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Magnetic iron particles with high magnetization useful for immunoassay

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ABSTRACT

TiO_2-encapsulated metallic Fe particles (Ti–O/Fe) were synthesized through a solid phase reaction. The Ti–O/Fe particles were non-toxic to tumor cells in a cell viability assay. After silica coating using a sol–gel method, streptavidin was covalently bound onto the Ti–O/Fe particles. Thus produced HMMI particles showed higher magnetization (114 Am^2/kg) and a larger specific surface area (15 m^2/g) than conventional streptavidin-immobilized magnetite particles. The high magnetization allowed for rapid magnetic separation, while the additional large specific surface area improved the detection of the adiponectin antigen both in terms of extended detection range and higher assay speed.

1. Introduction

Magnetic particles have been used in a variety of biomedical applications such as DNA isolation, cell separation, magnetic hyperthermia, MRI contrast agents, and targeted drug delivery [1–4]. The magnetic components used in these applications are typically iron oxides (γ-Fe_2O_3, Fe_3O_4) because of their chemical stability and biocompatibility. One drawback of these iron oxides is their lower intrinsic magnetization compared to magnetic metal particles, such as Fe, Co, and Fe–Co alloys. Magnetic metal particles would thus be expected to perform better in biomedical applications because they can respond more rapidly to magnetic fields. Metal particles, however, oxidize easily, resulting in the deterioration of their high magnetization.

To counteract the corrosion (i.e., oxidation) problem in biologically important aqueous solutions and buffers, metal nanoparticles have been prepared with nanocoatings. In a first method, metallic Fe particles with polymer coatings were synthesized by a microwave plasma polymerization method [5]. In another method, the sol–gel process was employed to coat Fe particles with SiO_2 [6]. In a third method, carbon-encapsulated Fe particles were synthesized by an arc discharge method [7]. Although all these Fe particles were successfully coated, their resistance to corrosion in aqueous solutions has yet to be examined. Moreover, it is not known if Fe particles with nanocoatings are biocompatible. In previous work, we developed TiO_2-encapsulated metallic Fe particles (Ti–O/Fe), which exhibited high corrosion resistance in a chaotropic solution [8]. Here we investigate the biocompatibility of Ti–O/Fe particles and their performance in an immunoassay after immobilizing streptavidin onto their surfaces.

2. Experimental

2.1. Preparation of magnetic Ti–O/Fe particles

Iron particles encapsulated by titanium oxide (Ti–O/Fe) were obtained by annealing a mixture of α-Fe_2O_3 and TiC in N_2 [8]. The average particle size was 0.8 μm. The Ti–O/Fe particles were coated with silica through a conventional sol–gel method as detailed in Ref. [9].

2.2. Immobilization of streptavidin

Streptavidin was covalently coupled to the silica-coated Ti–O/Fe particles (HMMI particles) by first incubating 10 mg of the particles with 50 μl of succinic anhydride at 50 °C for 1 h under stirring. After driving a condensation reaction using 48 mg of *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide hydrochloride, the carboxylated Ti–O/Fe particles were incubated with 1000 ml of a 2 mg/ml streptavidin solution (Wako) at 25 °C for 4 h.

2.3. Characterization of magnetic particles

Particle size and size distribution were measured by means of a laser scattering analyzer (HOLIBA, LA-920) and confirmed by scanning electron microscopy (SEM). The specific surface area of the particles was measured by a Brunauer–Emmett–Teller (BET) method (Mountech, Macsorb Model-1201). Magnetic properties were measured by a vibrating sample magnetometer (VSM, Toei, VSM-5) at room temperature up to a field of 1.6 MA/m.

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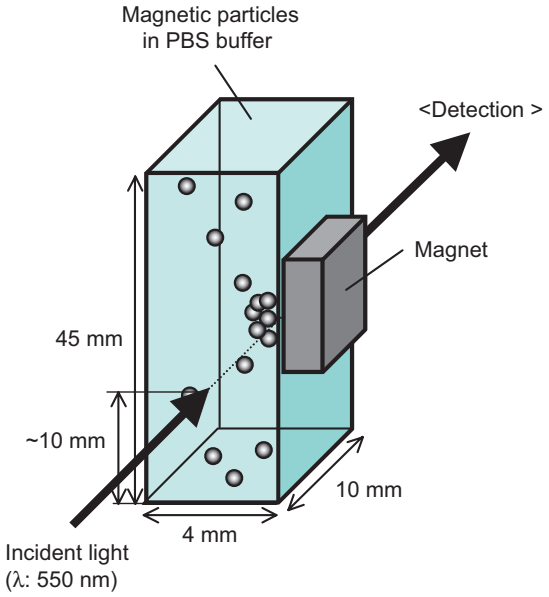

Fig. 1. Schematic diagram of magnetic separation test.

