Synthesis fluorescent magnetic nanoparticles in a microchannel using the La Mer process and the characterization of their properties

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Abstract This study presents a stable and controllable synthesis of fluorescent magnetic nanoparticles (FMNs) in a flow-through microchannel for the bi-modal use of magnetic activated cells sorting and fluorescence-activated cell sorters. The La Mer process is carried out to synthesize magnetic nanoparticles using co-precipitation. Then, the magnetic nanoparticles are coated with conjugation of chitosan and fluorescent isothiocyanate with two different concentrations. The chemical composition of the magnetic nanoparticles is determined by comparing the standard X-ray diffraction peaks of Fe3O4, and their sizes are also examined by using field emission scanning electron microscopy and dynamic light scattering measurement. The magnetic property of saturation magnetization and coercive field is characterized in a vibrating sample magnetometer. Also, the possibility of external manipulation in the synthesis of the magnetic particles is demonstrated by separating the synthesized fluorescent magnetic nanoparticles into a non-reacting laminar flow. Finally, their fluorescence property is determined by measuring the fluorescence adsorption spectra and the photoluminescence emission spectra in UV–Vis spectroscopy.

Introduction

Recent developments in chemistry, material science, and biology have allowed the generation of a range of novel nanoparticles targeted at applications in the fields of medical science, information technology, and environmental science [1]. An area of intense development has been magnetic iron oxide nanoparticles (MNPs) for use in medicine [2]. The MNPs have been designed to facilitate in vitro and in vivo drug delivery, magnetic resonance imaging (MRI), anticancer treatment, cell treatment, and diagnostic applications [3–6].

For in vitro applications, the magnetic property of MNPs has been used as an important characteristic in biosensing [7]. The binding of MNPs to targeted cells is rapid, and MNPs-bound cells can be labeled quantitatively without the influence of nanoparticles on optical properties [8]. MNPs bound to the surface of cells can be also used to manipulate the kinematic movement of cells by controlling the direction and intensity of the magnetic field [9]. In addition, MNPs do not aggregate even when exposed to a magnetic field and are easily sterilized [10]. These advantages have been commercialized to isolate specific cells as an in vitro platform, known as magnetic activated cells sorting (MACS) [11].

The fluorescence property of nanoparticles has been widely studied to isolate specific eukaryotic cells from the complex cell mixture by labeling the cells of interest with fluorescent dyes [12]. The process is carried out in specifically designed equipment, called as fluorescence-activated cell sorters (FACSs). However, FACS is limited by relatively small separation capacity with a sorting population of ~107 cells per hour [9]. The method has further disadvantages of low sensitivity, poor quality of separation, loss of labeled cells, a large amount of reagent, and long process time [13].
M. Geens et al. investigated a combined MACS and FACS approach to remove cancer cells from urine and human testicular cell suspensions. The series of MACS and FACS procedure enriched the proportion of spermatogonial cells while reducing the number of cancer cells. Spermatogonia cells increased from 3.94 to 76.55 %, and the cancer cells were reduced from 10.35 to 0.39 % [14]. The cell sorting using both MACS and FACS can be a promising protocol to enrich and to decontaminate cells, if suitable fluorescent magnetic nanoparticles (FMNPs) are prepared.

The development of the FMNPs is still at an early development stage so that the characterization of the materials is difficult and arbitrary [15]. Most of the MNPs have core–shell nanostructures coated with a polymer functionalized with a fluorescent dye. The polymer coating is advantageous because it makes the solid MNPs soft and can prevent oxidation. Also, it can improve biocompatibility [16, 17]. Recent developments to improve the biocompatibility of the fluorescent MNPs have used chitosan of a biocompatible compound modified with fluorophores to coat the MNPs [18].

The La Mer model of monodisperse collision growth explains major MNPs growth [19]. The method to prepare Fe3O4 nanoparticles includes wet chemical co-precipitation of ferrous and ferric ions with ammonia. An ammonia solution is slowly dropped into a stirred solution of ferrous and ferric ions. The Fe3O4 molecules are formed and grow into nanoparticles as nuclei aggregate with time [20]. The MNPs continuously increase in size at a rate linked to the initial precursor concentration. Thus, it is difficult to control the size of nanoparticles and uniformity.

