

Carboxymethyl-dextran/magnetite hybrid microspheres designed for hyperthermia

Toshiki Miyazaki¹, Shota Anan¹, Eiichi Ishida² and Masakazu Kawashita³

¹Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, Kitakyushu, Japan

²Faculty of Engineering, Kyushu Institute of Technology, Kitakyushu, Japan

³Graduate School of Biomedical Engineering, Tohoku University, Sendai, Japan

Corresponding author:

Toshiki Miyazaki, Associate Professor

Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, 2-4 Hibikino, Wakamatsu-ku, Kitakyushu 808-0196, Japan

Tel & Fax: +81-93-695-6025

E-mail: tmiya@life.kyutech.ac.jp

Abstract Recently, organic–inorganic hybrids composed of derivatives of dextran, a polysaccharide, and magnetite nanoparticles have attracted much attention as novel thermosteeds. If they can be fabricated into microspheres of size 20–30 μm , they are expected to show not only hyperthermia effects but also embolization effects in human liver and kidney cancers. In this study, we examined the fabrication of carboxymethyl-dextran/magnetite microspheres using a water/oil emulsion as the reaction medium. Improvement of the chemical stability of the microcapsules by coating with silica using a sol–gel process was also investigated. The obtained hollow microspheres contained particles of size 20–30 μm . Silica coating using an appropriate catalyst for hydrolysis and polycondensation of alkoxy-silanes was found to be effective for preventing dissolution and collapse in simulated body environments.

Keyword; Carboxymethyl-dextran; Magnetite; Organic–inorganic hybrid; Microsphere; Silica coating; Chemical stability

1 Introduction

Hyperthermia is attracting much attention as a novel low-invasive cancer treatment. Hot water, infrared light, and microwaves have been used, but these methods do not heat deep-seated cancers effectively. Hyperthermia using magnetic particles as thermosteds has been investigated [1,2]. In this treatment, magnetic particles injected into the tumor through a catheter or syringe achieve heat generation to efficiently kill cancer cells in an alternating magnetic field.

Recently, organic–inorganic hybrids composed of carboxymethyl dextran (CM-dextran) or alkali-treated dextran, which are polysaccharide derivatives, and superparamagnetic magnetite nanoparticles have been attracting much attention as novel thermosteds [3,4,5]. They are already used clinically as contrast media in magnetic resonance imaging. In these hybrids, ionic bonds are formed between carboxyl groups in the dextran derivatives and Fe ions in the magnetite, improving magnetite dispersion.

If the hybrids can be fabricated into solid microspheres, not only hyperthermia effects but also embolization effects caused by cutting off nutrition supplies to the cancer tumors could be achieved. Minamimura et al. reported the preparation of microspheres of average size 6 μm by emulsification of a mixture of alkali-treated dextran and magnetite nanoparticles with a cross-linking agent [6]. Embolization-assisted hyperthermia has been observed in rat liver. In addition, Viroonchatapan et al. prepared microspheres of magnetoliposomes of average size 1 μm by emulsification of an alkali-treated dextran/magnetite hybrid and a phospholipid [7]. However, these microspheres are too small for treating human liver and kidney cancers because they are liable to pass through the organs and enter the circulatory system. It has been reported that a suitable microsphere size for human liver and kidney cancer treatments is 20–30 μm [8]. The implanted microspheres also need to maintain their shape, without collapsing or dissolving, until the tumor has disappeared. However, the chemical stability of the microspheres has not yet been fully evaluated.

In this study, we examined the fabrication of CM-dextran/magnetite microspheres of particle size 20–30 μm using a water/oil (W/O) emulsion as the reaction medium. We also investigated improvements in the chemical stability of the microcapsules in physiological environments by coating the microcapsules with silica using a sol–gel process. We examined the effects of the addition of colloidal silica to the microspheres on their chemical stability.