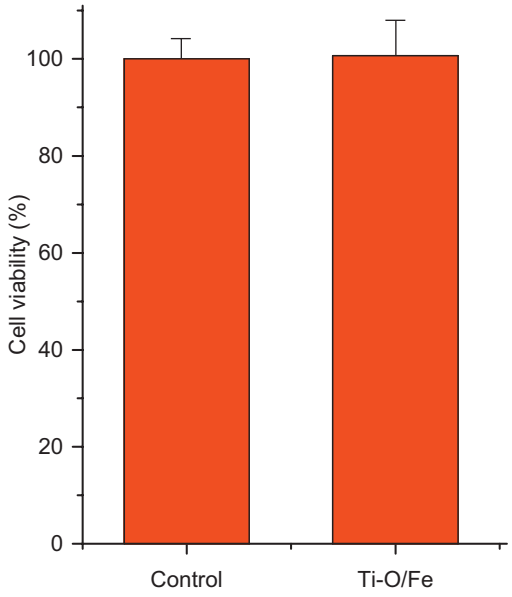

Fig. 2. Cell toxicity test of Ti–O/Fe particles determined in mesothelioma H226 cells.

2.4. Magnetic separation test

Magnetic particles were dispersed in phosphate buffered saline (PBS) at a concentration of 0.25 mg/ml. One milliliter of the suspension was placed in a 1.5 ml cuvette, a magnet attached to the cuvette wall, and the absorbance at 550 nm measured by UV–visible spectrophotometry (Hitachi High-Technologies, U0080D). The schematic diagram of the measurement setup is shown in Fig. 1.

2.5. MTT assay

The toxicity of Ti–O/Fe particles was evaluated with an MTT cell viability assay, which is a colorimetric method using 3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide. Mesothelioma H226 cells (20,000 cells/well) were incubated for 2 days with 0.38 mg of the Ti–O/Fe particles suspended in media. Twenty microliters of a 5 mg/ml MTT solution prepared in PBS was then added, and the particles incubated for 3 h. All media was removed, 200 μl of dimethyl sulfoxide (DMSO) added and shaken at 37 °C for 1 h. Finally, the optical density was measured at 540 nm on a Multiscan Ascent plate reader.

2.6. Immunoassay

In order to evaluate the antigen binding capacity of the HMMI particles, a colorimetric method was performed using an adiponectin ELISA kit (Otsuka). One hundred micrograms of the HMMI particles was used for each test. Commercially available streptavidin-coupled magnetic particles (Invitrogen, MyOne™; 1 μm, M280; 2.8 μm) were also used for comparison. After reaction of magnetic particles conjugated to adiponectin antibody with the adiponectin antigen, the binding was detected spectrophotometrically at 450 nm.

3. Results and discussions

Ti–O/Fe particles were not toxic to tumor cells, as seen in an MTT assay, even after the incubation of the rather large amount of 3.8 mg/ml of particles for 2 days (Fig. 2). The cell viability of tumor cells with added Ti–O/Fe particles was 100±5%, whereas the control cell viability without particles was 100±3%. This suggests that the Ti–O/Fe particles are biocompatible. The biocompatibility is very likely due to the inner Fe core of the particles being completely encapsulated by TiO_2, a material which is known to be biocompatible on its own [10]. Therefore, the Ti–O/Fe particles in this work are promising for biomedical applications.

Table 1
Physical properties of magnetic HMMI particles.

Sample	Average particle size D_{50} (μm)	Specific surface area (m^2/g)	Saturation magnetization Ms (Am^2/kg)
Silica-coated Ti–O/Fe	0.8	15	114
MyOne™	1.0	10	27
M280	2.8	6	12

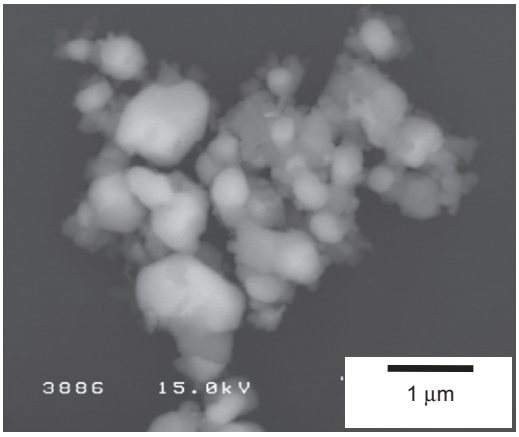

Fig. 3. SEM image of the Ti–O/Fe particles.