In this paper, we present continuous and stable microfluidic synthesis of MNPs using a multilayered lamination flow in a microchannel. This method has a potential to control size and its distribution of MNPs by varying the inlet flow rates and chemical compositions. Also, by placing a permanent magnet near the reaction microchannel, the synthesized MNPs can be collected when they reach a target size. As a result, the control of size and distribution is possible. We also added fluorescence onto the MNPs by using the fluorescent compound of isothiocyanate (FITC) coated with chitosan (CS). The CS–FITC was functionalized on the surface of the MNPs. Finally, the synthesized FMNPs were characterized by examining their size, magnetic, and fluorescent properties.

Synthesis of FMNPs in microchannel

The reaction microchannel was fabricated by bonding a cast polymethylsiloxane (PDMS) and a transparent slide glass. A 100-μm-thick layer of the SU-8 (SU-8 3050, MicroChem) photosist was spin-coated onto a polished silicon wafer with the speed cycle ramping up to 500 rpm over 5 s and sequentially to 2000 rpm and was maintained for 40 s. Two stages of soft baking to evaporate the solvent and increase the density of the film were conducted at the condition of 3 min at 65 °C and 9 min at 95 °C in a baking oven. The SU-8 photosist was then patterned by UV exposure under the 1-line of 365 nm for 30 s and was cross-linked. Finally, the SU-8 patterned mold was prepared by developing in the SU-8 developer for 3 min and then rinsing with isopropanol alcohol. Especially, to reduce the agglomeration of nanoparticles on the microchannel wall, the cross-sectional size of the microchannel was designed to be 100-μm thick and 50-μm thick, which was the aspect ratio of 2:0. [21]

The PDMS pre-polymer was mixed with the curing agent at a volumetric ratio of 10:1. The mixture was then degassed in a vacuum, until air bubbles were removed completely. The PDMS was then poured onto the prepared SU-8 mold and cured at 65 °C for 30 min. The patterned PDMS was detached from the SU-8 mold. The PDMS layer was treated by oxygen plasma (Plasma cleaner PDC-32G, Harrick Plasma) and immediately brought in contact with the slide glass for bonding. The plasma power used was 10.5 W, while the chamber pressure was set to 200 mTorr. The plasma exposure was maintained for 2 min. Finally, the PDMS layer was bonded with the slide glass to form the reaction microchannel [21, 22].

For the co-precipitation of the MNPs, 2.705 g of FeCl3·6H2O and 0.995 g of FeCl2·4H2O were dissolved in a 100 ml dilute water to give a final concentration of 0.1 M FeCl3 and 0.05 M FeCl2, respectively. The FeCl3 was acidified by adding 10 ml of 0.1 M HCl to prevent the oxidation of FeCl3. The 5 % NH4OH solution was prepared from 30 % NH4OH. All solutions were stored at room temperature. Fluorescein isothiocyanate (FITC) of 1.28 mM concentration was produced by dissolving 5 mg of FITC in 50 ml ethanol and was covered to prevent bleaching. A 0.5 g chitosan was dissolved in 2 % acetic acid of 100 ml to obtain 0.5 % chitosan solution. Analytical grade PBS, 0.1 M HCl, and dichloromethane (DCM) were prepared, and all chemicals were purchased from Sigma Aldrich.

The MNPs were synthesized by using the co-precipitation method.

\[ 2\text{FeCl}_3 + \text{FeCl}_2 + \text{NH}_4\text{OH} \rightarrow \text{Fe}_3\text{O}_4 \downarrow + \text{NH}_4\text{Cl} \]

The prepared chemicals were introduced into three inlets, respectively, as shown in Fig 1. The FeCl3 and FeCl2 were mixed with a molar ratio of 2:1 and introduced into Inlet 1. The NH4OH solution was injected through Inlet 3. As soon as two reactants met, the synthesis of the magnetic nanoparticles was initiated rapidly on the
interfacial surface between the layers [23–25]. The synthesized magnetic nanoparticles appeared on the wall surface because zero velocity was imposed on the wall resulted from non-slip condition, and the fluidic force was insufficient to flush the produced magnetic nanoparticles attached to the wall. The aggregation of the MNPs on the wall surface occurred and eventually led to the blockage of the microchannel.

To prevent the attachment of the MNPs on the surface of the microchannel, the DCM of a non-polar organic solvent was also introduced to form finally a five layered laminar flow in the microchannel. The flow rate of the DCM through Inlet 2 was optimized to retard the explosive MNPs formation and to reduce the MNPs attachment to the wall surface. The optimum inlet flow rate of the DCM was 80 μl/min. The inlet flow rates of the mixture of FeCl₃ and FeCl₂ and NH₄OH were 15 and 15 μl/min, respectively. Also, in Fig. 1, the dark color in the microchannel indicates the formation of the MNPs, and serious aggregation to block the microchannel was not observed.