2 Materials and methods

2.1 Materials

$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 2-ethyl-1-hexanol, dibutyltin dilaurate (DBTDL), sorbitan monooleate (Span80), and NH_3 aqueous solution were purchased from Wako Pure Chemical Industries (Osaka, Japan), CM-dextran and a colloidal silica suspension (LUDOX) were purchased from Sigma-Aldrich Co. (St Louis, MO, USA), and the other reagents were purchased from Nacalai Tesque Inc. (Kyoto, Japan).

2.2 Preparation of hybrids

An aqueous solution (25 mL) containing 0.047 M $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ and 0.093 M $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was heated at 75 $^\circ\text{C}$, and mixed with an equivolume of CM-dextran sodium salt aqueous solution at 4 mass%. Then 1 M NaOH was slowly added to the mixed solution until the pH of the solution reached 12. The solution was then centrifuged at 1500 rpm to remove large particles, transferred to a cellulose tube, and dialyzed for 24 h to remove remaining water-soluble salts. The sol was freeze-dried to obtain a hybrid powder using a freeze-drier (FRD-830M, AGC Techno Glass Co., Ltd., Tokyo, Japan).

2.3 Preparation of CM-dextran/magnetite microspheres, and silica coating for different treatment times

The obtained hybrid sol (10 mL, 10 mass%) was mixed with 40 mL of 2-ethyl-1-hexanol containing Span80 at 5 mass%. The mixture was emulsified at 3000 rpm for 2 min using a rotary homogenizer (Homo Mixer Mark II, Tokushu Kika Co., Osaka, Japan). The formed W/O emulsion was immediately poured into 150 mL of 1-butanol and stirred for 1 min. The formed precipitate was filtered and washed with ethanol. The precipitate (70 mg) was dispersed in 50 mL of ethanol and then 5 mL of tetraethyl orthosilicate (TEOS), and DBTDL as a dehydration catalyst, at 2.2 mass%, were added to the solution. The solution was stirred at room temperature for various periods ranging from 1 to 50 h.

The microstructure of the obtained powder was characterized using X-ray diffraction (XRD; MXP3V, Mac Science Ltd., Yokohama, Japan), scanning electron microscopy (SEM; S-3500N, Hitachi Co., Tokyo, Japan), and energy-dispersive X-ray microanalysis (EDX; EMAX Energy, Horiba Ltd., Kyoto, Japan). In the XRD measurements, Cu K α X-rays with a voltage and current of 40 kV and 30 mA, respectively, were used. The measurements were performed in step scanning mode, with 0.02° steps, at 1 s per step. The particle sizes of the specimens were calculated geometrically from the SEM photographs and using a laser diffraction particle size analyzer (LA-950, Horiba Ltd., Kyoto Japan).

The chemical stability of the hybrid microspheres was examined as follows. Microspheres (50 mg) were soaked in 20 mL of simulated body fluid (SBF) with inorganic ion concentrations of Na⁺ 142.0, K⁺ 5.0, Mg²⁺ 2.5, Cl⁻ 147.8, HCO₃⁻ 4.2, HPO₄²⁻ 1.0, and SO₄²⁻ 0.5 mM at pH 6 for 5 d [9]. The pH of the SBF was fixed at 6 because the pH around tumors has been reported to be weakly acidic as a result of secretion of lactic acid [10]. The solution was continuously shaken at 120 strokes/min, and the temperature was kept at 40 °C. The concentration of Fe, which dissolved slowly from the microspheres into the surrounding fluid, was determined using inductively

coupled plasma atomic emission spectroscopy (Optima 4300DV Cyclon, Perkin-Elmer Co., Cambridge, UK).

2.4 Preparation of CM-dextran/magnetite/colloidal silica microspheres and silica coating using different catalysts

A mixture of 300 mg of the obtained CM-dextran/magnetite hybrid powder and 2 mL of colloidal silica suspension (LUDOX, Type LS, silica concentration 30 mass%) was prepared as the water phase of the W/O emulsion. The solution was mixed with 2-ethyl-1-hexanol containing Span80 at 5 mass% as a surfactant. The mixture was emulsified at 3000 rpm for 2 min using a rotary homogenizer. The formed W/O emulsion was immediately poured into 100 mL of 1-butanol and stirred for 1 min.

