MICROFLUIDIC SYNTHESIS OF FLUORESCENT MAGNETONANOPARTICLES AND CHARACTERIZATION

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ABSTRACT

This study presents a stable and controllable synthesis of the fluorescent magnetic nanoparticles in a flow-through microchannel. La Mer process is carried out to synthesize the magnetic nanoparticles using co-precipitation. Then, the magnetic nanoparticles are coated with conjugation of chitosan and fluorescent isothiocianate. The chemical composition of the magnetic nanoparticles and their sizes are determined. The magnetic property of saturation magnetization and coercive field is characterized. Also, the possibility of external manipulation by separating the synthesized fluorescent magnetic nanoparticles into non-reacting lamination flow is demonstrated. Finally, their fluorescence property is determined.

KEYWORDS

Fluorescent, Magnetic, Nanoparticles, Synthesis, Microfluidic.

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]. MNPs have been designed to facilitate in vitro and in vivo drug delivery, magnetic resonance imaging (MRI), anticancer treatment, cell treatment and in vitro diagnostic applications [3].

For in vitro applications, the magnetic property of the nanoparticles has been used as an important component of many biosensors [4]. Binding of MNPS to targeted cells is rapid and the cells treated by MNPs can be labeled quantitatively without changing the optical properties of the nanoparticles [5]. In addition, MNPs do not aggregate even when exposed to a magnetic field, and are easily sterilized. 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 external magnetic fields. These advantages allow to isolate specific cells and have been commercialized as an in vitro platform, known as magnetic activated cells sorting (MACS) [6-8].

Fluorescence property of nanoparticles has been widely studied to isolate specific eukaryotic cell from complex cell mixtures by labeling the cells of interest with fluorescent dyes [9]. This process is carried out in specifically designed equipment, fluorescence-activated cell sorters (FACSs). However, the use of FACS is limited by relatively small separation capacity, sorting population of approximately $l0^7$ cells per hour and instrutment [6]. The method has further disadvantages, including low sensitivity, poor quality of separation, loss of the labeled cells, and amount of reagent and time required [10].

M. Geens et al. investigated a combined MACS/FACS approach to remove cancer cells from murine and human testicular cell suspensions. The series of MACS & FACS procedures enriched the proportion of spermatogonial cells while reducing the number of cancer cells. Spermatogonia cells increased from 3.94 to 76.55% while the cancer cells were reduced from 10.35% to 0.39% [11]. The cell sorting by MACS and/or FACS may be a promising solution to obtain both enrichment and decontamination .

The development of fluorescent magnetic nanocomposites is still at an early developmental stage so that the characterization of the materials is difficult and arbitrary to inconsistencies [12]. Most fluorescent magnetic nanocomposites are core-shell nanostructures coated with a polymer functionalized with a fluorescent dye. The polymer coating is advantageous because it makes the solid MNPs soft, prevents oxidation, and can improve biocompatibility [13]. Recent developments to improve biocompatibility of the fluorescent MNPs have used chitosan of a biocompatible compound modified with a fluorophore to coat the MNPs [14].

The La Mer model of monodisperse collision growth explains major MNPs growth [15]. The method to prepare Fe_3O_4 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 Fe_3O_4 molecules are formed and grow into nanoparticles as nuclei aggregate with time [16]. 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 at 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) coupled with Chitosan (CS). The CS-FITC was functionalized on the surface of the MNPs. Finally, the synthesized fluorescent magnetic nanoparticles (FMNPs) were characterized by examining their size, magnetic and fluorescent properties.

MICROFLUIDIC SYNTHESIS OF FMNPS

Fabrication of the Reaction Microchannel

The microfluidic devices were fabricated by bonding the cast Polydimethylsiloxance (PDMS), and a transparent slide glass. A 100 μm-thick layer of the SU-8 (SU-8 2050,

MicroChem) photoresist was spin-coated onto a polished silicon wafer with the spread cycle ramping up to 500 rpm over five seconds and sequentially to 2000 rpm and was maintained for 30 seconds. Two stages of soft baking to evaporate the solvent and increase the density of the film were conducted at the condition of three minutes at 65 ºC and nine minutes at 95 ºC in a baking oven. The SU-8 photoresist was then patterned by UV exposure under I-line of 365 nm for 30 seconds and was cross-linked. Finally, the SU-8 patterned mold was prepared by developing in the SU-8 developer for three minutes and then rinsing with isopropyl alcohol.

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 ºC 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 two minutes. Finally, the PDMS layer was bonded with the slide glass to form the reaction microchannel.

Preparation of Chemicals

For co-precipitation of MNPs, 2.705 g of $FeCl_3.6H_2O$, and 0.995g of $FeCl_2.H_2O$ was dissolved in a 100 ml dilute water to give a final concentration of 0.1M $FeCl_3$ and 0.05M $FeCl_2$, respectively. The $FeCl_2$ was acidified by adding 10 ml of 0.1M HCl to prevent the oxidization of $FeCl_2$. The 5% NH_4OH solution was prepared from 30% NH_4OH. All solutions were stored at room temperature. Fluorescein isothiocyanate (FITC) of 1.28 mM concentration was produced by dissolving 5 mg of FITC in a 10 ml ethanol and was covered to prevent bleaching. A 0.5 g of Chitosan was dissolved in a 2% acetic acid of 100 ml to obtain a 0.5% Chitosan solution. Analytical grade PBS, 0.1M HCl, ethanol, and dichloromethane (DCM) were prepared and all chemicals were purchased from Sigma Aldrich.