The CS–FITC was produced by reacting the thiocyanate groups of FITC with the primary amino group of chitosan. Figure 2 shows the structure of the CS–FITC molecule with the chemical bonding between (–S–C≡N) group of FITC and (–NH₂) group of CS. The reaction was carried out in a stirred flask in the dark. A 10 ml FITC/ethanol was added to a 50 ml solution of 0.5% chitosan in the 25°C acetic acid and stirred at a high speed of 1000 rpm for 10 min at room temperature. The reaction was kept overnight with stirring.

After functionalizing the chitosan with FITC, a 10% solution of the MNPs in water was added by incubating them for 5 h under stirring at room temperature to coat the surface of MNPs with CS–FITC. The FITC–CS–MNPs were separated from the unbound CS–FITC components by using a static magnet and washed several times by de-ionized water. The CS–FITC coating reaction was prepared by polyelectrolyte adsorption and complex formation processes [26, 27]. The MNPs were functionalized with the free –NH₂ and/or –COOH group of the CS–FITC bound to the surface of the MNPs [28]. The CS–FITC coating remained attached to the MNPs by the combination of electrostatic and steric attachment forces. In the experiment, by considering the ratio of the CS–FITC to MNPs, two samples of M1 and M2 were synthesized with the 5 and 10 ml of the CS–FITC solution to a 10 ml solution of MNPs.

Property characterization of FMNPs

Currently, in situ characterization tool has been developed to examine the growth of nano-scale materials. By using a simple multifluide device, the real time analysis of morphology and dimension-controlled growth of gold nanoand microstructures with a time resolution of 5 ns [29]. However, since the formation of the FMNPs occurred explosively, the MNPs and the FMNPs were collected at each preparation state, and they were characterized in the external instruments.

The chemical composition of the MNPs was determined by examining the X-ray diffraction (XRD) of the MNPs (D8 Advance diffractometer, Bruker) using CuKα (1.5406 Å) radiation at room temperature in the range of 20–90° with a scanning speed of 0.02°/min. Figure 3 shows the XRD pattern of the synthesized Fe₃O₄ nanoparticles. The diffraction peaks of (220), (311), (400), (511), and (440) were observed and could be indexed to a cubic spinel structure [30, 31]. The XRD peaks of the Fe₃O₄ were compared with the standard data in the ICDD file (PDF No. 01-076-2151). All peaks were in good agreement with the standard diffraction pattern of Fe₃O₄.

The mean diameters of the MNPs and the FMNPs of M1 and M2 were obtained using the image analysis program of the FESEM images (Fig. 4) and they were measured to be 35, 50, and 55 nm, respectively. The size of FMNPs of M1 and M2 was as large as the thickness of the CS–FITC layer.
compared with that of the MNP. In Fig. 5, the TEM images of M1 and M2 show the different thickness of the CS-FITC layer of M1 and M2 with 2 and 3 nm, respectively. However, the dynamic laser scattering (Model malvern ZEN 1600) measurement showed the increased sizes as shown in Fig. 4. The sizes of MNPs, M1, and M2 were measured to be 250, 200, and 180 nm, respectively. The size discrepancy between FESEM and DLS indicated the clustering of the nanoparticles in the solution. Although the CS-FITC modification introduced polydispersity, all samples of the MNPs, M1, and M2 were reasonably monodisperse.

Magnetic property of the MNPs, M1, M2 of CS-FITC/MNPs was characterized by using a vibrating sample magnetometer (VSM) with the physical property measurements system (PPMS) of Quantum Design. Saturation magnetization (Ms) and coercive field (He) measurements were carried out at room temperature, with a magnetic field in the range from −10000 to 10000 Oe as shown in Fig. 6. A magnetic hysteresis loop was not observed in the magnetization curve of the MNPs and CS-FITC/MNPs. The ferrofluid showed superparamagnetic behavior with the saturation magnetization of 69.86, 58.1, and 56.2 emu/g for the MNPs, M1, and M2, respectively. For the single-domain particles, there were no domain walls so that in the superparamagnetic state, the whole magnetic moment of the particle was in the same direction as in the paramagnetic material.