The obtained precipitate (70 mg) was dispersed in 50 mL of ethanol and 5 mL of TEOS were added. Various catalysts for hydrolysis and polycondensation of TEOS were then added, i.e., 30 mL of 1 M HCl, 30 mL of 1 M NH₃, or 1.1 mL of DBTDL, and the mixture was stirred for 25 h at room temperature. Surface structural analyses and chemical tests in SBF were performed as described in Section 2.3.

3 Results

It was found that the obtained hybrid powder gave XRD peaks assigned to magnetite (JCPDS#19-0629) at 2θ values of 30, 35, 43, and 57°. This confirms that the obtained hybrid contains magnetite particles. Figure 1 shows SEM photographs of the surface and cross-section of the CM-dextran/magnetite microspheres before and after silica coating. We can see that hollow microspheres are obtained, and that the spherical shape is maintained even after the silica coating. Si distributed not only on the top surface of the hollow microsphere but also inside the shell, showing that the incorporated TEOS penetrates the shell. The Si/(Si + Fe + O + C) mass ratio increased with increasing treatment time. The mass ratio was 0.25% after the treatment for 50 h.

The obtained microspheres before the silica coating had particle sizes ranging from 5 to 60 μm , confirmed by the particle size analysis. Figure 2 shows the relative Fe concentrations in SBF after soaking the specimens subjected to TEOS treatments for different periods. The Fe concentration decreased significantly with increasing treatment time, and had been reduced by about 95% after TEOS treatment for 50 h. Figure 3 shows SEM photographs of the surfaces of CM-dextran/magnetite/colloidal silica microspheres treated with TEOS solutions containing various catalysts. The microspheres had smooth surfaces when DBTDL was used, whereas rough surfaces were observed for the other catalysts. In addition, spherical nanoparticles of size about 200 nm were observed on the microspheres when NH_3 was used as the catalyst. Figure 4 shows the relative Fe concentrations in SBF after soaking the CM-dextran/magnetite/colloidal silica microspheres treated with TEOS solutions containing various catalysts for 25 h. Fe release from the microspheres was significantly suppressed by HCl and DBTDL catalysts, but this effect was not observed in the case of NH_3 .

4 Discussion

In the present study, hollow microspheres composed of a CM-dextran/magnetite hybrid were successfully prepared by an aqueous process using a W/O emulsion as the reaction medium, as shown in Fig. 1. The CM-dextran/magnetite sol, dispersed as the water phase in the emulsion, was dehydrated in butanol and solid microspheres were precipitated, because the miscibility of butanol and CM-dextran is quite low. We previously reported the preparation of ceramic microspheres such as magnetite and yttrium oxide using a sol–gel reaction in a W/O emulsion [11,12]. The present results indicate that organic–inorganic hybrids can also be prepared by this method. It is known that the thickness of hollow microspheres increases with increasing precursor concentration in the water phase [13]. This suggests that the magnetite content could be

increased by making the microspheres fully dense, resulting in higher heat generation in an alternating magnetic field. Measurements of heat generation in an alternating magnetic field and optimization of the shell thickness of the microspheres will be performed in a future study. Although the particle size ranged from 5 to 60 μm , microspheres suitable for embolization-assisted hyperthermia, of size 20–30 μm , could be obtained by appropriate sieving.

The chemical stability of the CM-dextran/magnetite microspheres in SBF was significantly improved by sol–gel silica coating in the presence of DBTDL, as shown in Fig. 2. The formed silica film prevented dissolution of the microspheres in a simulated body environment. The Si content of the microspheres increased with increasing treatment time with TEOS. This means that larger amount of TEOS penetrated into the microspheres to form a denser silica coating layer with increasing treatment time.

