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In vitro apatite formation and drug loading/release of porous TiO_2 microspheres prepared by sol–gel processing with different SiO_2 nanoparticle contents

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ABSTRACT

Bioactive titania (TiO_2) microparticles can be used as drug-releasing cement fillers for the chemotherapeutic treatment of metastatic bone tumors. Porous anatase-type TiO_2 microspheres around 15 μm in diameter were obtained through a sol–gel process involving a water-in-oil emulsion with 30:70 SiO_2/H_2O weight ratio and subsequent NaOH solution treatment. The water phase consisted of methanol, titanium tetraisopropoxide, diethanolamine, SiO_2 nanoparticles, and H_2O, while the oil phase consisted of kerosene, Span 80, and Span 60. The resulting microspheres had a high specific surface area of 111.7 $m^2 \cdot g^{-1}$. Apatite with a network-like surface structure formed on the surface of the microspheres within 8 days in simulated body fluid. The good apatite-forming ability of the microspheres is attributed to their porous structure and the negative zeta potential of TiO_2. The release of rhodamine B, a model for a hydrophilic drug, was rapid for the first 6 h of soaking, but diffusion-controlled thereafter. The burst release in the first 6 h is problematic for clinical applications; nonetheless, the present results highlight the potential of porous TiO_2 microspheres as drug-releasing cement fillers able to form apatite.

1. Introduction

Lung cancer, breast cancer and prostate cancer often metastasize to bone [1]. Metastatic bone tumors are not fatal, but often cause skeletal pain and pathological fractures resulting in a marked decrease in patients' quality of life. Metastatic bone tumors are usually treated with a combination of surgical operations and chemotherapy. Firstly, the cancer-affected region is extirpated by surgery and the excision site is sometimes filled with injectable calcium phosphate cement in order to achieve early postoperative ambulation [2–4]. After the surgical operation, chemotherapy is performed in order to prevent cancer recurrence and metastasis. However, chemotherapy often carries a risk of side effects because the drugs are often administered systemically.

Polymethylmethacrylate (PMMA) and calcium phosphate cements are useful fillers for the excision site because of their excellent mechanical properties. PMMA cement is usually encapsulated by fibrous tissues and cannot bond to living bone, i.e. show bioactivity, if the matrix does no contain titania (TiO_2) nano- or microparticles [5,6] whose apatite-forming ability is responsible for the bioactivity of the cement. TiO_2 particles able to release drugs would therefore confer both bioactivity and chemotherapeutic abilities to PMMA cement. Previously, Yamashita et al. reported that porous TiO_2 microspheres approximately 5 μm in diameter can be obtained using a sol–gel method with a water-in-oil (W/O) emulsion [7,8]. In this sol–gel approach, colloidal silica (SiO_2) nanoparticles are firstly incorporated into TiO_2 microspheres and then leached out with a sodium hydroxide (NaOH) aqueous solution leaving behind a porous structure. We recently investigated the effects of SiO_2 nanoparticle size (15, 50, or 200 nm) on the apatite-forming ability of porous TiO_2 microspheres in simulated body fluid (SBF), and found that pore size and pore shape are important factors influencing the formation of apatite [9]. However, the effects of the SiO_2 nanoparticle content on the formation of apatite on the porous TiO_2 microspheres in SBF and the drug-releasing ability of the porous TiO_2 microspheres were not investigated. In this study, in order to evaluate the potential of porous TiO_2 microspheres as apatite-forming, drug-releasing cement fillers, we investigated first, the apatite-forming ability of porous TiO_2 microspheres prepared with different SiO_2 nanoparticle contents, and second, the drug-releasing behavior of the porous TiO_2 microspheres by using rhodamine B as a model for a hydrophilic drug [10,11].

