Double Emulsion Droplets as Microreactors for Synthesis of Mesoporous Hydroxyapatite

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Double Emulsion Droplets as Microreactors

ABSTRACT We introduce a novel approach for synthesizing mesoporous hydroxyapatite (HAp, (Ca)_{10}, (PO_4)_6(OH)_2) using double emulsion droplets as microreactors. By using capillary microfluidic techniques, the size and the geometry of the droplet microreactors can be tuned easily. Double emulsion droplets offer the combined advantages of both shielding the reactant and on-demand addition of reactant; this makes them highly...
versatile microreactors. Such droplet microreactors also enable simple visualization of the HAp formation process as well as control over the porosity in the HAp that is synthesized. Powder formed with our technique demonstrates a remarkable microstructure, as well as significantly enhanced BET specific average surface area and nanoscale porosity. Our results present a novel synthesis approach for controlling the nanoscale porosity and the morphology of inorganic particles using double emulsion droplets.

Introduction

The biocompatibility and chemical similarity to bone makes calcium phosphate (CaP) based ceramic materials, especially hydroxyapatite (HAp), a widely used material of choice for orthopedic, dental and other bio-related applications.1-3 To mimic the functions of their counterparts in natural biological systems, CaP-based materials are often used in conjunction with different biomolecules to provide surfaces for adsorption and catalysis of biochemical reactions.3-5 The adsorption of biomolecules onto the surface of these CaP-based materials depends critically on their structural properties such as microstructures, surface area and porosity.6 For example, introduction of nanosized pores in these materials can not only significantly enhance the total surface area, but can also promote integration of biomolecules and drugs with the particles. Various synthesis and processing techniques have been developed for these CaP-based materials, including solid-state-reaction 7, coprecipitation 8, 9, sol-gel 10-16, hydrothermal 17-19 and surfactant-templated 20-22 methods. While CaPs prepared with these techniques show improved
mechanical and surface properties as well as good bioactivity, agglomeration of final powders cannot be easily controlled.\textsuperscript{10} To control its morphology and shape, HAp nanopowder has been synthesized inside microemulsion droplets or reverse micelles, which act as microreactors separated from each other by an organic continuous phase.\textsuperscript{23-27} However, it remains difficult to produce HAp nanopowder with very high surface area, as determined by the Brunauer-Emmett-Teller (BET) techniques; this is advantageous for both densification as well as bone morphogenic protein or drug delivery applications when synthetic CaPs are used in treatments for bone related disorders. More importantly, most conventional methods involve intermediate processing steps that prevent in-situ visualization of formation of the inorganic nanoparticles. As a result, the exact mechanism for the formation of CaPs from its precursor materials is still not well understood.

A novel strategy to fabricate Hap nanopowder is to utilize droplets as reactors, each of which can be monitored individually. The homogeneity of the final powders is governed by the uniformity of the droplets. By flowing fluids through microchannels of hundreds of microns in size, microfluidic emulsification offers a means to prepare emulsions with a narrow size distribution by providing a high degree of control over the emulsion generation process.\textsuperscript{28, 29} The resulting single emulsion droplets have been demonstrated to be ideal templates for fabricating monodisperse particles, highlighting the versatility and controllability of the technique.\textsuperscript{30-34} Moreover, due to relatively larger droplet size compared with conventional emulsion techniques, subsequent processing of the droplets can be easily monitored with microscopy techniques. The high degree of control afforded by the microfluidic apparatus makes it possible to prepare not only single
emulsion droplets, but also multiple emulsions.\textsuperscript{28, 29, 35} For instance, water-in-oil-in-water (W/O/W) double emulsions have been used as templates for assembling amphiphiles to form vesicles with high size uniformity and with high encapsulation efficiency.\textsuperscript{36, 37} While controlled W/O/W double emulsion droplets have been used as templates for fabricating core-shell structures such as colloidosomes \textsuperscript{38} and gel shells \textsuperscript{39}, their use as microreactors has not been extensively explored. Double emulsion droplets are advantageous as microreactors because droplets are prevented from agglomerating with neighboring droplets by the middle oil shell surrounding the reacting core. Additional reactants can also be added to the microreactors by diffusion through the middle oil shells. The abilities to segregate reacting droplets from each other and also to allow additional reactants to be added to the droplets highlight the versatility of double emulsion droplets as microreactors.

