Bisphosphonate Release Profiles from Magnetite Microspheres

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Abstract

Hyperthermia has been suggested as a novel, minimally invasive cancer treatment method. After implantation of magnetic nano- or microparticles around a tumor, irradiation with alternating magnetic fields facilitates the efficient creation of *in situ* hyperthermia for deep-seated tumors. Building on this idea, if the microspheres are capable of delivering drugs, they could be promising multifunctional biomaterials effective for hyperthermia as well as chemotherapy. In the present study, magnetite microspheres were prepared using emulsion methods. The release behavior of alendronate, a typical bisphosphonate, from the microspheres was examined *in vitro* as a model of the bone tumor prevention and treatment system. Prevention of tumor metastasis to bone tissues is an urgent issue because it often leads to a significant reduction in patient survival rates. Porous magnetite microspheres around 30 μm in diameter were obtained without calcination. They maintained their original spherical shapes even after shaking in ultrapure water for 3 days, suggesting that they have sufficient mechanical integrity for clinical use. The microspheres showed slow release of the alendronate *in vitro*. The release rate was well controlled by the alendronate concentration.

Keywords: microspheres; drug delivery/release; alendronate; tumor; magnetite

Introduction

Hyperthermia is a minimally invasive cancer treatment that is based on the finding that cancer cells have lower heat resistivity than normal cells.[1] Tumors can be heated using several techniques such as infrared radiation, radiofrequency ablation, and using hot water. However, tumors located deep inside the body cannot be effectively treated with these techniques because their applications are external; the tumor is heated from outside the body.

Therefore, novel cancer treatments using ferromagnetic ceramic particles such as magnetite (Fe_3O_4) and γ-hematite (Fe_2O_3) may be useful. Even deep-seated tumors can be effectively heated and killed if particles are implanted around them and an alternating magnetic field is applied from outside the body. Various magnetic nanoparticles have been investigated as thermoseeds for this process.[2, 3] Implantation of ferromagnetic microspheres of 20–30 μm diameter into blood vessels around tumors facilitates the induction of more efficient hyperthermia combined with embolization to cut off the nutrient supply to the tumors.[4–7] This type of embolization therapy is especially effective for liver and kidney cancers.[8]

If these microspheres are also capable of delivering drugs, they could be promising multifunctional biomaterials effective for both hyperthermia and chemotherapy. Alendronate, a typical bisphosphonate, was chosen as a characteristic drug in the present study. It is clinically used for bone tumor treatment and inhibition of osteoporosis.[9, 10] Prevention of tumor metastasis to bone tissues is an urgent issue because it often leads significant reduction in patient survival rates. Although oral administration is widely used to deliver alendronate, there are several drawbacks to this method such as low biological availability (less than 1%) and side effects due to excess

dosages.[11] Therefore, the development of a local and sustainable drug delivery system is needed to address these limitations. While the preparation of bisphosphonate-modified magnetite nanoparticles has been previously reported,[12] those particles were designed for adsorption and removal of toxic uranyl ions from blood. The release behavior of bisphosphonate from these nanoparticles has not yet been reported.

In this study, magnetite microspheres were prepared using emulsion methods. Alendronate was incorporated into microspheres and its release was quantitatively assessed as a model of bone tumor prevention and treatment.

Materials and Methods

Preparation of microspheres

All the chemical reagents were purchased from Wako Pure Chemical Industries, Ltd., Japan. An aqueous volume of 37.5 mL of 37 mM $FeCl_2$ and 49 mM $FeCl_3$ was mixed with 8 mL of 26 wt% NH_3 solution. The precipitate that formed was collected by centrifugation, washed with ultrapure water, and dispersed in 22.5 mL of 0.2 M HCl. The solution was then heated at 80°C for 3 hours to form a sol. A water-in-oil (W/O) emulsion was then obtained by mixing 2-ethyl-1-hexanol containing 3 wt% Span80 and the sol at weight ratio of 3:1 at a rotation speed of 3400 rpm. A precipitate was immediately formed by addition of the emulsion to 1-butanol. Further details of this preparation technique and mechanism of the microsphere formation were described previously.[6]

The microstructure of the microspheres was evaluated by scanning electron microscopy (SEM; S-3500N, Hitachi Co., Tokyo, Japan), X-ray diffraction (XRD; MXP3V, Mac Science Ltd., Yokohama, Japan), and Brunauer, Emmett, and Teller

(BET) surface area and pore size analyzer (Autosorb-1C/MS, Quantachrome Instruments, Boynton Beach, FL, USA). A thin film of Au-Pd was deposited on the surfaces of the specimens using an ion sputter coater (E-101, Hitachi Co., Tokyo, Japan) for SEM observations. To evaluate mechanical properties, 10 mg of the microspheres was soaked in 30 mL of ultrapure water and shaken in a water bath (H-10, Taitec Co., Koshigaya, Japan) at a speed of 100 strokes/min for 3 days. Morphological changes in the microspheres were then observed by SEM.