The average particle size (D_{50}) and specific surface area of the Ti–O/Fe particles after silica coating is shown in Table 1. SEM observation confirmed these size measurements (Fig. 3). The

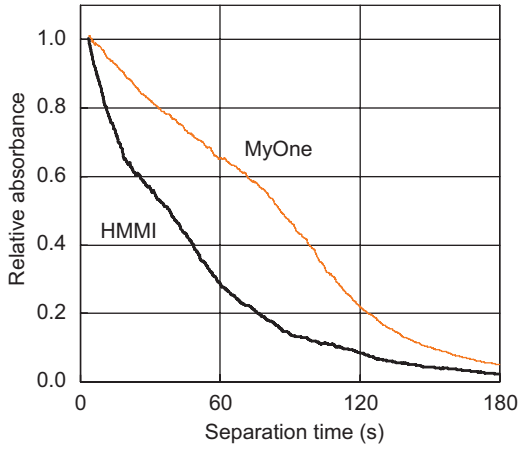

Fig. 4. Change in absorbance as a function of magnetic separation time.

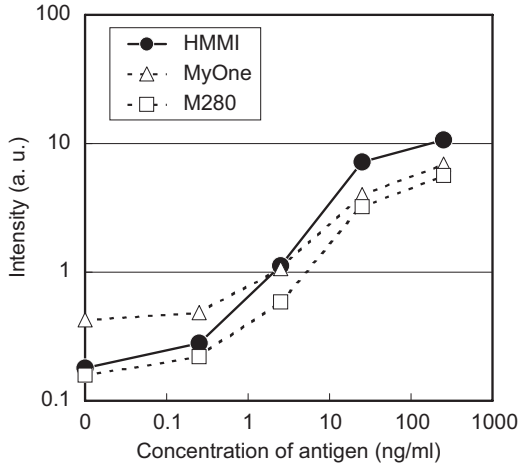

Fig. 5. Immunoassay sensitivity to antigen adiponectin.

particles are also smaller than and have specific surface areas 1.5 and 2.5 times greater than the conventional magnetic particles MyOne^{TM} and M280, respectively (15 m^2/g). The smaller size and larger surface area is advantageous for linker immobilization.

The saturation magnetization (*Ms*) of the silica-coated Ti–O/Fe particles was 114 Am^2/kg, which is 4–10 times higher than that of the conventional tested magnetic particles (Table 1). Such highly magnetic particles are very useful for an immunoassay, because faster separations and thus a faster assay become possible. This was shown nicely in Fig. 4, where the magnetic separation of HMMI particle suspensions was significantly faster than that of the conventional MyOne^{TM} particles, confirming the higher magnetization of the HMMI particles.

The immunoassay sensitivity to detect the adiponectin antigen using different magnetic particles is shown in Fig. 5. The background reading (i.e., the intensity without adiponectin) of the HMMI particles was lower than the one of MyOne^{TM}, and similar to the one of M280. HMMI particles thus show low non-specificity, and will thus allow for the detection of low concentrations of antigen (0.25 ng/ml) with high accuracy. The

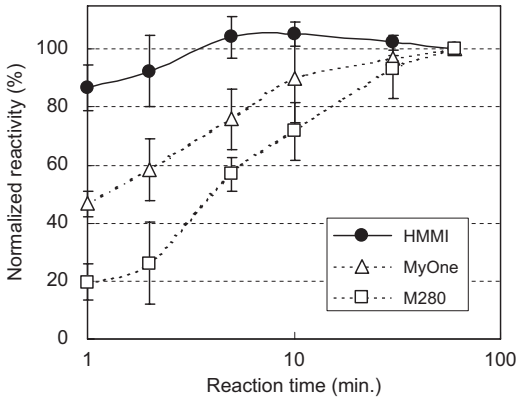

Fig. 6. Antigen binding time for different magnetic particles. Each reactivity is normalized to the 60 minutes time point.

HMMI particles can also detect antigen with higher signal intensity than other conventional magnetic particles (for instance M280). The higher signal intensity for the same antigen amount might be due to small particle size and large surface area, which allows for binding of biotinylated antibody at high density.

Fig. 6 shows reactivity of different magnetic particles with adiponectin antigen. The normalized reactivity in HMMI particles was saturated in about 5 min, while for conventional particles saturation took about 60 min. HMMI particles thus react about 10 times faster with the adiponectin antigen which is likely attributed to their smaller particle size, leading to slower sedimentation in suspension, larger specific surface area, and allowing for high-density conjugation of antibody. Together, the reaction between conjugated antibody on the magnetic particles and antigen in an aqueous solution is facilitated.

4. Conclusion

Biocompatible Ti–O/Fe particles with immobilized streptavidin were successfully prepared. Their small size (0.8 μm), large specific surface area (15 m^2/g), and high magnetization (114 Am^2/kg) allowed for rapid magnetic separation, and fast performance in the detection of antigen. The Ti–O/Fe particles are thus promising magnetic particles applicable to magnetic separation for *in vitro* analyses, including immunoassays, DNA isolations [9], and cell separations.

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