Fabrication of Yttrium Phosphate Microcapsules by Emulsion Route for *In Situ* Cancer Radiotherapy

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Abstract

Radiotherapy is a novel, non-invasive cancer treatment. Radioactive hollow microspheres, i.e., microcapsules, are attractive for *in situ* cancer radiotherapy because they can effectively reach tumors without settling in blood vessels. In particular, microcapsules 20-30 μm in size are expected to exhibit not only a radiotherapy effect but also embolization that blocks the nutrient supply to cancer cells. β-ray irradiation is the most suitable source for *in situ* radiotherapy because of its moderate range. Several kinds of β-emitting yttria (Y_2O_3) microcapsules have therefore been developed. Yttrium phosphate (YPO_4) should have a longer irradiation effect than that of Y_2O_3 because the half-life of ^{31}P (14.3 days) is longer than that of ^{90}Y (64.1 hours). However, the preparation of YPO_4 microcapsules has not been reported to date. In the present study, YPO_4 microcapsules were fabricated using a water/oil (W/O) emulsion prepared by first dispersing a YPO_4 sol into toluene containing a surfactant, with mechanical homogenization. The emulsion was then added into butanol to dehydrate the water phase and precipitate microcapsules. These were then heat-treated to improve their mechanical strength and chemical stability. Microcapsule fragility at low YPO_4 sol concentrations in the water phase was attributed to the thinness of the microcapsule shell. The size of the microcapsules decreased with increasing emulsification speed. The chemical stability of the prepared microcapsules is similar to those of previously reported YPO_4 and Y_2O_3 microspheres in weakly acidic conditions. Thus, little leakage of radioactive species into nearby healthy tissues is expected. The obtained microcapsules are expected to be highly effective for cancer radiotherapy.

Keywords: Yttrium phosphate, Microcapsule, Cancer treatment, Radiotherapy, Emulsion, Chemical durability

1. Introduction

Cancer has recently become the main cause of death in Japan [1]. Therefore, the development of efficient cancer treatments is urgently needed. Surgical excision is widely chosen as a treatment course. However, some organs do not recover their functions after surgery. The development of a non-invasive cancer treatment is needed to improve quality of life.

Radiotherapy is a non-invasive cancer treatment but it is difficult to treat deep-seated cancer with low side effects by irradiation from outside the body. Recently, novel radiotherapies using ceramic microspheres imparting β-ray irradiation have been developed [2]. As an irradiation source, a γ-emitter is liable to penetrate into the surrounding healthy tissues and consequently induce side effects, while an α-emitter can convert another surrounding element into a γ-emitter. Therefore, a β-emitter, which has an average range of 2.5 mm, is suitable for *in situ* radiotherapy. Implanted microspheres reach tumors through blood vessels and can thus locally irradiate a deep-seated tumor. Hollow microspheres (microcapsules) are expected to effectively reach tumors without settling in blood vessels, unlike dense microspheres. In particular, those with a size of about 20-30 μm can shut off the supply of nutrients to cancer cells by embolization [2]. In addition, they are attractive as carriers of anti-cancer agents in drug delivery.

It is known that non-radioactive ^{89}Y, with a natural abundance of 100%, can be converted into β-emitting ^{90}Y, whose half-life is 64.1 hours, by neutron bombardment [3]. Based on this, microspheres composed of Y_2O_3-Al_2O_3-SiO_2 glass [4-6], Y_2O_3 ceramics [7-10], and ^{90}Y-containing resin [11] have been proposed. However, the half-life of ^{90}Y is somewhat brief for effective radiotherapy. Non-radioactive ^{31}P, with a natural abundance of 100%, can be converted into β-emitting ^{32}P, whose half-life is 14.3 days, by neutron bombardment. Therefore, yttrium phosphate (YPO_4) is expected to possess a longer irradiation effect *in vivo* than that of Y_2O_3. Although the preparation of dense YPO_4 microspheres

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has previously been reported [7,12], that of YPO_4 microcapsules has not.

In the present study, YPO_4 microcapsules were prepared via the precipitation of YPO_4 from a water/oil (W/O) emulsion and subsequent heat treatment. To evaluate the optimal conditions for the preparation of YPO_4 microcapsules suitable for radiotherapy, the effects of YPO_4 concentration in the water phase and emulsification speed on the shape and diameter of the particles were investigated. In addition, the chemical stability of the microcapsules was evaluated in a simulated body environment.