In vitro drug release profiles

A mass of 30 mg of the prepared microspheres was soaked in 20 mL of sodium alendronate trihydrate ($NH_2(CH_2)_3COH(PO_3H_2)(PO_3HNa)\cdot 3H_2O$) solution at various concentrations for 1 hour under vacuum at 0.03 MPa, gently washed with ultrapure water, and dried at 60°C. Next, 10 mg of the drug-loaded microspheres was soaked in 20 mL of ultrapure water at room temperature for various times. The alendronate concentration was quantitatively determined by inductively coupled plasma atomic emission spectroscopy (Optima 4300DV Cyclon, Perkin-Elmer Co., Cambridge, UK).

Results

Figure 1 shows an SEM image of the prepared microspheres. Porous microspheres with a diameter of approximately 30-40 μm were obtained. The XRD pattern of the microspheres is shown in Figure 2. Broad diffraction peaks assigned to the magnetite (JCPDS#19-0629) were detected at 30, 35, 43, 54, 58, and 63° in 2θ. The BET surface area was 82.3 m^2/g and the average pore diameter was 17.6 nm.

Figure 3 shows an SEM photograph of the prepared microspheres after being

shaken in ultrapure water for 3 days. Their original spherical shapes were well maintained even after the shaking.

Figure 4 shows the content of alendronate incorporated into the microspheres as a function of the solution alendronate concentration. The incorporated mass increased with the increasing alendronate concentration in solution.

Figure 5 shows the alendronate released from drug-loaded microspheres into ultrapure water over time as a function of the initial alendronate concentration. The microspheres with initial concentrations of 100 and 1000 ppm gradually released alendronate over time. The degree of alendronate release increased with increasing initial alendronate concentration. The released concentration was plotted as a function of the square root of soaking time (Fig. 6) showing highly linear correlations for the 100 and 1000 ppm concentration samples. These results indicate that the process of alendronate release is diffusion-controlled.

Discussion

Magnetite microspheres were prepared by dehydration of a W/O emulsion containing the magnetite sol derived from Fe^{2+} and Fe^{3+}. In a prior study by the present authors, non-magnetic microspheres of iron (III) hydroxide were converted into magnetite by hydrothermal treatment in an aqueous solution containing ethyleneglycol and urea.[6] In the present study, magnetite microspheres were obtained without further thermal or hydrothermal treatments. Based on the present results, this streamlined fabrication process is effective. Magnetite nanoparticles can easily aggregate in aqueous conditions through van der Waals and weak magnetic attractions.[13] The present microspheres are assumed to be constructed by dehydration and aggregation of the

magnetite nanoparticles in butanol. Because the prepared microspheres were not broken after being shaken in ultrapure water (Fig. 3), they are expected to have a low risk of injuring the surrounding blood vessels *in vivo* caused by broken fragments. The high aggregation potential of the magnetite nanoparticles likely contributes to the high mechanical integrity of the microspheres.

The prepared microspheres were able to incorporate and release alendronate, and that release rate was controlled by the alendronate concentration. Iron oxide and phosphate can construct a complex via formation of an Fe-O-P bond.[14] Therefore, the phosphate group of the alendronate may construct a similar complex with magnetite crystals as shown schematically in Figure 7. The released portion of alendronate from the microspheres after 7 days was estimated to be 8% based on the results in Figures 4 and 5. Tight bonding between the magnetite and the alendronate would contribute to the prevention of an initial burst and increase continuous drug release.

The release of bisphosphonate from hydroxyapatite-based microparticles has been reported by several research groups. Seshima *et al.* investigated the release of pamidronate, a type of bisphosphonate, from calcined hydroxyapatite granules of 300–500 μm in diameter.[15] After soaking the granules calcined at 400°C for 3 days, the pamidronate concentration was 0.3 mM and the survival rate of osteoclasts was decreased to 40%. This indicates that bone resorption can be suppressed by pamidronate release.

In addition, Shi *et al.* prepared composite microspheres of poly(DL-lactic acid-co-glycolic acid) and hydroxyapatite, incorporated alendronate through emulsion methods, and examined its release behavior.[16] After 3 days, the alendronate concentration was 5.4 μM, but there was no significant change in osteoblast

proliferation compared with negative controls. Conversely, after 7 days, the concentration had increased to 8.6 μM and significant osteoblast proliferation was observed.

