Unique preparation method of self-assembled spherical particles consisting of hydroxyapatite nanocrystals modified by amino groups

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Hydroxyapatite (HAp) has been very useful biomaterial for many orthopedic [1] and maxillofacial [2] applications in medical fields. Some researchers, recently, have been focusing on bio-ceramics as a carrier for drugs or biomolecules in drug delivery systems (DDS) [3–6]. Gautier *et al*. demonstrated that the difference in specific surface area of apatite granules 200–500 μm in diameter influenced human growth hormone loading and release [5]. Itokazu *et al.* stated that DDS of porous ceramics incorporating chemotherapeutic agents against tumor cells could be valuable form of local chemotherapy [6]. It is thus important in terms of particle size and specific surface area for a drug carrier to hold, deliver and release drugs to destinations in the living body.

It is well known that the spray drying method is the most common and commercially used preparation procedure for spherical HAp, which are secondary particles aggregated with minute needle-like crystals 200–300 nm in length [7]. The range of particle size is also relatively narrower compared to that of spray pyrolysis or other methods [7]. It is not, however, suitable as a drug carrier in DDS due to its dense structure, indicating a lower specific surface area. We developed self-assembled spherical and porous HAp particles with nanocrystals as the drug carrier. The particles can be prepared only by mixing HAp nanocrystals in an organically mixed solvent. The self-assembled HAp particles display a porous structure and very narrow range of particle size. This short communication presents the unique preparation method and properties of the spherical HAp particles characterized by scanning electron microscopy (SEM) and the Brunnaure–Emmett–Teller (BET) method.

Calcium hydroxide [$Ca(OH)_2$], potassium dihydrogen phosphate (KH_2PO_4) and phentaethylene glycol dodecyl ether as the surfactant were obtained from WAKO Pure Chemical Industries Ltd., Osaka, Japan. γ-Aminopropyltriethoxysilane [γ-APS; $(C_2H_5O)_3SiC_3H_6NH_2$] was kindly donated by Shin-Etsu Chemical Industries Co., Tokyo, Japan. Toluene and methanol (WAKO Pure Chemical Industries Ltd.), used in surface modification and assembled particles preparation, were dehydrated with molecular sieves and purified by distillation. HAp nanocrystals were prepared by the modified emulsion system described in previous reports [8, 9]. Briefly, 10 ml of an aqueous suspension of 2.5 M $Ca(OH)_2$ was poured into 40 ml of oil phase containing 0.5 g of the surfactant at 50 °C. Subsequently, 10 ml of 1.5 M KH_2PO_4 aqueous supersaturated solution was added into the W/O emulsion. After reaction, the product was purified by centrifugation and washed with ethanol and water. The powder was calcined at 800 °C for 1 h at a heating rate of 10 °C/min. HAp nanocrystals (1.0 g), after drying at 120 °C for 24 h, were added into 50 ml of anhydrous toluene under a nitrogen atmosphere in a three-necked flask with a reflux condenser. To prepare amino groups-modified HAp nanocrystals (HAp-NH_2), 5.0 ml of γ-APS was injected into the reaction system and refluxed at 120 °C for 24 h. The reactant was purified by centrifugation with toluene to remove any unreacted reagent. The HAp-NH_2 was added into toluene/methanol (9/1 of volume ratio) in an Erlenmeyer flask with a concentration of 10 mg/ml. The flask was immersed in an ultrasonic bath (20 kHz, 35 W) for 1 min and subsequently quietly put on a desk at ambient temperature. The solution was removed using a PTFE membrane filter (ADVANTEC, Florida, USA) with a pore size of 2 μm. The self-assembled HAp particles were finally obtained by heating at 120 °C and sintering at 800 °C

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for 6 h. SEM (JSM-6301F, JEOL, Japan) was used in order to observe the assembled particles employing gold coating. The specific surface area of the self-assembled HAp particles was determined by the BET method using a NOVA-1200 type High Speed Surface Area and Pore Size Analyzer (Quantachrome Co., Florida, USA).

The characteristics of nano-HAp crystals are as follows: rod-like morphology with high crystalinity, particle size (a axis $= 78 \pm 16$ nm, c axis $= 154 \pm 48$ nm, aspect ratio $= 1.98$ determined from transmission electron micrograph image), Ca/P $= 1.61$ calculated from inductively coupled plasma (ICP) spectrometry, Ca deficient HAp containing carbonate analyzed by Fourier transform infrared (FT-IR) spectrometer [8, 9]. The amino group donation ratio to a HAp-NH_2 crystal was estimated to be approximately one molecule per 1.0 nm^2, calculated by a sensitive spectrophotometric method [10]. It was implied that the donation value of amino groups to the nanocrystal surface was almost at maximum. The spherical and self-assembled HAp particles before sintering were constructed from the HAp-NH_2 crystals.

