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Molecular integration on phospholipid polymer-coated magnetic beads for gene expression analysis in cells

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ABSTRACT

Magnetic polymer beads, composed of a polystyrene core and hydrophilic poly(2-methacryloyloxyethyl phosphorylcholine (MPC)) graft layer, containing magnetic nanoparticles were prepared for analyzing genes in the cells. The initiator group for atom transfer radical polymerization (ATRP) was provided on the surface of the beads and polymerization of MPC created the poly(MPC) graft layer. The terminal bromine group of poly(MPC) chains converted to a reactive group. Streptavidin was immobilized using the terminal reactive group in the poly (MPC) chains for capturing the biotinated DNA primer of the polymerase chain reaction (PCR). After selective binding of three kinds of messenger RNA from the cell lysate, PCR was carried out to increase complementary DNA. The polymer beads were stable even under the PCR thermal cycling conditions, and dispersed again easily. The magnetic polymer beads can be candidate solid support for PCR of cell lysates.

1. Introduction

Recently, various functional particles have been developed for biological and biomedical applications [1–4]. In the biomedical analysis field, the purification and concentration processes are very important to realize high signal/noise ratio analysis. Magnetic particles with large surface area can be applied for the purification of biomolecules. Moreover, the magnetic particles can be easily collected from the reaction solution without using centrifugation, and thus, they had been receiving attention for inclusion in the automation process of biomolecule analysis [5,6]. Furthermore, the magnetic particles are advantageous because they can be used repeatedly for various bioanalytical processes as the desired washing buffer can easily wash them. In particular, when polymerase chain reaction (PCR) is performed using magnetic particles to amplify a specific gene region, the particles should have good dispersion stability against repeated thermal cycles during the reaction and resistance to non-specific adsorption of biomolecules for achieving excellent isolation, purification, and concentration. During the PCR process, the advantage of constructing complementary DNA (cDNA) libraries on the magnetic particles is considered to allow multiple analyses of precious materials and small samples. These recovered cDNA libraries can be used for further gene expression analysis anytime in the future if necessary. Although the construction of cDNA libraries is a fundamental step in most molecular biology techniques, the protocol is very complicated. In general, extraction and purification are important steps for high-efficiency PCR. Therefore, the magnetic particle system is very useful because it does not require a centrifugation step. In addition, the solid phase approach using magnetic particles is greatly favorable as they can be used repeatedly to quantify even small amounts of DNA because of the easy of separation of the solid phase from the reaction solution [7].

Magnetic particles covered with functional polymers for obtaining multifunctional particles have been reported [8–10]. In particular, magnetic particles covered with hydrophilic polymers can be easily manipulated by an external magnetic field and have good dispersion in aqueous medium [3,4,11,12]. For example, magnetic nanoparticles can be embedded in poly(2-hydroxyethyl methacrylate (HEMA)) microbeads crosslinked with ethylene glycol dimethacrylate [13,14]. Given that the surface properties of poly(HEMA) are not ideal (e.g., its lack of hydrophilicity, non-specific binding of biomolecules, and induction of complement activation), considerable functionalization of the surface is strongly recommended [15–17]. Another important surface design for magnetic nanoparticles is direct grafting of certain water-soluble polymers on their surface [18,19]. One of the candidate methods to construct precisely designed polymer chains grafted on the surface, namely surface-initiated atom transfer radical polymerization (ATRP), has been widely considered after pretreatment of the surfaces with the ATRP initiator [14,20–23]. ATRP has advantages such as control of molecular weight, narrow polydispersivity, easy synthesis of block copolymer, and preparation of high-density polymer brush under

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mild conditions [24–29]. The monomers used in these studies are HEMA, acrylic acid, and glycidyl methacrylate. This polymerization process can be applied to modify the surface of nanoparticles.

Previously, we have demonstrated the synthesis of poly(MPC) chains on the surface of magnetic ferric oxide particles by ATRP with an immobilized initiator that included a bromine (Br) atom in the ATRP moiety [30]. Poly(MPC) is water soluble because of the extremely hydrophilic nature of the MPC unit, and the properties of the poly(MPC) do not depend on biological conditions such as salt concentrations, pH, and temperature [31,32]. In this state, the poly(MPC)-grafted magnetic ferric oxide particles cannot be used as adsorbents for a specific protein or for collection using a magnetic field because the poly(MPC) chains surrounding the nanoparticles suppress spontaneous protein adsorption. To introduce bioaffinity toward the protein, *p*-nitrophenyloxycarbonyl poly(ethylene glycol) methacrylate (MEONP), which has an active ester group, was polymerized as a binding block segment [33–36]. The poly(MPC-*block*-MEONP)-grafted magnetic ferric oxide particles could immobilize proteins through a reaction between the active ester group and the amino group of the protein. However, on careful consideration, this immobilization method was not ideal for capturing proteins because the immobilization occurred on the side chain and not on the outermost surface. Hence, we focused on the Br atom located on the end group of the poly(MPC) brush, which was probably located on the outermost surface of polymer beads [37–39]. Click chemistry based on the Huisgen cycloaddition reaction between an azide and alkyne group is the best-known method to introduce a functional group [40,41]. At first, the Br atom was converted to an azide group using sodium azide. The purification of magnetic nanoparticles with terminal group-functionalized poly(MPC) chains was easily achieved using an external magnetic interaction.

In this study, we embedded magnetic nanoparticles in the core of polymer beads and then grafted poly(MPC) chains onto the surface of the beads. To allow molecular integration on the surface, the functional group at the terminus of the poly(MPC) chains was then sequentially converted. Finally, we evaluated the performance of the beads in capturing DNA from a cell lysis solution and then amplified the DNA by PCR.