The chemical stability of the CM-dextran/magnetite/colloidal silica microspheres was quite low without subsequent sol–gel silica coating (see Fig. 4). This means that simply adding colloidal silica to the CM-dextran/magnetite system is insufficient for improvement of the chemical stability, and that subsequent silica coating with an appropriate catalyst is essential. The colloidal silica was not sintered at high temperature. It is therefore assumed that the added colloidal silica is merely dispersed in a matrix of microspheres and does not form a continuous protective film. NH_3 was not effective as a catalyst for improving the chemical stability, although it has catalytic potential. When TEOS is hydrolyzed in the presence of HCl or DBTDL, partially hydrolyzed TEOS is dominant and therefore a linear or planer siloxane network is readily constructed [14,15]. In contrast, when TEOS is subjected to hydrolysis and polycondensation under alkaline conditions, fully hydrolyzed $\text{Si}(\text{OH})_4$ has been reported to be dominant [14]. The polycondensation therefore proceeds three-dimensionally to readily form silica nanoparticles. This is supported by the SEM photographs showing formation of spherical nanoparticles (see Fig. 3). The silica nanoparticles do not form a

continuous film, as shown schematically in Fig. 5(a); therefore no protection against penetration by the surrounding water would be obtained, unlike in the cases of the other catalysts, as shown in Fig. 5(b).

5. Conclusions

CM-dextran/magnetite hybrid microspheres were prepared using an aqueous process in a W/O emulsion. The chemical stability of the microspheres can be improved by coating with silica using a sol–gel process, with an appropriate catalyst for hydrolysis and polycondensation of alkoxysilanes. These microspheres have potential as thermoseeds for cancer treatments using hyperthermia with embolization.

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Figure captions

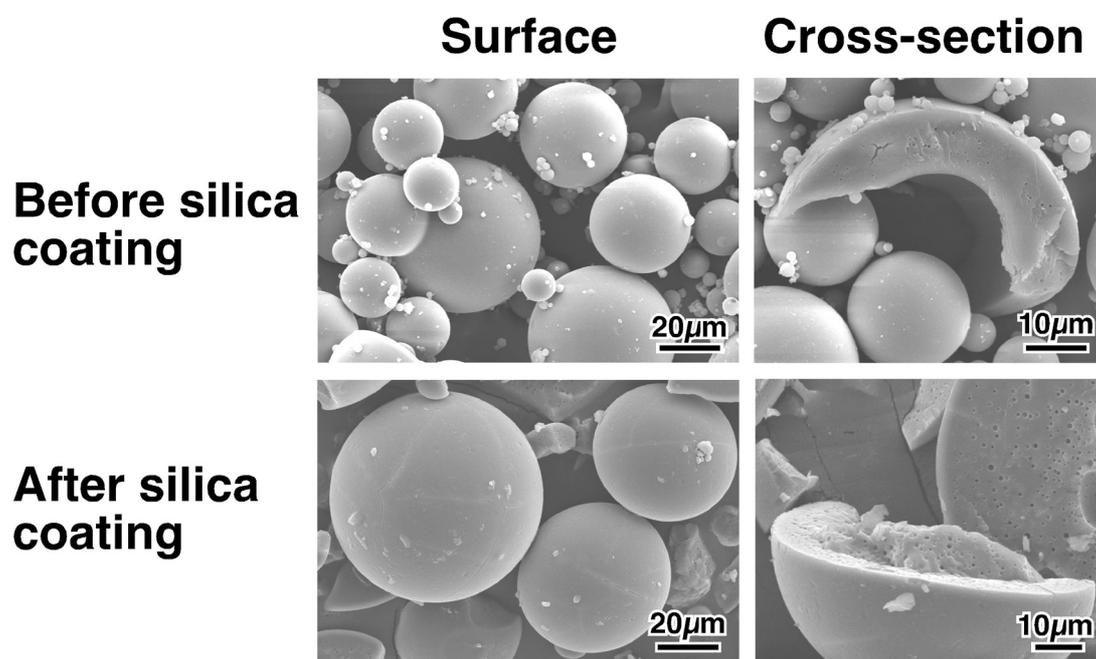


Fig. 1 SEM photographs of surface and cross-section of CM-dextran/magnetite microspheres before and after silica coating (catalyst: DBTDL, TEOS treatment time: 50 h).

