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Synthesis of nanosized hydroxyapatite/agarose powders for bone filler and drug delivery application

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ABSTRACT

Drug-loaded bioactive composite powders are used for the treatment of orthopedic diseases and prevention of infection or inflammatory reaction after surgical implantation. Nanosized ($80 \times 23\ nm^2$) and porous (17 ± 1 nm) hydroxyapatite (HAp)/agarose composite rods were prepared by sol-gel synthesis and subjected to microwave and conventional heating. Microwave heating increased the degree of crystallinity and the thermal stability and produced calcium-deficient HAp/agarose composite powders. There was a considerable reduction (by 39%) in the size of rods on microwave heating whereas the conventional heating at 700 °C rendered the samples porous and agglomerated with a significant decrease in the specific surface area. The agarose contents in as-synthesized and microwave heated samples were ~14% and 4%, respectively. The samples were partially degradable upon immersion in SBF, and later exhibited calcium phosphate deposition which was confirmed by gravimetry. An antibiotic (amoxicillin) and anticancer (5-fluorouracil) drug-loaded microwave-heated nanosized HAp/agarose composite powder gave an extended drug release when compared to the as-synthesized and the conventionally heated samples. The composite powders showed a negative zeta potential, hemocompatibility and better antimicrobial efficacy than pure HAp (conventional heated sample). The microwave heating retained the organic phase (agarose) along with a reduction in particle size. In addition, this technique is simple, fast and cost-effective to produce mesoporous, bioactive and resorbable nanocomposite (HAp/agarose) powders which could find application as bone filling materials and drug delivery systems.

1. Introduction

Nowadays, there is an increasing incidence of bone-related diseases such as osteomyelitis (infection) and cancer [1]. Osteomyelitis is an inflammation of bone or bone marrow, usually caused by bacteria or fungi. These microorganisms infect and spread to the bones and adjacent areas through the blood stream [2]. The treatment of these diseases consists of chemotherapy, tissue transfers, bone grafting and the implantation of antibiotic-loaded biocompatible materials (beads, paste or solids). Infection is a major problem in the medical field due to poor accessibility of the infected site by systemically administered antibiotics [3]. Hence, an efficient and controlled local drug delivery system has to be developed, and current research efforts are directed to develop novel drug storage and release systems [3]. The use of nanobiomaterials such as biodegradable polymers [4], xerogels [5], hydrogels [6], mesoporous silica [7], calcium phosphate (hydroxyapatite) [8] and polymer composites [9] as drug carriers leads to greater efficiency, safety, biocompatibility and provides a better therapeutic response due to controlled and prolonged release of the drugs [4].

Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, HAp, is the major inorganic component of bone and teeth. Nanosized HAp has a considerable potential to be used as implant, prosthetic bone replacement and as protein and drug delivery system [8,10]. Heated HAp has a lower bioactivity and is brittle in nature; hence it is not suitable for load bearing applications. The powder form of HAp is good for filling bone cracks and small irregular defects. However, there will be a migration of HAp particles from the implants. It is difficult to handle and keep the implant compact in the defect site [11,12]. Thus, it is necessary to mix a suitable biocompatible polymer with the HAp granules to overcome these drawbacks. HAp composites have attracted much attention as the presence of HAp in the

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composite material enhances the proliferation of osteoblasts, resulting in a better osteoconductivity [13,14]. Composites based on degradable biopolymers such as collagen [15], fibrin glue, gelatin, chitosan, alginate and hydroxy-propyl-methyl cellulose with inorganic powders were reported as bone fillers [16].

Agarose is a natural, thermally responsive polysaccharide that is widely used in biological sciences (*e.g.*, microbial cultivation and gel electrophoresis) [17]. It is a linear polymer behaving like a hydrogel, allowing rapid room temperature polymerization [18,19]. Studies have demonstrated the suitability of agarose scaffolds for promoting stem cell differentiation into chondrocytes [20]. Tabata et al. [21], and Suzawa et al. [22] reported that HAp/agarose and calcium carbonate/agarose scaffolds had better healing properties than pure HAp.

The semi-synthetic orally administered broad-spectrum antibiotic drug amoxicillin (AMX) is extensively used against bacterial infections. A slow and continuous release of the drug during bone implantation is essential to prevent infectious diseases. A slow and continuous release from the drug-loaded calcium phosphates, porous HAp blocks and HAp coated on metals was previously reported [23–25]. 5-Fluorouracil (5-Fcil) is an antineoplastic drug which is used in the treatment of cancer. It is an acidic, water-soluble and hydrophilic drug used in chemotherapy for the treatment of solid tumors [26,27]. To the best of our knowledge, there are no reports on the synthesis of nanocrystalline HAp/agarose composite powders by microwave heating. Here, we report the synthesis of nanosized HAp/agarose composite by a pH-controlled sol-gel technique. In addition, the effects of microwave and conventional heating on the biological and drug release properties were investigated.