2. Experimental

2.1. Materials

MPC was purchased from NOF Co. (Tokyo, Japan), where it was synthesized using a previously reported procedure [42]. Styrene (St), divinylbenzene (DVB), HEMA, poly(vinylpyrrolidone) (PVP), lauryl peroxide (LPO), 2, 2′-azobisisobutyronitrile (AIBN), 2-bromoisobutyryl bromide (BIBB), ethyl 2-bromoisobutyrate (EBIB), potassium persulfate (KPS), and sodium dodecylbenzene sulfonate (SDBS) were commercially available reagents. Hydrophobic coated Fe_3O_4 magnetic nanoparticles (10 nm in diameter) (EMG1400) were purchased from FerroTec Co. (Tokyo, Japan). Other reagents and solvents were of extrapure grade and used without further purifications.

2.2. Preparation of magnetic poly(St-co-DVB) core beads

Poly(St-*co*-DVB) (PS) core beads containing magnetic nanoparticles were prepared by a miniemulsion polymerization procedure [43]. In 50 mL of deionized water, PVP (1.25 mg), NaCl (2.5 g), and $NaNO_2$ (5.0 mg) were dissolved to form an aqueous phase. St (3.0 g), DVB (150 mg), and LPO (60 mg) were mixed to form an organic phase, and then 1.0 g of magnetic nanoparticles was dispersed in the mixture by sonication in an ice bath. The two mixtures were mixed with gentle shaking and then emulsified using a probe-type sonicator for 3 min. The obtained emulsion was transferred to a 100-mL two-neck flask equipped with stainless steel paddles and heated at 70 °C with stirring at 300 rpm for 20 h. The resulting magnetic polymer beads were collected by centrifugation at 9000 rpm for 10 min and separated by filtration using stainless steel meshes of 150 μm pore size and then 20 μm pore size, before being resuspended in ethanol. Finally, the suspension was centrifuged at 5000 rpm for 5 min. The precipitated polymer beads were collected by filtration and washed three times with ethanol.

2.3. Surface modification of magnetic core beads with the ATRP initiator

Seeded emulsion polymerization was used to coat the magnetic bead surface with the ATRP initiator. At first, a monomer with the ATRP initiator moiety 2-(2-bromoisobutyryloxy)ethyl methacrylate (BIEM) was synthesized by a condensation reaction between HEMA and BIBB [44,45]. PS beads containing the magnetic nanoparticles (1.0 g) were suspended in 100 mL of aqueous solution containing 1.0 mg/mL SDBS and transferred to a 200-mL three-necked flask equipped with Teflon paddles. BIEM (500 mg) and DVB (100 mg) were added dropwise to the suspension and stirred at 200 rpm for 2 h to adsorb monomers onto the core beads. Then, KPS (30 mg) was added to the suspension, and the polymerization reaction was carried out with stirring for 16 h at 70 °C. The obtained beads (PS-Br beads) were centrifuged and sufficiently washed with water and methanol.

2.4. Grafting of poly(MPC) chains onto PS-Br beads by surface-initiated ATRP

Grafting of poly(MPC) was performed using surface-initiated ATRP (SI-ATRP) [18,26,46]. The polymerization degree of the MPC unit was controlled at 100. That is, 2.0 mL of methanol containing MPC (2.1 g) was used. Then, Cu(I)Br (10 mg) and 2,2′-bipyridyl (22 mg) were added to the solution under Ar gas flow. To this solution, 5.0 mL of methanol solution containing 200 mg of PS-BIEM beads was added, and then Ar gas was bubbled through for 30 min to remove oxygen. EBIB (14 mg) was added to the solution, which was again bubbled with Ar gas for 5 min; the tube was sealed carefully and rotated using a rotary mixer for 24 h at room temperature. After the reaction, the magnetic beads covered with poly(MPC) chains (PS-PMPC-Br[100]) were centrifuged and washed three times with methanol. The conversion of MPC, determined by a ^{1}H NMR (JEOL JNM-GX270, Tokyo, Japan) analysis, was > 95%. PS-PMPC-Br(10), for which the polymerization degree of the MPC units was 10, was prepared using the same procedure.

2.5. Introduction of azide group into the terminus of poly(MPC) chains

Br atoms at the termini of poly(MPC) chains were converted to azide groups as follows [30]. Sodium azide (130 mg) was dissolved in 20 mL of methanol/acetonitrile mixture (50/50 vol%), and the PS-PMPC-Br (200 mg) beads were suspended in the solution. The reaction mixture was rotated for 24 h at room temperature. The beads (PS-PMPC-N_3) were collected by centrifugation, filtration, and washing three times with water.

2.6. Cycloaddition reaction between PS-PMPC-Br beads and carboxylated alkyne

Azide groups at the termini of the polymer chains were reacted with alkyne compounds using the Huisgen cycloaddition reaction [30]. PS-PMPC-N_3 (100 mg) was dispersed in 20 mL of water/ethanol mixture (50/50 vol%) along with $CuSO_4{\cdot}5H_2O$ (5.0 mg), sodium L-ascorbate (40 mg), and 4-pentynoic acid (98 mg). The reaction mixture was rotated for 24 h at room temperature. The beads (PS-PMPC-COOH) were collected by centrifugation, filtration, and washing three times with water. To remove any residual Cu catalyst, the beads were dispersed in 0.10 M *N*, *N*, *N′*, *N′*-ethylenediaminetetraacetic acid (EDTA) disodium salt aqueous solution and rotated overnight, followed by centrifugation and washing with 1.0 mM HCl aqueous solution and water. The density