2. Materials and methods

NaOH and 1-butanol were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan). 80 mL of 0.56 M $Y(CH_3COO)_3$ and 20 mL of 2.3 M KH_2PO_4 aqueous solutions were mixed. The resulting precipitates were collected by a centrifugal separator (CN-2060, As-one Co., Osaka, Japan) and washed with ultrapure water. They were then dispersed in 15 or 30 mL of 0.1 M HNO_3 and stirred at room temperature for 24 h to obtain a YPO_4 sol. 5 mL of the sol were then mixed with 50 mL of toluene containing nonionic surfactant (Span80) at 0.5 mass% and emulsified at various speeds, ranging from 2000 to 3000 rpm, for 10 min using a rotary homogenizer (Homo Mixer Mark II, Tokushu Kika Co., Osaka, Japan). The resultant W/O emulsion was immediately added to 150 mL of 1-butanol and stirred for 1 min. The precipitates were filtered and dried at 60 ℃ for 24 h, heated to 1000 ℃ at a rate of 5 ℃/min, and kept at that temperature for 2 h.

The microstructure of the powder product was characterized by X-ray diffraction (XRD; MXP3V, Mac Science Ltd., Japan), scanning electron microscopy (SEM; S-3500N, Hitachi Co., Japan), and an energy-dispersive X-ray analyser (EDX; Model EX-400; Horiba Co., Kyoto, Japan). The particle size distribution was characterized by measuring the size of each particle from SEM images. The chemical stability of the microcapsules was examined as follows. 50 mg of microcapsules were kept in 20 mL of 50 mM 2-(*N*-morpholino) ethanesulfonic acid (MES) buffer for 10 days at 36 ℃ under vigorous stirring. The pH of the MES solution was buffered at 6 by the dropwise addition of 1 M NaOH because the pH around tumors is reported to be sometimes weakly acidic through the secretion of lactic acid [13]. Y and P concentrations released from the microcapsules into the buffer were measured by inductively coupled plasma (Optima 4300DV Cyclon, Perkin-Elmer Co., England) atomic emission spectroscopy (ICP-AES). The fractions of Y and P released from the microcapsules were calculated using the following equation:

$$\text{Released fraction} = \frac{\text{Molar quantity of Y or P released from the sample into MES (mol)}}{\text{Total molar quantity of Y or P contained in the sample (mol)}} \quad (1)$$

3. Results

Figure 1 shows SEM images of the non-heated particles prepared from YPO_4 sol mixed with either 15 or 30 mL of HNO_3 solution. Microspheres with good spherical shapes were obtained in the former, while almost all of the particles were broken in the latter. A YPO_4 sol containing 15 mL of HNO_3 solution was used for subsequent sample preparation. Figure 2 shows SEM images and the size distribution of the particles obtained at various emulsification speeds after heat treatment at 1000 ℃. The particle sizes ranged from 5 to 50 μm, and tended to decrease with increasing emulsification speed. Figure 3 shows a SEM image of cross-sections of the particles after heat treatment at 1000 ℃. The obtained microspheres were found to have a hollow structure. Figure 4 shows XRD patterns of the microcapsules after heat treatment at 1000℃. The sample was amorphous before heat treatment (Data not shown), while peaks assigned to crystalline tetragonal xenotime-type YPO_4 (JCPDS card no. 11-0254) were observed after heat treatment. The crystallinity of the samples increased with increasing heat treatment temperature (Data not shown). Fractions of Y and P released from the microcapsules into MES buffer during stability tests were 1.4×10^{-5} and 6.6×10^{-4}, respectively. Y/P molar ratios of the microcapsules before and after soaking in MES buffer were 1.27 ± 0.07 and 1.54 ± 0.18, respectively ($n = 5$).

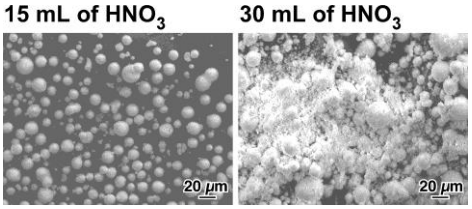

Figure 1. SEM images of non-heated particles prepared from a YPO_4 sol mixed with 15 or 30 mL of HNO_3 solution (emulsification speed of 3000 rpm).

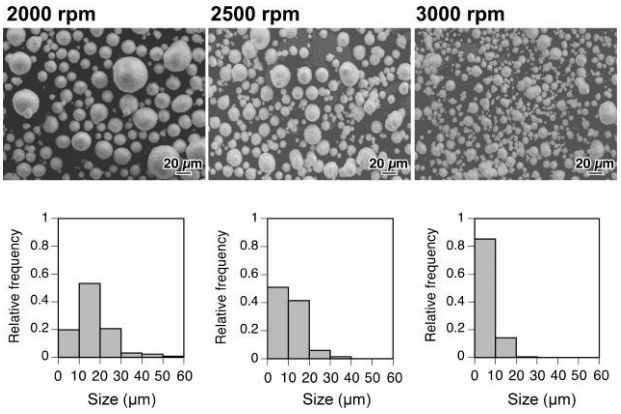

Figure 2. SEM images and size distribution of particles fabricated at various emulsification speeds after heat treatment at 1000 ℃.