Fig. 1 shows SEM images of assembled HAp particles observed by higher (20 000×; (a)) and lower (2000×; (b)) magnification. In the higher magnification (Fig. 1a), the edges of rod-like HAp particles were shown to be fused with each other due to calcination at 800 °C. Some pores were observed in the particle from the external view. Spherical particle seems to be formed by the assembling of individual nanocrystals in a disorderly way. The structure of the particle is different from the dense structure of spray-dried solid-sphere particles [11]. It is suggested that there is a larger space in an assembled spherical particle compared to that of spray-dried solid-sphere particles. Fig. 1b shows the lower magnification image of the particles. The particles with a narrow range of diameter are well separated. Table I shows the properties of the self-assembled HAp particles determined from SEM image and the BET method. The average diameter and standard deviation of the self-assembled particles calculated from 100 of them in the SEM images indicate 1.93 ± 0.35 μm. Luo and Nieh prepared HAp particles having a narrow particle size range of 0.33–2.30 μm (1.37 μm of average diameter) by spray-drying at a pressure of 5 kg cm^{-2} [11]. The size of our assembled HAp particles shows a narrower range compared to that of the spray-dried solid-sphere particles. The specific surface area of the self-assembled HAp particles, on the other hand, is 6.99 $m^2\,g^{-1}$, determined by the BET method. The specific surface area of an ideal solid sphere of HAp with a 1.93 μm in diameter was estimated to be 0.98 $m^2\,g^{-1}$ as 3.16 g cm^{-3} of the density [12]. Our self-assembled HAp particles had a larger specific surface area from these data. It is thus suggested that the particle has a porous structure, which agrees with the SEM image. The self-assembled HAp spherical particles could not be prepared in solvent mixture ether than the volume ratio of 9/1(toluene/methanol). The self-assembling sphere morphology seems to depend on a delicate balance of the ratio of the solvent mixture. The electrostatic repulsive force between amino groups on the HAp crystals and the solvent is assumed to be the driving force forming the self-assembling spheres. It is thought that the crystal size of HAp also might be associated with the particle formation. The self-assembled particle is very unique as a DDS carrier because it can hold drugs in the space of the particle and also fracture into smaller parts with single crystals going to an object organ or tissue in the living body by physical stimulations, such as, ultrasonic waves.

In conclusion, self-assembled spherical particles consisting of HAp nanocrystals modified by amino groups were prepared in organic mixture of solvents. The particles show a porous structure and a narrow range of diameter size. The preparation method is very unique because a large-scale apparatus is not necessary. We are now examining in detail the mechanism of the formation of spherical self-assembling HAp nanocrystals modified by amino groups.

TABLE I Characterization of assembled HAp particles

Mean diameter (μm)[a]	Standard deviation of diameters (μm)[a]	Specific surface area ($m^2\,g^{-1}$)
1.93	0.35	6.99

[a]The data were determined from 100 particles by TEM observation.

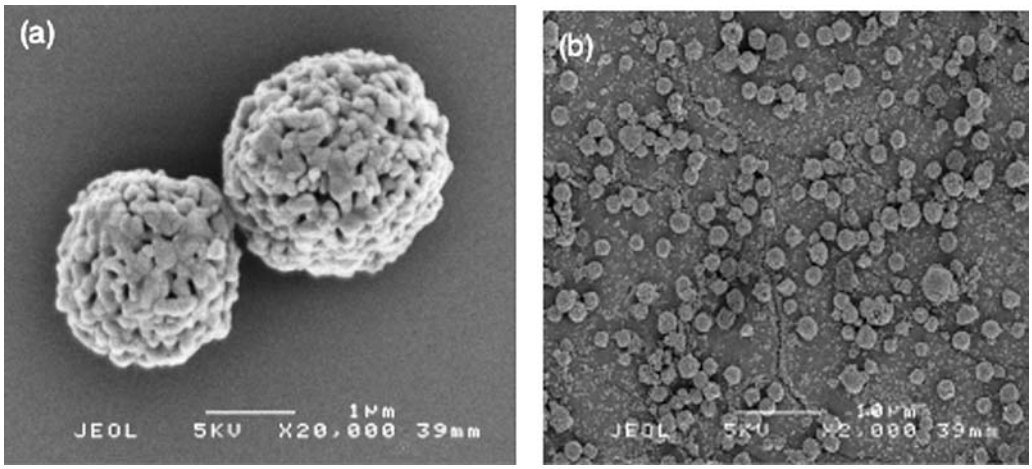

Figure 1 SEM images of self-assembled HAp particles observed by higher (20 000×; (a)) and lower (2000×; (b)) magnification.

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