Also, after coating the MNPs with CS-FITC, the saturation magnetization decreased. The decrease of the saturation magnetization was most likely attributed to the existence of the coated materials on the surface of the MNPs, probably because the electronic structure of the outer layer of iron atoms was disrupted [32]. The decrease of saturation magnetization might depend on the thickness of the polymer shell or the amount of modified FITC on the surface of the MNPs in M1 and M2 samples due to the surface anisotropy upon coating and external morphology transformation [33].

The fluorescence property of the MNPs was determined by using UV–Vis spectroscopy (Model U-2900/2910, Hitachi). Figure 7a shows the fluorescence adsorption spectra of samples M1 and M2. It is likely that after coating the MNPs with CS-FITC, the core of magnetic nanoparticle could affect the fluorescent properties of the bound CS-FITC. In the absorbance spectra, there were 2 peaks at 300 and 495 nm wavelength.

PL spectra of the samples M1 and M2 were measured at the excitation wavelength of 300 and 495 nm. In Fig. 7b, we show the emission spectra at 365 and 525 nm, respectively, for the samples M1 and M2. The effect of the MNPs core to the CS-FITC shell did not change absorbance and emission features, but the amount of CS-FITC coated on the surface of MNPs affected the intensity of the MNPs. Coating the surface of the MNPs with a high amount of FITC of the M2 sample showed a large increase in the PL intensity. It seems to be evident for the high amount of FITC which was already conjugated with the MNPs through the CS polymer.

To examine the feasibility of collecting the MNPs during growing with reaction, we experimented by manipulating the MNPs in the flow-through microchannel. Pure DI water and the MNPs suspensions were introduced in inlet 1 and 2. Their inlet flow rates were 5 and 1 µl/min, respectively. A permanent magnet was placed near the upper wall of the microchannel. As shown in Fig. 8, the MNPs introduced along inlet 1 moved toward the upper channel because of the attraction force by the permanent magnet. Finally, most of the MNPs were collected.

Recently, the growth of the magnetic nanoparticles in a microreactor has been reported by using a droplet
Fig. 5 TEM images of the MNPs, M1, and M2.

Fig. 6 Magnetization curves of the MNPs and the FMNPs samples M1 and M2 prepared with different FITC concentrations.

Phase flow and a continuous flow due to the advantages of rapid and well-controlled mixing, reliable operation, minimum consumption of reagents, environmental friendliness, and so on. Droplet pairs from two spatially separated nozzles were generated with stable size control. These droplets contained the reagents for the precipitation of iron oxide and electrocoalesced to synthesize iron oxide nanoparticles [34]. The principle of forming the iron oxide nanoparticles used the coprecipitation reaction in a microchannel. In our study, the coprecipitation reaction of iron oxide was conducted with alkali on the interface of two laminar flows in the microchannel.

Also, a coaxial flow in a millichannel was used to synthesize the iron oxide nanoparticles. In the laminar coaxial flow, the interface was formed between a reagent of iron salts and OH⁻ and tetramethylammonium hydroxide (TMAOH) stream. On that interface, the coprecipitation reaction occurred. An excess of the TMAOH stream was used to improve the dispersion of nanoparticles after the synthesis and to avoid their adsorption on the channel wall [17]. In our study, the DCM was used to prevent the attachment of the MNPs on the inner surface of the microchannel because the DCM is a nonpolar solvent.
Fig. 7 Absorbance spectra and the PL emission spectra of FMNPs samples M1 and M2

(a) Absorbance spectra
(b) PL emission spectra

Fig. 8 External manipulation of the FMNPs flowing along the microchannel from bottom edge to top edge by the permanent magnet (a). The FMNPs positioned near the bottom edge of the microchannel at the inlet (b, c). Transverse movement of the FMNPs as they flow along the microchannel in the presence of the magnet (d). Most of the FMNPs moved toward the top edge of the microchannel at the outlet.

Conclusions

The FMNPs with a small size distribution have been stably synthesized in the multi-layered laminar flow by using the La Mer process. Based on the experimental evaluations, they have exhibited characteristic of the magnetic and fluorescent properties, which are required for the multimodal use of MACS and FACS. Also, the external manipulation of MFNPs flowing along the microchannel was carried out. They moved from the bottom edge to the top edge by the permanent magnet placed near the top edge of the microchannel. This implies that the magnetic nanoparticles can be fractionated when they are grown to a target size.

The microfluidic synthesis using the multi-layered laminar flow in the microchannel may offer a new solution of complex issues in chemistry and biology. Also, future work will focus on the feasibility test of the application of FMNPs to MACS and FACS to perform cell assays with high specificity and selectivity.

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