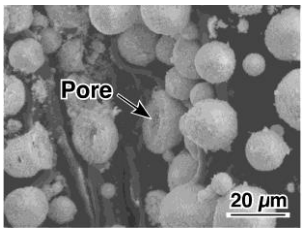

Figure 3. SEM image of cross-sections of particles after heat treatment at 1000 ℃ (emulsification speed of 2000 rpm).

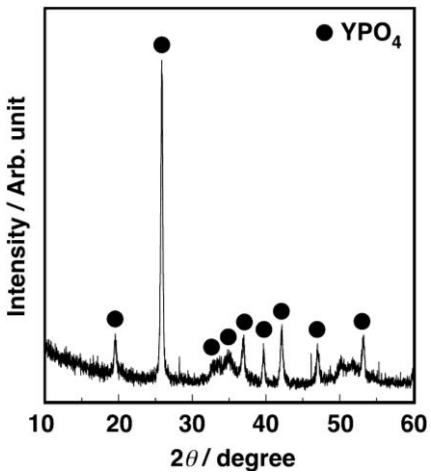

Figure 4. XRD patterns of microcapsules after heat treatment at 1000℃ (emulsification speed 2000 rpm).

4. Discussion

It was determined that YPO_4 microcapsules can be efficiently obtained using a W/O emulsion. During this process, microcapsule formation is thought to progress as follows [14]. YPO_4 sol is dispersed as a water phase in toluene to form the W/O emulsion. The sol is dehydrated by diffusion of 1-butanol from the oil phase to the water phase. A YPO_4 gel then precipitates at the W/O interface because it is sparingly soluble in 1-butanol. Consequently, the shell of the YPO_4 microcapsule is created. When the volume of HNO_3 solution in the water phase is too high, the resulting particles are too brittle to maintain a spherical shape (see Fig. 1). The mechanical strength of the microcapsules is low because a very thin shell forms at low YPO_4 concentration. A similar relationship between precursor concentration and wall thickness was reported for silica microcapsules [15].

The microcapsule size decreased with increasing emulsification speed. The droplet size of the water phase in the W/O emulsion generally decreases with increasing rotation speed because of increased shear stress [16]. It is assumed that the droplet size of the water phase strongly governs the size of the microcapsules produced. The relative frequency of particle sizes suitable for radiotherapy (20-30 μm) was the highest at an emulsification speed of 2000 rpm (See Fig. 2).

The release fraction of Y of the present microcapsules was similar to that from dense YPO_4 microspheres obtained by heat treatment of gel spheres at 1100 ℃ [12], and much lower than that from dense YPO_4 microspheres created by plasma melting and subsequent quenching [7]. For the latter, Kawashita *et al*. found that acid-soluble impurities accumulated at the grain boundary of the microspheres, causing low chemical stability [7]. The present results support the view that normal heat treatment is more suitable than quenching for the fabrication of microspheres with high chemical stability.

The released fraction of P was 47 times higher than that of Y, as shown in Table 1. This means that the Y/P molar ratio of the released chemical species differed from that of the original YPO_4 microspheres, which had a molar ratio of about one [7,12]. It has been reported that $AlPO_4 \cdot 1.5H_2O$ dissolves unevenly, releasing a larger amount of P than Al in weakly acidic conditions of around pH 6, because the released Al^{3+} is hydrated and re-precipitated in this pH region [17]. A similar phenomenon may occur in the case of the present YPO_4 microcapsules. The increase in the Y/P molar ratio of the microcapsules after soaking in MES buffer supports this assumption (see Table 2).

Table 1. Fractions of Y and P released from samples into MES buffer at pH 6 at 36 ℃ for 10 days (emulsification speed of 2000 rpm).

Y	P
1.4×10^{-5}	6.6×10^{-4}

Table 2. Y/P molar ratios of microcapsules before and after soaking in MES buffer at pH 6 at 36 ℃ for 10 days (emulsification speed of 2000 rpm, n = 5).

Before	After
1.27 ± 0.07	1.54 ± 0.18

5. Conclusion

YPO_4 microcapsules with diameters in the range of 20-30 μm were obtained by a wet process in a W/O emulsion. The size of the microcapsules can be controlled well by the emulsification speed. The chemical stability of the microcapsules in buffer solutions was as high as those previously reported for YPO_4 microspheres. These microcapsules are expected to be useful for the *in situ* radiotherapy treatment of cancer.

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