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Synthesis of nanocrystalline hydroxyapatite using surfactant template systems: Role of templates in controlling morphology

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ABSTRACT

Hydroxyapatite (HA) nanopowder was synthesized by reverse microemulsion technique using calcium nitrate and phosphoric acid as starting materials in aqueous phase. Cyclohexane, hexane, and isooctane were used as organic solvents, and Dioctyl sulfosuccinate sodium salt (AOT), dodecyl phosphate (DP), NP5 (poly (oxyethylene)$_5$ nonylphenol ether), and NP12 (poly(oxyethylene)$_{12}$ nonylphenol ether) as surfactants to make the emulsion. Effect of synthesis parameters, such as type of surfactant, aqueous to organic ratio (A/O), pH and temperature on powder characteristics were studied. It was found that the surfactant templates played a significant role in regulating the morphology of the nanoparticle. Hydroxyapatite nanoparticle of different morphologies such as spherical, needle shape or rod-like were obtained by adjusting the conditions of the emulsion system. Synthesized powder was characterized using X-ray diffraction (XRD), BET surface area and transmission electron microscopy (TEM). Phase pure HA nanopowder with highest surface area of 121 m^2/g were prepared by this technique using NP5 as a surfactant. Densification studies showed that this nanoparticle can give about 98% of their theoretical density. *In vitro* bioactivity of the dense HA compacts was confirmed by excellent apatite layer formation after 21 days in SBF solution. Cell material interaction study showed good cell attachment and after 5 days cells were proliferated on HA compacts in OPC1 cell culture medium. The results imply this to be a versatile approach for making hydroxyapatite nanocrystals with controlled morphology and excellent biocompatibility.

1. Introduction

Hydroxyapatite (HA) is a compound of great interest in many fields. Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) is a calcium phosphate based inorganic material and a major mineral component of human bones and teeth [1]. Synthetic HA has excellent biocompatibility and bioactivity; and widely used in non-load bearing application such as implants and coating onto prostheses [2,3]. For successful application of HA based ceramics as bone grafts, higher strength and toughness are desirable. Nanostructured material can improve the sinterability due to high surface energy and, therefore, improves mechanical properties [4]. However, sintering behavior not only depends on the particle size but also on particle size distribution and morphology of the powder particle [5]. HA has several other applications such as filters for heavy metals from aqueous solution [6], in high performance liquid chromatography (HPLC) for separation of protein and nucleic acid [7], and in gas sensors as well as in catalysts [8]. The performance of HA in these areas is largely determined by specific surface area and therefore nanostructured HA with high surface area is highly desirable for these applications.

HA can be synthesized by a variety of processes including solid-state reaction [9] and wet chemical methods such as sol–gel [10–12], coprecipitation [13,14], and hydrothermal [15]. Recently, surfactant based emulsion systems have shown a lot of promise for the synthesis of nanoparticle with controlled morphology [16–21]. Emulsion is a stable suspension of two immiscible liquids such as water and oil. Addition of surfactant stabilizes the emulsion by reducing the surface tension of the immiscible liquid and by formation of nanosized liquid droplets. Synthesis of nanopowder takes place within these droplets. Since each droplet acts as an independent micro or nanoreactor, the droplet size and shape can control the morphology and particle size of the final powder. The emulsion process strongly depends on the type of surfactant used and concentration of the surfactant present in the liquid medium. Several attempts have been made to control morphology of the HA nanopowder using ionic [22,23] and nonionic [24] surfactant based systems. In our previous study [25], we have reported synthesis of HA nanopowder with controlled morphology using mixed surfactant system of NP5 and NP12 surfactants. The study revealed that the morphology of HA nanopowder can be controlled by controlling synthesis parameters and HA particle less than 100 nm were synthesized. Cao et al. have reported HA nanofiber with ultrahigh aspect ratio of above 1000, using cetyltrimethyl ammonium bromide (CTAB) surfactant [26]. Uota et al. have reported synthesis

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of HA nanopowder with a very high specific surface area of 364 m^2 g^{-1} using nonaoxyethylene dodecyl ether and polyoxyethylene [20] sorbitan monostearate [27]. Lim et al. [28,29] have reported synthesis of HA nanopowder with particle size less than 100 nm using nonionic Empilan KB6Z and mixed poly(oxyethylene)$_5$ nonyl phenol ether (NP5) and poly(oxyethylene)$_9$ nonyl phenol ether (NP9) as surfactant.

In the present study, we are reporting the synthesis of HA nanopowder using different surfactant systems to examine the influence of the type of surfactant (ionic or nonionic) on the formation of calcium phosphates. Dioctyl sulfosuccinate sodium salt (AOT), dodecyl phosphate (DP), NP5, and NP12 were used as surfactants. The ability to manipulate the morphology of HA nanopowder by promotion and inhibition of crystal growth has been investigated in detail by studying the effects of parameters such as different surfactants, aqueous to organic ratio, aging time, and Ca^{2+} concentration on phase formation. The effects of interactions between the head group of different surfactants and inorganic ions/crystal surfaces in controlling the nanopowder morphological features were also discussed. We have performed preliminary studies on densification, mineralization in SBF and cytotoxicity test with osteoblast cell (OPC1) with the compacts made with synthesized HA nanopowder.