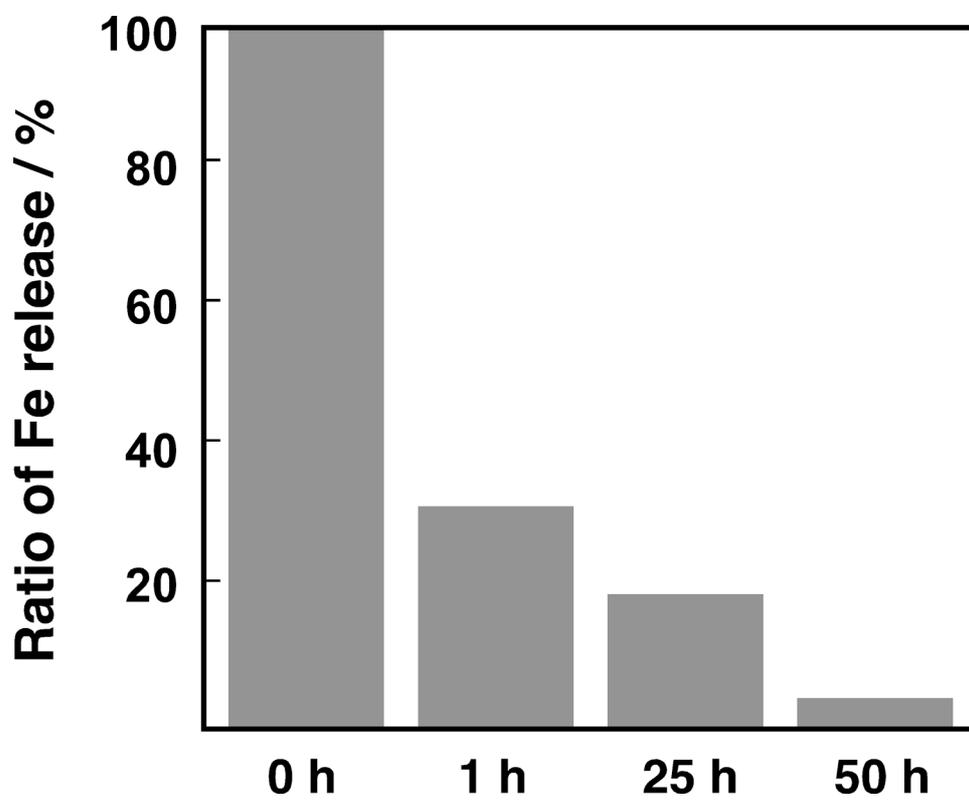


Fig. 2 Relative Fe concentrations in SBF after soaking specimens subjected to TEOS treatment for various periods. Total Fe contained in the microspheres is calculated as 100%.

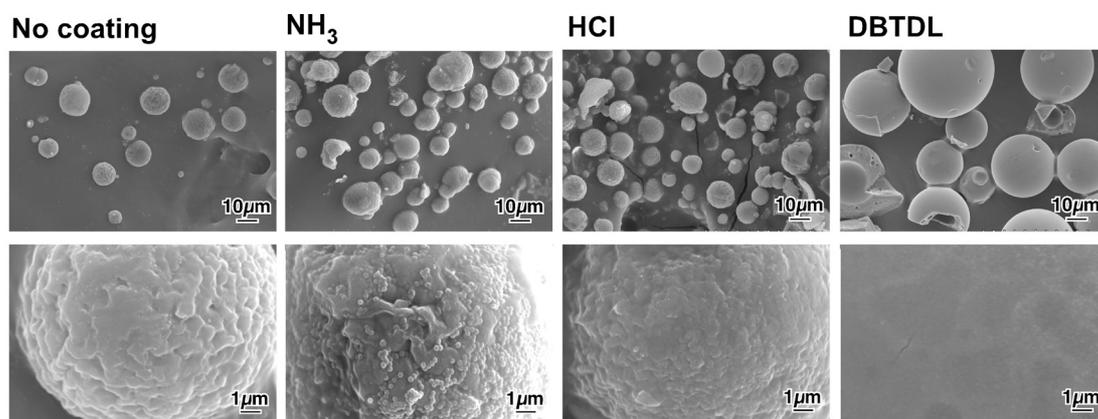


Fig. 3 SEM photographs of surfaces of CM-dextran/magnetite/colloidal silica microspheres treated with TEOS solutions containing various catalysts.