Synthesis of Magnetic Nanoparticles (MNPs)

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

$$2FeCl_3 + FeCl_2 + NH_4OH = Fe_3O_4 + NH_4Cl$$

The $FeCl_3$ and $FeCl_2$ were mixed with molar ratio of 2:1 and were reacted with the NH_4OH solution in the microchannel. The chemicals were introduced into three inlets, respectively and formed a strong five layered lamination flow as shown in Fig.1A. As soon as the reactants were introduced, the synthesis of the magnetic nanoparticles was initiated on the interfacial surface between the layers. The continuous MNPs formation in the interface formed between the reactant solutions was obtained and the MNPs grew.

The synthesized magnetic nanoparticles appeared on the wall surface because of the zero velocity on the wall under no-slip condition. The fluidic force was insufficient to flush the magnetic nanoparticles attached on the wall and aggregation occurred from the wall surface. This growth of MNPs on the wall surface eventually led to blocking of the microchannel. To prevent the attachment of the MNPs on the surface wall of microchannel, dichloromethane (DCM) as a non-polar organic solvent would create additional layer on the wall surface of the microchannel. The flow rate of the DCM in the middle of microchannel was optimized to retard the rapid diffusion of the MNPs formation and to prevent the MNPs attachment to the wall surface. The optimum inlet flow rate of DCM was 80 µl/min. The inlet flow rates of $FeCl_3/FeCl_2$ through the inlet 2 and NH_4OH through the inlet 3 were 15 and 15 µl/min, respectively. Fig.1B shows the stable lamination flow in the microchannel with the synthesis reaction occurring at the interface of two laminar flows. The dark color observed in Fig. 1B indicated the formation of the MNPs.

Synthesis of Fluorescent Magnetic Nanoparticles (FMNPs)

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 ($-NH_2$) 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 2% acetic acid and stirred at 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 dilute water was added by incubating them for 5 hours under stirring at room temperature to coat surface of MNPs with CS-FITC. The FITC-CS-MNPs were separated from the unbound CS-FITC components by using static magnet and washing them several times in de-ionized water. The CS-FITC coating reaction was prepared by polyelectrolyte adsorption and complex formation processes. Magnetic core–shell particles were functionalized with free $-NH_2$ and/or –COOH group of the CS-FITC bound to the surface of MNPs. The CS-FITC coating remained attached to the MNPs by the combination of electrostatic and steric attachment forces. By considering the ratio of the CS-FITC to MNPs, two samples of M1 and M2 were synthesized with the 5 ml and 10 ml of the CS-FITC solution to a 10 ml solution of MNPs.

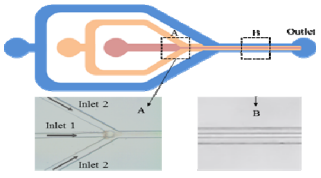

Figure 1: Five layered laminar flow formed to synthesize MNPs in the flow-through microchannel.

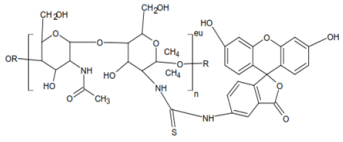

Figure 2: Molecule structure of the CS-FITC.

CHARACTERIZATION OF FMNPS

The chemical composition of the MNPs was determined by examining the X-ray diffraction (XRD) of MNPs (Bruker D8 Advance diffractometer) using CuKα (1.5406 Å) radiation at room temperature in the range of 20 to 90° in the 2θ scale, with a scanning speed of 0.02°/min. Fig. 3 shows the XRD pattern of Fe_3O_4 nanoparticles. The XRD peaks of the Fe_3O_4 were compared with those of standard one in JCPDS file (PDF No. 65-3107) The diffraction peaks of (220), (311), (400), (511), and (440) were observed and could be indexed to a cubic spinal structure. All peaks were in good agreement with the known standard diffraction pattern of Fe_3O_4.

The mean diameters of MNPs, and FMNPs of M1 and M2 were obtained using the image analysis program of the FESEM image and they were 35, 50 and 55 nm, respectively. The size of FMNPs of M1 and M2 was large as much as thickness of the CS-FITC layer.

The DLS measurement showed the increased sizes as shown in Fig. 4. The sizes of MNPs, M1 and M2 were measured to be 250 nm, 200 nm 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 step introduced polydispersity, all the samples of MNPs, M1 and M2 were reasonably monodisperse.

