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Formation of nanostructured fluorapatite via microwave assisted solution combustion synthesis

Maryam Nabiyouni [a], Huan Zhou [b,c,*], Timothy J.F. Luchini [d], Sarit B. Bhaduri [c,e]

[a] Department of Bioengineering, The University of Toledo, Toledo, OH, USA
[b] Institute of Biomedical Engineering and Health Sciences, Changzhou University, Changzhou, Jiangsu, China
[c] Department of Mechanical, Industrial and Manufacturing Engineering, The University of Toledo, Toledo, OH, USA
[d] Composite Vehicle Research Center, Michigan State University, East Lansing, MI, USA
[e] Division of Dentistry, The University of Toledo, Toledo, OH, USA

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ABSTRACT

Fluorapatite (FA) has potential applications in dentistry and orthopedics, but its synthesis procedures are time consuming. The goal of the present study is to develop a quick microwave assisted solution combustion synthesis method (MASCS) for the production of FA particles. With this new processing, FA particles were successfully synthesized in minutes. Additionally, unique structures including nanotubes, hexagonal crystals, nanowhiskers, and plate agglomerates were prepared by controlling the solution composition and reaction time. In particular, the as-synthesized FA nanotubes presented a "Y" shape inner channel along the crystal axis. It is supposed that the channel formation is caused by the crystal growth and removal of water soluble salts during processing. The as-synthesized FA nanotubes showed good cytocompatibility, the cells cultured with a higher FA concentration demonstrated greater growth rate. With this new and easily applied MASCS processing application, FA nanoparticles have increased potential in dental and orthopedic applications.

1. Introduction

Apatitic mineral phase makes up 95–96 wt.% of dental enamel along with water (3 wt.%), and organic matter (1 wt.%) [1]. Though, calcium phosphate salts like hexagonal hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HA) account for the majority of the apatite forms in human enamel, natural teeth always contain some fluoride (F), in the form of fluorapatite ($Ca_{10}(PO_4)_6\ F_2$, FA) or fluor-hydroxyapatite ($Ca_{10}(PO_4)_6(OH,F)_2$, FHA) [2,3].

Both HA and FA crystals are composed of six PO_4^{3-} groups surrounded by ten Ca^{2+} ions with two OH^- or F^- ions located along the c axis. The distinguishing features of two apatites are the presence of either ion along the c axis, the position and orientation of the ion with respect to the nearby calcium atoms, and the cell volume [4]. The F^- ion has a smaller size than OH^-, 1.28 Å compared to 1.37 Å. As a result, F^- ions can pack more closely resulting in smaller cell volume [5].

Synthetic FAs are harder and have slightly better thermal stability than the original apatite phase of human teeth [6,7]. Great stiffness, significant resistance to acid damage, and suitable biocompatibility of FAs make them potential candidates in restorative dentistry. The application of FA could come in different forms like crowns, inlays, dentin simulators, coatings and cements [7–10]. In addition to dentistry, it is also worth noting that FA is important in orthopedics to promote bone regeneration [11–15].

In reviewing the literature, it is reported that FA can be synthesized via different techniques including the sol–gel process [16–18], wet-chemical processing [19,20], solid-state reaction [21,22], and the hydrothermal process [9,19,23]. Details of all synthesis techniques and resulting features of the as-synthesized FA are summarized in Table 1. However, the common limitation of all reported technologies is that they are time consuming.

Our group has made great strides in synthesizing calcium phosphate whiskers via a rapid microwave-assisted solution combustion synthesis (MASCS) process [24–26]. In the process, aqueous solutions containing $NaNO_3$, HNO_3, $Ca(NO_3)_2.4H_2O$, KH_2PO_4 and different additives were irradiated in a household microwave oven for 5 min. The solidified substances were then simply stirred in water at room temperature to obtain the whiskers of the desired CaP phase including HA, tricalcium phosphate ($Ca_3(PO4)_2$, TCP), biphasic calcium phosphate (BCP), and chlorapatite ($Ca_{10}(PO_4)_6Cl_2$, CA) [24–26]. In the present work, MASCS was used to efficiently produce FA nanoparticles with unique features.