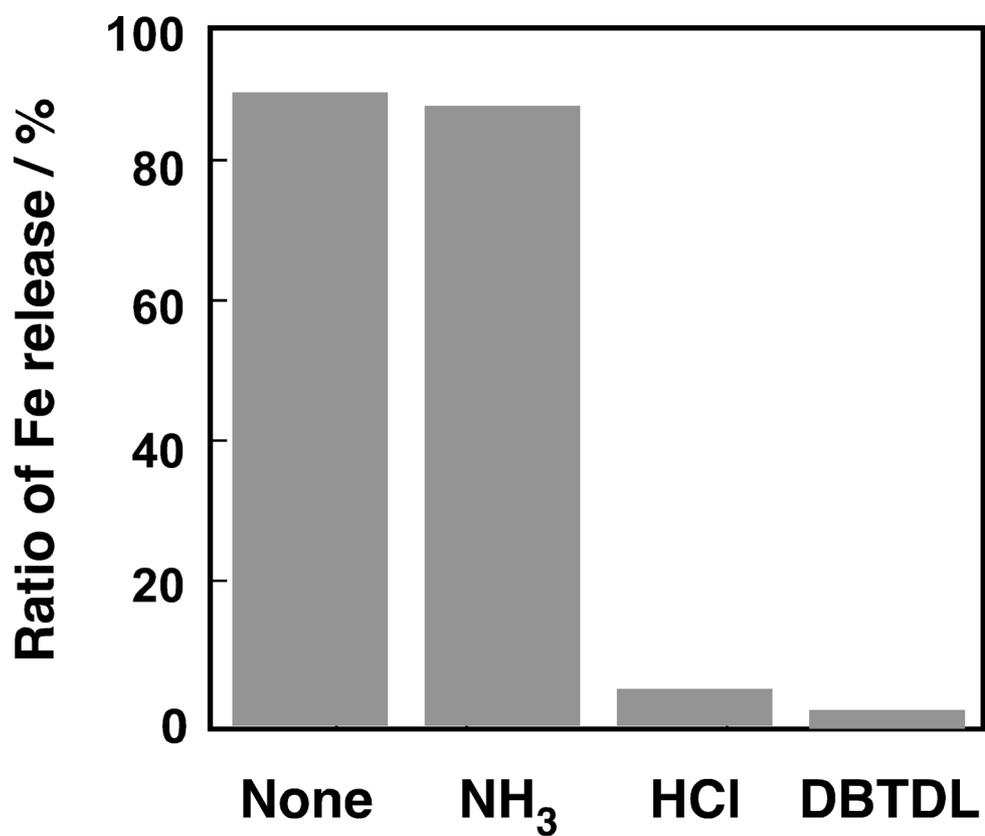


Fig. 4 Relative Fe concentrations in SBF after soaking CM-dextran/magnetite/colloidal silica microspheres treated for 25 h with TEOS solutions containing various catalysts. Total Fe contained in the microspheres is calculated as 100%.

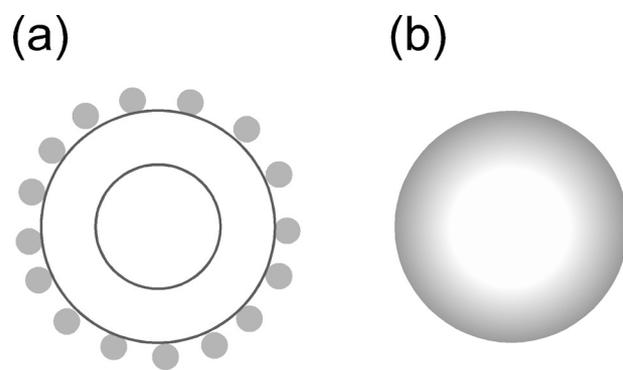


Fig. 5 Schematic representation of distribution of sol-gel silica coating by addition of (a) NH_3 and (b) other catalysts.