2. Experimental methods

2.1. Material preparation

The powder form of nanosized HAp/agarose composite was prepared by an ethanol-based sol-gel technique, followed by microwave treatment. Calcium nitrate tetrahydrate $Ca(NO_3)_2{\cdot}4H_2O$ (Merck), diammonium hydrogen phosphate $(NH_4)_2HPO_4$ (Merck), agarose (SRL), ethanol (Merck) and aqueous ammonia solution (Merck) were used for the synthesis without further purification. 0.3 M diammonium hydrogen phosphate (9.91 g) was dissolved in 250 mL of ethanol, heated to 85 ± 5 °C and subjected to vigorous stirring. Then, 1 wt% of agarose was added to the phosphate solution. 0.5 M calcium nitrate tetrahydrate (29.51 g) was dissolved in 250 mL ethanol which was added to phosphate/agarose solution at constant flow rate (2 mL/min) under continuous stirring. After mixing, the solution was continuously stirred for 3 h. The pH of the solution was maintained at 10.5 by the addition of aqueous ammonia solution with a pH stat instrument (Radiometer analytical). A constant temperature of 85 ± 5 °C was maintained until the end of the reaction. The reaction mixture was refluxed until the completion of the reaction to avoid the evaporation of the solvent ethanol/water. The solution was vigorously stirred, aged for a day and final precipitates washed with deionized water. The colloidal precipitates were centrifuged at 3000 rpm and dried at 70 °C in air. The same procedure was applied to prepare colloidal precipitates and it was subjected to microwave heating at 900 W for 30 min (Whirlpool model magicook). The as-synthesized powder was subjected to conventional heating at 700 °C in air for 2 h. After heating, a color change was observed on the powders (brown to white) due to the decomposition of agarose. The schematic illustration for preparation of HAp/agarose composite by sol-gel synthesis and subjected to heating by microwave and conventional is shown in Fig. 1. The as-synthesized samples, microwave and conventionally heated samples are referred to as SAS, SMWT and SAS700, respectively in the following.

2.2. Characterization

Powder X-ray diffraction patterns were recorded with a Siemens D500 diffractometer (CuKα radiation, λ = 1.5406 Å, 40 kV, 20 mA, 5–70 degree 2θ, increment steps of 0.02 degree 2θ). The diffraction peaks were indexed and its full width half maximum was analyzed with the XRDA software [28]. Further, the crystallinity was examined by empirical relation between X_c and β_z *i.e.* $\beta_z \times (X_c)^{1/3} = K_A$ with X_c the degree of crystallinity, β_z the full width at half maximum of (002) plane in (°2θ), and K_A a constant set to 0.24 [29]. Fourier transform infrared (FTIR) spectra were recorded in the range of 400–4000 cm^{-1} with a Perkin-Elmer spectrometer RXI FTIR with KBr pellets. The surface morphology and the elements present in the samples were studied by scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDX) ESEM Quanta 400 FEG, FEI; gold-palladium [80:20]-sputtered samples; EDX detector: S-UTW-Si (Li) [30]. Transmission electron microscopy TEM was performed using a JEOL 2100 microscopy operating at 200 kV with 0.1 nm point resolution and equipped with a Gatan US1000, 2048 × 2048 pixel CCD camera. 10 mg of synthesized samples were dispersed in 5 mL ethanol using probe type sonicator for 5 min and deposited on an Augar

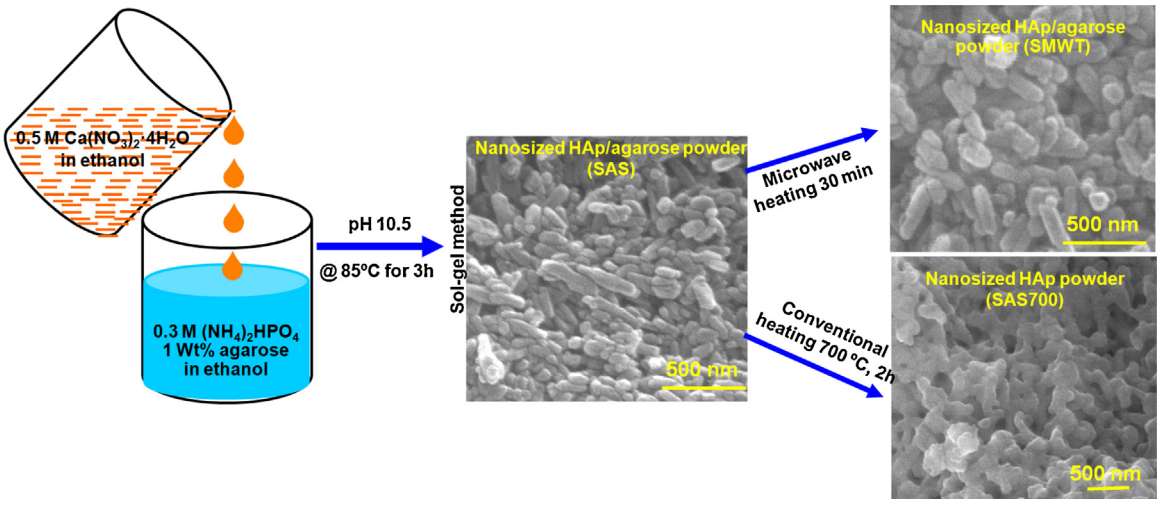

Fig. 1. Schematic illustration for synthesis of nanosized HAp/agarose powder by sol-gel synthesis and subjected to heating by microwave and conventional.