* Corresponding author at: Institute of Biomedical Engineering and Health Sciences, Changzhou University, Changzhou, Jiangsu, China. Tel.: +86 1 419 530 8223, fax: +86 1 419 530 8206.
E-mail address: Huan.Zhou@rockets.utoledo.edu (H. Zhou).

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Table 1
Summary of FA synthesis technologies.

Method	Reactants	Time	Structure	Ref.
Sol–gel	$(C_2H_5O)_3P$, NH_4F, $Ca(NO_3)_2.4H_2O$, ethanol	>8 days	100 nm spherical particles	[16]
Sol–gel	$(NH_4)_2HPO_4$, NH_4F, $Ca(NO_3)_2.4H_2O$, H_2O	21 h	N/A	[17]
Sol–gel	$CaCl_2.6H_2O$, $Na_2HPO_4.12H_2O$, KF, H_2O, octane, surfactant	52 h	20–180 nm particles	[18]
Sol–gel	$CaCl_2.6H_2O$, $Na_2HPO_4.12H_2O$, KF, H_2O, diethyleneglycol	>28 h	100–300 nm particles	[18]
Wet-chemical	$Ca_{10}(PO_4)_6(OH)_2$, NaF, H_2O, HNO_3, $NH_3.H_2O$	>5 days	20–50 nm nanorods/nanowires	[19]
Wet-chemical	$Ca(NO_3)_2$, KH_2PO_4, KF, acrylamide, polyethylene oxide, polyacrylamide, N,N,N′N′-tetramethylenediamine	>4 days	300–1000 nm hexagonal crystals	[20]
Solid-state	CaF_2, $Ca_3(PO4)_2$	>2 h	plate-like irregular nano particles	[21,22]
Hydrothermal	$CaHPO_4$, Na_2HPO_4, NaH_2PO_4, KF	>84 h	N/A	[9]
Hydrothermal	$Ca_{10}(PO_4)_6(OH)_2$, NaF, H_2O, HNO_3, EDTA-$Na_4.4H_2O$	>6 h	1–5 μm nanorods/nanowires	[19]
Hydrothermal	$Ca(NO_3)_2$, NaF, NaH_2PO_4, poly(acrylic acid)	1 h	152 (24) × 38 (6) nm nanospindles	[23]

Some of these features include FA nanoparticles with high aspect ratio and crystallinity and the nanoparticles can be synthesized using a simple process, not previously offered by other reported techniques.

2. Experiments

2.1. Synthesis

Reagents for whisker synthesis such as $NaNO_3$, NaF, $Ca(NO_3)_2.4H_2O$, and KH_2PO_4 were purchased from Fisher Scientific, USA. In addition, CaF_2 was purchased from Sigma–Aldrich, USA. Different experimental conditions, such as compositions and durations, were studied in order to find the best composition and optimum condition to produce FA whiskers (Table 2). CaF_2 and NaF were both used as sources for F^- in reaction. The difference is NaF is water-soluble and CaF_2 is insoluble. The reaction solution was prepared by adding chemicals one by one to 10 mL de-ionized water in a 30 mL beaker with 200 rpm stirring. The final mixture was a clear solution with salts in suspension, although excess CaF_2 particles were visible.

After 10 min of stirring, beakers containing the mixture were placed in a household microwave oven (700 W, Kenmore, USA) and were covered with a 250 mL inverted Pyrex beaker. The assembly was then heated at 100% power for 3, 5, or 7 min separately. Resulting substances were left to air-dry for 15 min. The 30 mL beakers were placed in a 250 mL Pyrex beaker containing 100 mL of water to cool and solidify completely. Air-dried substances were magnetically stirred at 400 rpm in 500 mL of de-ionized water to suspend formed particles and dissolve water-soluble salts. Finally, the solution was washed with approximately 2 L of de-ionized water and filtered using a filter paper (Whatman Grade 5, Fisher Scientific, USA). The filtrate was then placed in an 80 °C oven overnight for further characterization and evaluation.