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2. Experimental procedures

2.1. Sample preparation

All of the reagent-grade chemicals were purchased from Wako Pure Chemical Industries, Tokyo, Japan. Porous TiO_2 microspheres were prepared using a sol–gel method in W/O emulsion, based on our previous study [9]. For the oil phase, kerosene (54.0 g), Span 80 (9.0 g), and Span 60 (3.0 g) were mixed at 40 °C for 15 min in a homogenizer (HG-200, AS ONE Corp., Osaka, Japan) at 1800 rpm. For the water phase, methanol (2.7 g), titanium tetraisopropoxide (3.36 g), and diethanolamine (2.0 g) were mixed at 0 °C with a magnetic stirrer (HS-360, AS ONE Corp., Osaka, Japan).

Separately, SiO_2 nanoparticle suspensions with varying SiO_2 contents were prepared by adding different amounts of colloidal SiO_2 (Snowtex® XL, Nissan Chemical Industries, Ltd., Tokyo, Japan) to ultrapure water (H_2O). The average size and concentration of the SiO_2 nanoparticles in the colloid were 50 nm and 40 wt.%, respectively. The total H_2O content of the dispersion was fixed at 4.2 g by changing the amount of H_2O added. Table 1 gives the sample names, the corresponding SiO_2 and H_2O contents, and the SiO_2/H_2O weight ratios in the SiO_2 nanoparticle dispersions. The number in the sample name (S-*xx*) indicates its SiO_2 content (wt.%). The SiO_2 nanoparticle dispersion was mixed with the water phase and then dropped into the oil phase under continuous mixing in the homogenizer to achieve a W/O emulsion. The mixing was prolonged at 40 °C for 20 min at 1800 rpm, at 50 °C for 20 min at 1800 rpm, at 60 °C for 70 min at 1800 rpm, and finally at 60 °C for 70 min at 1700 rpm. After mixing, the W/O emulsion was centrifuged at 3000 rpm for 5 min (CN-1050, AS ONE Corp., Osaka, Japan) and the supernatant liquid was removed by decantation. The precipitates were twice washed with 30 mL of ethanol, dried at 60 °C for 12 h, and then heat-treated at 600 °C for 5 h in an electric furnace (FO100, Yamato Scientific Co., Ltd., Tokyo, Japan), with the temperature increased at a rate of 5 °C·min^{-1}.

To create porous structures, the heat-treated precipitates prepared with 0:100 or 10:90 weight ratios of SiO_2/H_2O were soaked in 4 mL of 3 mol·L^{-1} (M) sodium hydroxide (NaOH) solution, while those with SiO_2/H_2O weight ratios of 20:80 or 30:70 were soaked in 4 mL of 1 M NaOH solution at room temperature for 24 h.

2.2. Characterization of the samples

The morphology of the samples was observed by field-emission scanning electron microscopy (FE-SEM; JSM-6500F, JEOL Ltd., Tokyo, Japan). The measurements were performed in standard FE-SEM imaging mode, at an accelerating voltage of 15 kV and a working distance of 8.3 or 8.4 mm. The samples were not pre-coated with electrically conductive materials prior to observation. The crystalline phases of the samples were investigated by powder X-ray diffraction (XRD; MiniFlex600HDA, Rigaku Corp., Tokyo, Japan), with a Cu Kα source operating at 40 kV–15 mA and with a scanning rate of 10°·min^{-1}. The structures of the samples were investigated by Fourier-transformed infrared spectroscopy (FT-IR; FT/IR-6200, JASCO Corp., Tokyo, Japan), by recording transmission spectra using the potassium bromide (KBr) pellet method. The KBr content of the testing samples was kept at around 0.125 wt.%. The specific surface area (SSA) of the samples was measured with a gas absorption analyzer (Autosorb®-iQ-C, Quantachrome Corp., Florida, USA), and the pore size distribution in sample S-30 before and after NaOH solution treatment was derived from volumetric adsorption measurements (BELSORP-mini II, BEL Japan, Osaka, Japan). The samples were heated at 200 °C for 2 h prior to measurements in order to remove adsorbed water. The zeta potentials of the samples were measured by laser electrophoresis spectroscopy (ZS90, Malvern Instruments Ltd., Worcestershire, UK) at pH 7.4 in saline. The pH of the saline was adjusted using 10 mM aqueous solutions of HCl and NaOH.