In the present work, we synthesize HAp using double emulsion droplets as microreactors. A glass capillary microfluidic set-up is used for generating double emulsion microreactors with calcium and phosphorus precursors encapsulated in the inner aqueous droplet. Formation of HAp is triggered by adding an alkali to the continuous phase to adjust the pH. Since the alkaline solution is miscible with the continuous phase, no additional homogenization treatment is required. Powders obtained with this technique are shown to exhibit unique microstructures with very high surface area and mesoporosity. The double-emulsion-droplet technique represents a novel approach for synthesis of inorganic powders and offers a means to understand the formation mechanism through direct visualization. The technique also opens up a new synthesis
approach for inorganic powder particles in which the morphology and the nanoscale porosity can be controlled.

**Materials and methods**

Water-in-oil-in-water (W/O/W) double emulsion drops were produced using glass microcapillary devices. The inner phase consisted of 0.1-1 M calcium nitrate tetrahydrate (Ca(NO$_3$)$_2$·H$_2$O, ≥ 99.0%, SigmaUltra, St. Louis, Missouri, USA) and 0.071-0.71 M phosphoric acid (H$_3$PO$_4$, orthophosphoric acid, ≥ 85%, Fluka, St. Louis, Missouri, USA) in water. The osmality of the solutions was measured with a micro osmometer (Advanced Instruments, Inc., Model 3300). Unless otherwise noted, the middle oil phase consisted of 2 wt% surfactant (Dow Corning® 749 fluid, Midland, Michigan, USA) in a silicone oil (Dow Corning® 200 Fluid, 5 cSt, Midland, Michigan, USA). The outer phase was a 5 wt% poly vinyl alcohol aqueous solution (PVA; $M_w$: 13000-23000 g·mol$^{-1}$, 87-89% hydrolyzed, Aldrich, St. Louis, Missouri, USA). The surfactant stabilized the inner drops against coalescence with the exterior aqueous phase, while PVA prevented coalescence of the oil drops. The pH of the double emulsion formed was adjusted by adding 0.1 N ammonium hydroxide solution (NH$_4$OH, Fluka, St. Louis, Missouri, USA) to the continuous phase. The outer phase was prepared in some runs by dissolving PVA directly in 0.1 N NH$_4$OH solution to achieve a one-step synthesis of HAp particle aggregatates. A schematic of the process is shown in Fig. 1. Unless otherwise specified, all other chemicals were obtained from Aldrich. Water with a resistivity of 18.2 MΩ cm$^{-1}$ was acquired from a Millipore Milli-Q system (Millipore, Billerica, Massachusetts, USA).
Preparation of double emulsions

Monodisperse W/O/W double emulsions were prepared using glass microcapillary devices, as shown schematically in Fig. 2a. The round capillaries (World Precision Instruments, Inc., Sarasota, Florida), with inner and outer diameters of 0.58 mm and 1.0 mm, respectively, were tapered to desired diameters with a micropipette puller (P-97, Sutter Instrument, Inc.) and a microforge (Narishige International USA, Inc., East Meadow, New York, USA). Two tapered capillaries were aligned inside square glass capillaries (Atlantic International Technology, Inc., Rockaway, New Jersey, USA) with an inner dimension of 1.05 mm. A transparent epoxy resin (5 Minute Epoxy, Devcon, Danvers, Massachusetts, USA) was applied to seal the capillaries where necessary. The outer radii, $R_o$, of the double emulsions ranged from 40 to 80 μm, while the inner radii, $R_i$, ranged from 30 to 70 μm. These values were controlled by the size of the capillaries and the flow rates of the different phases. The calcium and phosphorus precursors were pre-dissolved in the inner phase for subsequent encapsulation in double emulsion drops. Positive syringe pumps (PHD 2000 series, Harvard Apparatus, Holliston, Massachusetts, USA) were used to deliver the different phases at desired flow rates. A typical set of flow rates for the outer, middle and inner phases was 10, 2.2 and 1.2 mL/h respectively and the droplet-generation frequency was about 1000 Hz. Samples were collected between a cover slip and a glass slide separated by a 0.5-mm-thick silicone isolator. The isolator was pre-filled with 0.1 N ammonium hydroxide solution for pH adjustment. The subsequent formation of HAp was monitored with optical microscopy.

Preparation of hydroxyapatite (HAp) powders
To prepare HAp powders, double emulsions with calcium and phosphorus precursors in the inner drops were collected in vials of 0.1 N ammonium hydroxide solution; this triggers the formation of hydroxyapatite. After the vials were completely filled, they were capped and sealed with parafilm. The vials were then left tumbling on a tumbler, during which water continuously diffused from the continuous phase to the inner drops due to an osmotic pressure difference. The double emulsion drops eventually destabilized, releasing the as formed HAp powders. To wash these HAp powders, the remaining oil phase in the vials was removed and the aqueous supernatant was replaced with fresh water. After repeating the washing step for 5 times, the remaining HAp was dried in an oven at 40 °C for 3-7 days to obtain a dry powder for further characterization. The dry powder was characterized both before and after calcination at elevated temperatures.

