for 2 h and observed by the confocal microscopy.

**Results:** The nanoparticles had the diameter of 150 nm and could be collected by neodymium magnet.

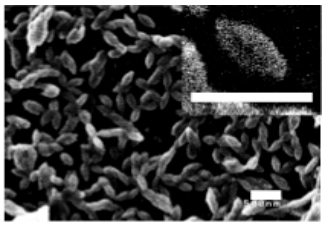

Fig. 1 SEM image of anisotropic magnetic polymer particles. (Scale bar : 500 nm)

The magnetic responsiveness was given to the nanoparticles successfully. After stretching, the major axis was enlarged to about 350 nm and the minor axis was shortened to about 130 nm (Fig. 1). The average aspect ratio of the particle calculated from the scanning electron microscope (SEM) image was 2.6 ± 0.5. The physical anisotropy was successfully given to the nanoparticles, and this would lead to improve the stirring efficiency. The nanoparticles with PT*b*M-0-100 did not enter the cell, while the nanoparticles with PT*b*M-90-10 chains showed cellular uptake (Fig. 2). It is due to the cationic property of TMAEMA interacted with the cell membrane. Furthermore, the nanoparticles with PT*b*M-90-10 inside the cells did not affect the morphology and proliferation of the cells. It is due to the cytocompatibility of MPC moiety at the outer layer of the nanoparticles. Thus, the ability to enter the cell and the cytocompatibility were both given to the nanoparticles by coating with zwitterionic polymers and cationic polymers on its surface.

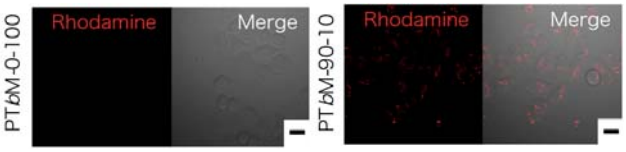


Fig. 2 Confocal microscopic images of HeLa cells incubated for 2 h in Dulbecco's modified Eagle's medium (DMEM) including 20 μg/mL particles after rinse with phosphate buffered saline (PBS). (Scale bar : 20 μm)

**Conclusions:** The methodology to fabricate the anisotropic magnetic nanoparticles with an ability to enter the cell nontoxically was established. Applying the magnetic field as an external stimulus by magnetic tweezers, the movement of the nanoparticles can be controlled non-invasively and remotely. This enables the quantitative analysis of the intracellular environment and the control its functions in the cells such as viscosity measurement, endosomal escape, and acceleration of diffusion speed of DNA or RNA.

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