2.2. Characterization

The as-synthesized particles were first visualized by scanning electron microscope (SEM, S4800, Hitachi, USA) to determine which composition resulted in the desired one-dimensional structure. Inner structures of FA particles were investigated using transmission electron microscopy (TEM, HD-2300, Hitachi, USA) with a voltage of 200 kV. The phase compositions of particles were characterized by X-ray diffraction (XRD, Ultima III, Rigaku, USA) with monochromated Cu Kα radiation, setting the operating conditions at a voltage of 40 kV and a current of 44 mA. Particles were examined at 2θ angles from 10° to 60° at a scanning speed of 1° per minute. To identify the phases present in the sample, the XRD pattern of the sample was compared with every calculated pattern in a Powder Diffraction File (PDF) database from the International Center for Diffraction Data (ICDD). Using the search-match capabilities of the JADE (MDI, USA) XRD software and the ICDD-PDF database, all phases present in the samples were identified. Fourier transform infrared spectroscopy (FTIR, UMA-600 Microscope, Varian Excalibur Series, USA) was applied for chemical analysis of FA particles. The transmittance of samples was recorded with 256 scans with resolution of 4 cm^{-1} between 4000 and 400 cm^{-1}. Zeta potential measurements were performed using a Zeta Potential/Particle Sizer (Nicomp 380ZLS, Particle Sizing Systems, USA) to measure the surface charge of samples. The measurements were performed using the multi-angle square cell setup and the photon source was set on photomultiplier tubes only. The zeta potentials were determined by measuring the electrophoretic movements of charged particles under the applied electric field.

In order to examine stability of FA particles in physiological conditions, the particles were incubated in simulated body fluid (SBF) at 37 °C environmental conditions. Composition of the as-prepared SBF is shown in Table 3 [27,28]. The solution was replenished every other day. After 7 days, the incubated FA particles were dried and characterized using SEM coupled with energy dispersive spectrometry (EDS).

2.3. Preosteoblast culture

Preosteoblast cells (MC3T3-E1, CRL-2593™, ATCC, USA) were used to study the indirect effect of FA on preosteoblast proliferation and differentiation. Centrifuge tubes containing 25, 50, 100, and 150 mg/mL FA in alpha minimum essential medium (α-MEM, Thermo Scientific HyClone) were incubated overnight prior to cell seeding. Cells were initially grown at 37 °C and 5% CO_2 in α-MEM, augmented by 10% fetal bovine serum (FBS, Thermo Scientific HyClone, USA). The culture medium was replenished every other day until the cells reached 90% confluency. To study cell proliferation, MC3T3-E1 cells were seeded to wells (Flacon™ 12 wells cell culture plates, BD Biosciences, USA) at a density of 10,000 cells/well. Immediately after seeding cells, 50 μL of FA containing α-MEM with respective FA concentration was added to the wells together with 500 μL cell culture medium. Cell density was measured after 24 h, and 7 days using CytoTox 96® Non-Radioactive Cytotoxicity Assay kit (Promega, USA). For statistical analysis, all

Table 2
Reaction solution compositions and microwave irradiation time for FA synthesis.

Experimental conditions	H_2O (mL)	$NaNO_3$ (g)	CaF_2 (g)	NaF (g)	$Ca(NO_3)_2.4H_2O$ (g)	KH_2PO_4 (g)	HNO_3 (mL)	MW time (min)
FA-1	10	5.00	1.00	–	1.00	0.384	1.20	5
FA-2	10	5.00	–	0.53	1.00	0.384	1.20	5
FA-3	10	5.00	1.00	–	1.00	0.384	1.20	7
FA-4	10	5.00	1.00	–	1.00	0.384	1.20	3
FA-5	10	5.00	0.70	–	1.00	0.384	1.20	3