**Direct visualization of HAp formation in double emulsions**

Optical-microscopy images were obtained with 10×, 40× and 63× objectives at room temperature using an inverted microscope (DMIRBE, Leica, Wetzlar, Germany), an inverted fluorescence microscope (DMIRB, Leica, Wetzlar, Germany) or an upright fluorescence microscope (DMRX, Leica, Wetzlar, Germany) equipped with a high speed camera (Phantom, V5, V7 or V9, Vision Research, Wayne, New Jersey, USA) or a digital camera (QICAM 12-bit, Qimaging, Surrey, British Columbia, Canada). All double-emulsion-generation processes were monitored with the microscope using a high-speed camera. The formation mechanism of HAp from double emulsions and the resulting HAp powders were imaged using a digital camera.
Characterization of HAp powders

Microstructural analysis: Scanning electron microscopic (SEM) images of dried powders were taken using a Zeiss Supra 55VP field emission scanning electron microscope (FESEM, Carl Zeiss, Germany) at an acceleration voltage of 20 kV. Transmission electron microscope (TEM) images were taken on a JEOL JEM-2010 TEM (JEOL, Tokyo, Japan) operated at 200 kV. TEM samples were prepared using a drop of suspension with high volume fraction of as formed HAp on a 200 or 300 mesh copper grid coated with Lacey carbon (Electron Microscopy Sciences, Hatfield, Pennsylvania, USA).

BET specific average surface area analysis: Adsorption and desorption measurements were performed using a Beckman Coulter SA 3100 surface area and pore size analyzer (Beckman Coulter, Fullerton, California, USA) with nitrogen as an adsorbate. The Brunauer-Emmett-Teller (BET) surface areas were calculated from $p/p_0 = 0.3$ in the adsorption curve using the BET equation. The pore size distributions were calculated from the desorption and the adsorption data using the Barrett-Joyner-Halenda (BJH) model. Prior to each sorption measurement, the sample was out-gassed for 1h at 300 °C under vacuum.

Phase analysis: Thermogravemetric analysis (TGA, Q50, TA Instruments, New Castle, Delaware, USA) was performed to determine the phase transformation temperatures for the as-formed powder. Phase analysis of the powders before and after calcinations were performed by powder X-ray diffraction (XRD) using a Scintag XDS2000 fixed sample
position powder diffractometer (Scintag, Cupertino, California, USA) with Cu Kα radiation at 40 kV and 30 mA. The XRD patterns were taken at room temperature in the range of $12° \leq 20 \leq 70°$ with a scan rate of $1°$/minute and step size of 0.02°.

Results and Discussion

Synthesis of mesoporous HAp in double emulsions

Monodisperse W/O/W double emulsions droplets are used as reactors where calcium nitrate and phosphoric acid are stored in the inner droplets for the synthesis of HAp. The synthesis is initiated after the addition of ammonium hydroxide according to the following equation:

$$10 \text{Ca(NO}_3\text{)}_2 + 6 \text{H}_3\text{PO}_4 + 20 \text{NH}_4\text{OH} \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 20 \text{NH}_4\text{NO}_3 + 18 \text{H}_2\text{O}$$

By adjusting the reactant concentration such that the osmotic pressure is always higher in the inner drop than in the continuous phase, water can continuously diffuse into the inner drops. This leads to swelling of the inner drops and a reduction in the thickness of the oil shells. The HAp formed inside the inner drop is eventually released after the oil shells become too thin to stabilize the double emulsion, as shown schematically in Fig. 1.

The reaction is completed immediately after pH adjustment by adding ammonium hydroxide, as suggested by the instantaneous precipitation shown in Fig. 2b. The increase of pH in the inner droplets is made possible by the addition of ammonium hydroxide, which does not dissociate completely in water. Therefore, the undissociated ammonia molecules can diffuse across the hydrophobic oil shell. The originally transparent inner
droplets become turbid as soon as the droplets come into contact with the alkaline solution. No precipitation is observed outside the inner droplets indicating that the timescale for the diffusion of the reactants through the oil shells is larger than that for the pH adjustment and precipitation reaction. The behavior is uniform for all observable droplets. For normal reaction vessels, such as round-bottom flasks and test tubes, reactants are added through an opening in the vessels. In the case of our droplet microreactors, we encapsulate all reactants with low permeabilities through the shell phase of the double emulsion drops in the step of microfluidic emulsification. Reactants that can permeate the shell of the double emulsion, for example, by diffusion, are added as a subsequent step for triggering the reaction.