Magnetic property of MNPs, M1 and 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 (Hc) measurements were carried out at room temperature, with a magnetic field in the range of -10000 to 10000 Oe as shown in Fig 5. A magnetic hysteresis loop was not observed in the magnetization curve of MNPs and CS-FITC/MNPs. Ferrofluid showed superparamagnetic behavior with saturation magnetization of 69.86 emu/g, 58.1 emu/g and 56.2 emu/g for MNPs, M1 and M2, respectively. For 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 MNPs with CS-FITC, the saturation magnetization decreased. The decrease of the saturation magnetization was most likely attributed to the existence of coated materials on the surface of MNPs, probably because the electronic structure of the outer layer of iron atoms was disrupted. The decrease of saturation magnetization might depend on the thickness of the polymer shell or the amount of modified polymer on the surface of MNPs in M1 and M2 samples due to the surface anisotropy upon coating and external morphology transformation.

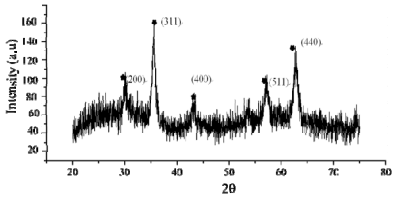

Figure 3: XRD spectra of MNPs.

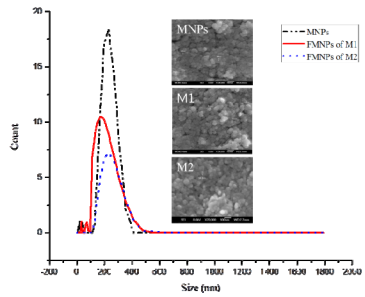

Figure 4: DLS spectra of MNPs, FMNPs samples of M1, and M2 and their FESEM images.

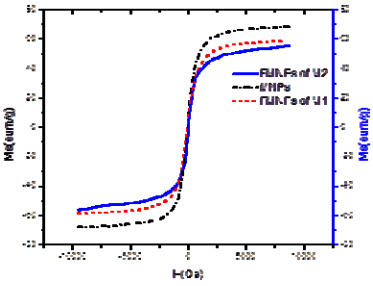

Figure 5: Magnetization curves of MNPs and FMNP samples of M1 and M2 prepared with different FITC concentrations.

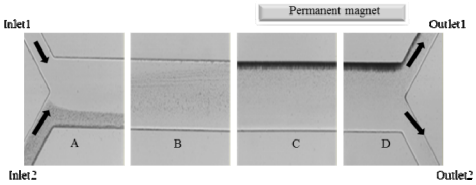

Figure 6: External manipulation of the FMNPs flowing along the microchannel from bottom edge to top edge by the permanent magnet; A. FMNPs positioned near the bottom edge of the microchannel at the inlet; B and C. Transverse movement of FMNPs as they flow along the microchannel in the presence of the magnet; D. Most of FMNPs moved toward the top edge of the microchannel at the outlet.

To collect the MNPs after the reaction completed, we examined the manipulation of FMNPs in the flow-through microchannel. DI water and CS-FITC/MNPs suspensions were introduced in inlet 1 and 2. Their inlet flow rates were 5 µl/min and 1 µl/min, respectively. A permanent magnet was placed near the upper wall. Figure 6 shows that the FMNPs infused through the inlet 1 moved toward the upper channel because of the attraction force by the permanent magnet. Finally, most of FMNPs were collected in the outlet 1(Fig. 6D).

The fluorescence property of the FMNPs was determined by using UV-VIS spectroscopy. Fig. 7A shows that the fluorescence adsorption spectra of samples M1 and M2. It is likely that after coating MNPs with CS-FITC, the effect of magnetic nanoparticle core could affect the fluorescent spectral properties of the bound FITC a. In absorbance spectra, there were 2 peaks at 300 and 495nm wavelength. For fluorescence studies samples were excited using a 300nm and 495nm laser. All the PL emission spectra (Fig. 7B) for the M1 and M2 were obtained by

fixing the excitation at 300 and 400 nm which were at 365 nm and 525 nm respectively. The effect of the MNPs core to CS-FITC shell does not change absorption or emission features but the amount of CS-FITC coated on the surface affects the emission intensity. Coating the surface of MNPs with high amount FITC in M2 shows a large increase in PL intensity (Fig. 7B). It seems to be an evident for high amount of FITC which already conjugated with MNPs through CS polymer.

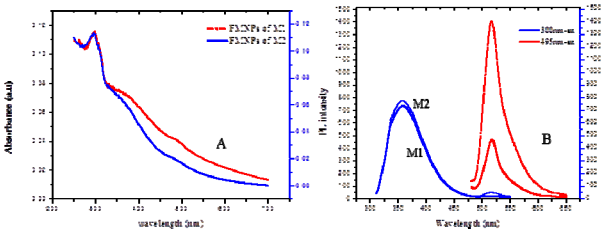

Figure 7: Absorbance spectra and the PL emission spectra of FMNP samples of M1 and M2

CONCLUSION

The fluorescent magnetic nanoparticles 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 characteristics of the magnetic and fluorescent properties, which are required for the bimodal use of MACS and FACS. Also, the external manipulation of FMNPs flowing along the microchannel was carried out. They moved from bottom edge to 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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