2. Experimental

2.1. Synthesis of HA

Calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$, Alfa Aeser, Ward Hill, MA, USA) and orthophosphoric acid (H_3PO_4, Fisher Scientific, PA, USA) were used as starting materials for preparing the precursors of HA powder. Isooctane (Fisher Scientific, PA, USA) was used as solvent to make the emulsion with AOT Dioctyl sulfosuccinate sodium salt ($C_{20}H_{37}NaO_7S$, Aldrich Chemical, WI, USA) as surfactant. All chemicals were used without any further purification. 0.02 M AOT solution in isooctane was prepared to make the organic phase. Hexane (Fisher Scientific, PA, USA) was used as solvent to make the emulsion with dodecyl phosphate (DP, $C_{12}H_{27}O_4P$, Alfa Aeser, Ward Hill, MA, USA) as surfactant. 0.02 M dodecyl phosphate solution in hexane was prepared to make the organic phase. Cyclohexane (Fisher Scientific, PA, USA) was used as an organic solvent to make the emulsion with NP5 (poly(oxyethylene)$_5$ nonylphenol ether, 4-$(C_9H_{19})C_6H_4$-$(OCH_2CH_2)_nOH$, n~5) and NP12 (poly(oxyethylene)$_{12}$ nonylphenol ether, 4-$(C_9H_{19})C_6H_4$-$(OCH_2CH_2)_nOH$, n~12) (Aldrich Chemical, WI, USA) as surfactant. 10 vol.% of surfactant (NP5 or NP12) was added to cyclohexane to make the organic phase. Aqueous solutions of calcium nitrate and phosphoric acid were made by dissolving the required amount in deionized water. Calcium nitrate concentration in the solution was maintained at 5.0, 3.0, and 1.0 M. Calcium to phosphorus molar ratio in the precursor solution was maintained at 1.67 according to the composition of HA. Aqueous phase and organic phase were mixed according to the volume ratio of 1:5, 1:10, and 1:15 to obtain the reverse micelle. The pH of the emulsion was adjusted to 7 and 9 by drop wise addition of ammonium hydroxide (NH_4OH, J. T. baker, NJ) with continuous stirring. The emulsion was kept at room temperature (~25 °C) for different time durations to study the aging effect. It was then evaporated on the hot plate at ~150 °C. Evaporated mass was then dried on the hotplate at ~400 °C for a couple of hours to obtain the precursor powder. Effect of calcinations temperature on powder characteristics was studied for 450 and 650 °C in a muffle furnace.

2.2. Phase analysis

Phase analysis was done by X-ray diffraction analysis using a fully automated Philips X-ray diffractometer with a Ni-filter using Co K_a radiation. 2θ angles ranged from 20 to 70° at a step size of 0.02° (2θ) and a count time of 0.5 s per step. Specific surface area analysis was done using a five-point BET surface area analyzer (Tristar Micromeritics, GA). Powder morphology was investigated using a transmission electron microscope (TEM, JEM 120, JEOL USA Inc., MA).

2.3. Densification study

Densification was carried out with compacts made with HA nanopowder after they were uniaxially pressed and then they were sintered at 1250 °C for 2 h in a muffle furnace. Microstructure analysis of the sintered compacts was done using scanning electron microscopy (SEM, Hitachi S570, Hitachi scientific Instrument, CA).

2.4. In vitro mineralization study by simulated body fluids (SBF) immersion

Mineralization study was performed with sintered HA nanopowder compacts in simulated body fluid (SBF) for 21 days. SBF was prepared by dissolving required amount of different salts followed by pH adjustment to 7.35 [30]. SBF solution was replaced with a freshly prepared solution at an interval of every 4 days.

2.5. Morphology of OPC1 cells on samples

In vitro analyses were carried on using a modified human osteoblast (HOB) cell line (OPC1). OPC1 is a conditionally immortalized osteoprecursor cell line derived from human fetal bone tissue [31]. OPC1 cells were cultured in a standard medium made of McCoy's 5A (with L-glutamine, without phenol red and sodium bicarbonate) [Sigma Chemical Co, Saint Louis, MO, USA], supplemented with 10% fetal bovine serum, 2.2 g/L sodium bicarbonate, 0.1 g/L penicillin and 0.1 g/L streptomycin and 8 μg/mL Fungizone (Gibco Laboratories, Grand Island, NY). Cells were removed from cell culture dishes with trypsin and split into 1:2 ratio, 3 days prior to use. Sintered compacts of HA nanopowder were sterilized via autoclaving and then seeded with OPC1 cells and cultured under standard aseptic conditions. After 5 days, samples were dried, stained and observed under SEM.

3. Results and discussion

In the presence of surfactants, mixing of aqueous and organic phases leads to the formation of transparent emulsion if the conditions are optimized. The ability of surfactant molecules to self-assemble into a well defined structure has provided researchers an advantage to design and synthesize inorganic materials with nanosized dimensions. The structures formed within emulsion by self-assembly of the surfactant molecules are used as a kind of template for the synthesis. However the final product can vary greatly with the ratios of inorganic cation to anion, ratios of water and oil, and the nature of surfactant. Ionic surfactants, such as AOT and DP and two nonionic surfactants NP5 and NP12, differing in the number of oxyethylene groups were used to evaluate the most suitable surfactant for the synthesis of crystalline monophasic HA nanopowder with high surface area. Experiments were conducted by varying synthesis parameters such as pH, aqueous/organic phase volume ratio (A/O ratio) in the reverse micelle, aging time and metal ion concentration in the aqueous phase to investigate the effect of various parameters on the morphology of the HA nanoparticle.

3.1. Effect of pH on crystallinity

The pH of the reverse micro emulsion was found to have a significant influence on the formation of crystalline HA. The emulsion pH after mixing aqueous and organic phases was found to be ~2.0. The resulting powder from this condition having any one of the surfactant exhibited average BET specific average surface area in the range of 5–15 m^2 g^{-1}. X-ray diffraction analysis of these powder indicated that