Although the surface area of the magnetite microspheres (82.3 m^2/g) prepared in the present study was larger than that of the hydroxyapatite prepared by Seshima *et al.* (46.2 m^2/g), the mass of released alendronate after 3 days (1.5 μM at maximum) was smaller than that reported in the above two papers. This might be attributed to a smaller powder to liquid ratio in the drug release measurement. In addition, magnetite is likely more positively charged than hydroxyapatite because the isoelectric point of the former (6.5) is higher than that of the latter (6.0).[17, 18] Thus, the tight ionic attraction of the magnetite with the negatively charged alendronate may also contribute to the slower release. However, further increasing the alendronate concentration loaded in the microspheres may increase the release up to the level necessary for effective enhancement of bone formation.

Porous magnetite microspheres were prepared using emulsion methods. These microspheres showed slow release of alendronate, a bisphosphonate drug. The release rate could be well controlled by the drug concentration loaded into the microspheres. The microspheres were able to release the drug over a long time and are therefore useful for novel hyperthermia and chemotherapy combined treatments.

Acknowledgments

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Figure legends

Figure 1: SEM image of the prepared microspheres.

Figure 2: XRD pattern of the prepared microspheres.

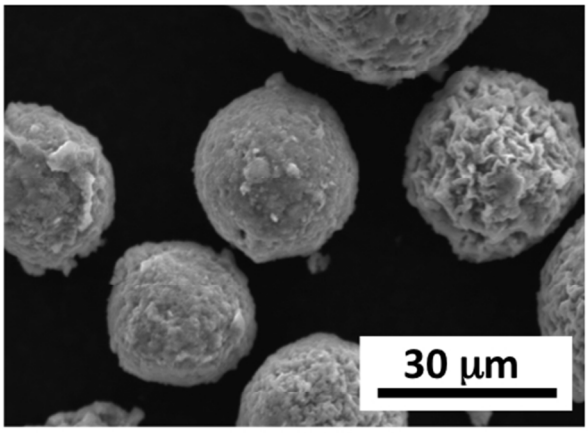
Figure 3: SEM image of the prepared microspheres after being shaken in ultrapure water for 3 days.

Figure 4: Mass of alendronate incorporated into the microspheres as a function of the solution alendronate concentration.

Figure 5: Alendronate concentration after soaking drug-loaded microspheres in ultrapure water over time as a function of the initial alendronate concentration.

Figure 6: Alendronate concentration after soaking drug-loaded microspheres in ultrapure water plotted as a function of the square root of time for different initial alendronate concentrations.

Figure 7: Schematic representation of binding of alendronate to magnetite.



Miyazaki *et al.*
Fig. 1

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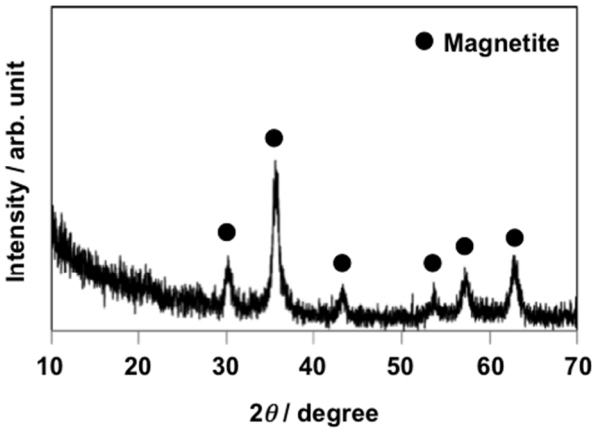

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Fig. 2

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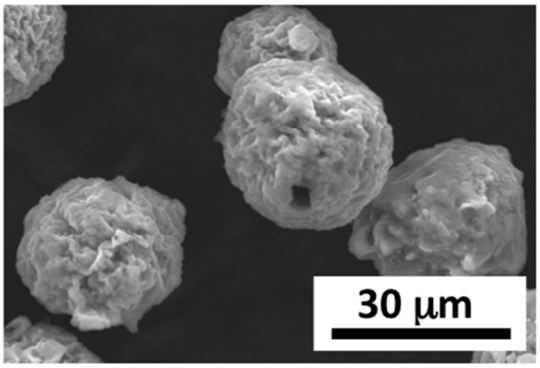