2.3. Soaking of the samples in SBF

The SBF was prepared by dissolving reagent-grade chemicals in ultrapure water according to the ISO23317:2012 protocol. All chemicals used for the preparation of SBF were purchased from Nacalai Tesque, Inc., Kyoto, Japan. Different amounts of the samples (S-0: 47.3 mg, S-10: 7.5 mg, S-20: 6.4 mg, S-30: 6.1 mg) were soaked statically in 50 mL SBF at 36.5 °C for 8 days. The different amounts used for each sample ensured matching surface areas. The SBF was refreshed every two days.

2.4. Evaluation of rhodamine B loading and release for sample S-30

Rhodamine B adsorption and release was evaluated for sample S-30 as outlined in a previous study [12]. A total of 2.5 mg of Sample S-30 was soaked in a microtube containing 1 mL of a 0.5 mg·mL^{-1} rhodamine B solution, whose concentration was determined by the solubility of rhodamine B in water. The microtube was rotated at 3 rpm for different periods ranging from 1 to 12 h at 36.5 °C, with a tube rotator (TR-350, AS ONE Corp., Osaka, Japan). After a given period, the sample was removed from the rhodamine B solution by centrifugation at 3000 rpm for 5 min (CN-1050, AS ONE Corp., Osaka, Japan) and the rhodamine B concentration in the supernatant was measured by spectrophotometry (PD-303S, APEL Co., Ltd., Saitama, Japan). The amount of rhodamine B loaded into sample S-30 was calculated from the rhodamine B concentration in the supernatant.

After washing the sample with 1 mL of ultrapure water at the end of the 12 h experimental period, the resulting rhodamine B-loaded S-30 sample was soaked in 1 mL of ultrapure water in a microtube that was then rotated with a tube rotator at 3 rpm for different periods ranging from 1 to 96 h at 36.5 °C. After a given period, the sample was extracted by centrifugation, and the amount of rhodamine B in the supernatant was measured by spectrophotometry. Six S-30 samples were subjected to these evaluations.

3. Results and discussion

Fig. 1 shows FE-SEM images of the different samples. Microspheres around 8 μm in diameter with smooth surfaces are observed for sample S-0 while marginally larger ones, approximately 10 μm in diameter with slightly rough surfaces are observed for samples S-10 and S-20. For sample S-30, the microspheres are around 15 μm in diameter with slightly rough surfaces. These results indicate that the incorporation of SiO_2 nanoparticles during preparation increases the size of the resultant microspheres, possibly due to the increased viscosity of the sol solution. The rough surfaces of samples S-10, S-20, and S-30 are formed by the dissolution of SiO_2 nanoparticles in the NaOH solution.

Fig. 2a and b respectively shows XRD patterns obtained from samples before and after NaOH solution treatment, in which strong diffraction peaks ascribed to anatase (PDF: 21-1272) are observed for all samples. In addition, small diffraction peaks associated with rutile (PDF: 21-1276) are visible in the spectrum for sample S-0, while for samples S-10, S-20 and S-30, a broad peak ascribed to amorphous colloidal SiO_2 [13] is observed around $2\nu = 22°$ before NaOH treatment (Fig. 2a) and also after (Fig. 2b) for the latter two samples. This suggests that for samples S-20 and S-30, some of the additive SiO_2 nanoparticles still remain even after soaking in a 1 M NaOH solution. Higher

Table 1
Sample names and the corresponding colloidal SiO_2 contents, H_2O contents and SiO_2/H_2O ratios in the SiO_2 nanoparticle suspensions.

Sample name	S-0	S-10	S-20	S-30
Colloidal SiO_2 [g]	0.000	1.167	2.625	4.500
H_2O [g]	4.200	3.500	2.625	1.500
SiO_2/H_2O [weight ratio]	0/100	10/90	20/20	30/70