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Fig. 3

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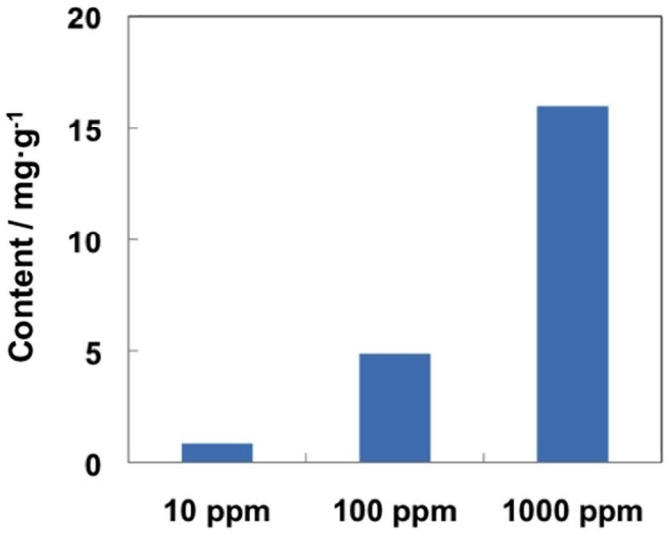

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Fig. 4

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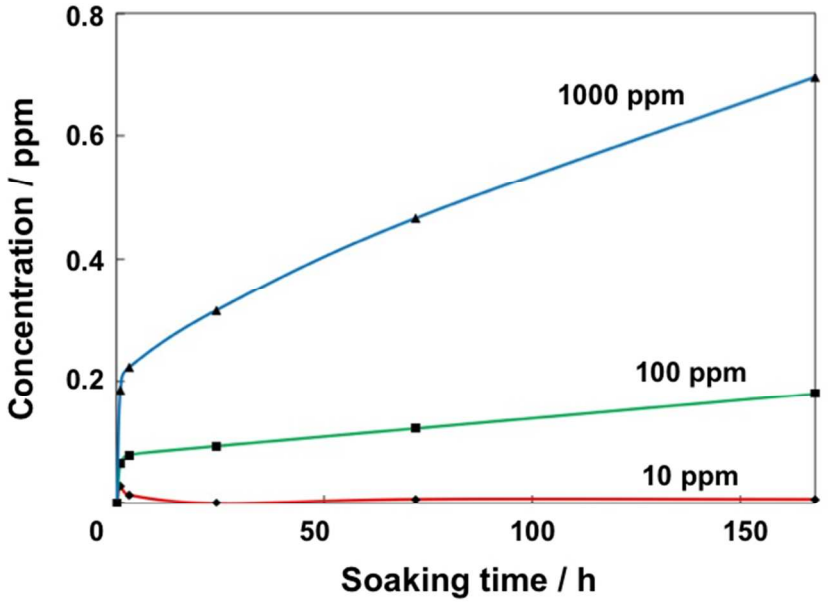

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Fig. 5

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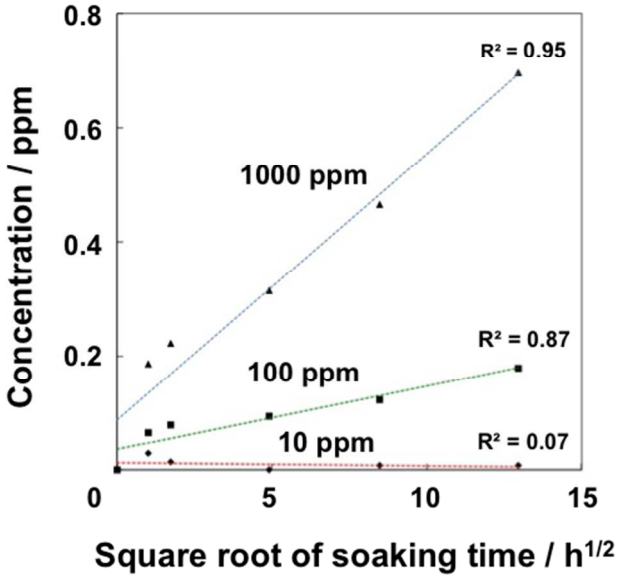

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Fig. 6

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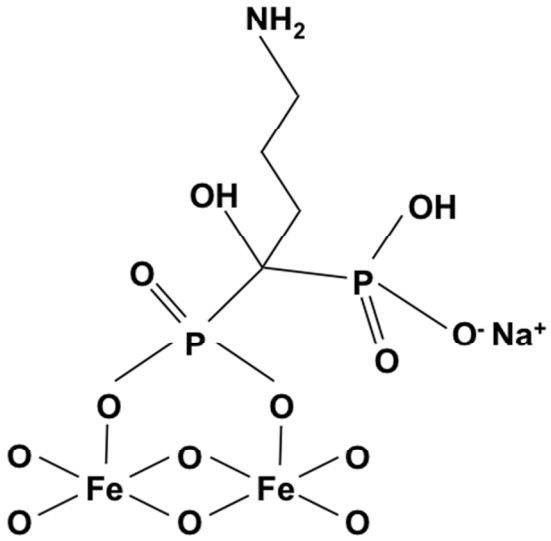

Miyazaki *et al.*
Fig. 7

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