The size uniformity of the droplet microreactors enables visualization of multiple precipitation reactions simultaneously. Due to high concentration of the reactants in the inner drops, the osmolality is typically much higher in the inner drop. An inner solution with calcium and phosphorus precursor concentrations of 0.5 M and 0.3 M, respectively, has an osmolality of ~ 1600 mOsm compared to ~ 100 mOsm for the outer continuous phase. The resulting osmotic pressure difference causes water to diffuse from the continuous phase to the inner drops, leading to their swelling. The large osmotic pressure difference may also contribute to the slow diffusion of reactants out of the inner drops and the fast pH adjustment in the inner drop due to the addition of ammonium hydroxide in the continuous phase. The solid precipitated structures remain compact without any change in size after swelling, as shown in Fig. 2c. The parts of the precipitates that were bound by the oil-water interface are now freely suspended in the inner aqueous phase, as shown in Fig. 2d. Since the volume of the oil shells, $V_{\text{shell}}$, surrounding the inner drops
remains constant, the thickness of the oil shell, $l$, decreases with the increase in radius of the inner drop, $R$, as $l = \frac{V_{\text{shell}}}{4\pi R^2}$ for small shell thickness. The thickness of the oil shell in Fig. 2e decreased from 18 μm to 4.8 μm after 91 h. As the shell gets thinner, it becomes more likely for the double emulsion droplets to destabilize, releasing the precipitates.

The versatility of the technique can be illustrated by the ease in tuning the micro- and nanoscale porosity of the precipitate structures; these can be adjusted by changing the concentrations of calcium and phosphorus precursors. The precipitate aggregate has an open porous structure when an inner solution with calcium and phosphorus precursor concentrations of 0.1 M and 0.06 M are used, as shown in Fig. 3a. All the individual precipitate particles that form the overall aggregate and their attachment points to one another can be clearly seen. As the calcium precursor concentration is increased to 0.5 M while maintaining the same calcium to phosphorus ratio, the aggregate formed has a denser structure, as shown in Fig. 3b. Individual particles cannot be distinguished anymore except for those near the edge of the precipitate, where the particles span outwards into the solution. As the precursor concentrations are increased further to 1 M and 0.6 M for calcium and phosphorus, respectively, the aggregate structure becomes highly dense with the lowest level of porosity. Based on these results, we believe that the precipitates form when ammonium hydroxide diffuses into the inner droplets. These precipitates then stick with each other to form a larger aggregate. If the starting reactant concentration is low, the collisions that lead to the aggregation are diffusion-limited, leading to the more open structures, as shown in Fig. 3a. By contrast, with a sufficiently high reactant concentration, diffusion-induced collisions are less important in the
formation of the aggregates, and a more dense aggregate structure results, as shown in Fig. 3b. Apart from the porosity, the size of the precipitate formed can also be easily adjusted, by varying the size of the double emulsion droplet reactors, which is controlled with the flow rates and the geometry of the glass capillary microfluidic devices.\textsuperscript{29} Even though only glass microcapillary devices are used in the present study, the concept of using double emulsion droplets as microreactors is applicable to double emulsions generated by other techniques.\textsuperscript{42}

**Properties of HAp powders processed with double emulsions**

Microstructural analysis using SEM and TEM: The powder obtained after washing and drying of the precipitates exhibits unique microstructures with high surface area. After repeated washing of the precipitates with water and then drying at 40 °C, the original spherical aggregates of the precipitates are not retained in the dried powder shown in Fig. 4a. At higher magnification, the powder appears to be made up of 1-µm spherical particles that form clusters as shown in Fig. 4b. It is most likely these spherical particles that are the precipitates that stick with each other to form the aggregates shown in Fig. 3. Small aggregate particles, about 1 µm in diameter, can be seen in the loose aggregate in Fig. 3a and also on the edges of the denser aggregates in Figs. 3b and 3c. At higher magnification, each spherical particle appears to assemble from needle-like nanoparticles connected to each other at the center of the sphere, forming a feathery morphology, as shown in Fig. 4c. The needle-like nanoparticles can be clearly observed in the TEM images shown in Fig. 5. These nanoparticles, which have a length of about 50 nm and a
typical aspect ratio of 10 or more, overlap with each other to form a network as shown in Fig. 5b. The precise nanostructures of the hydroxyapatite formed likely vary with different reaction conditions, such as pH and temperature, as demonstrated in synthesis techniques using surfactants as template.\textsuperscript{27}

BET surface area analysis: The unique morphology of the HAp powders synthesized in the double emulsion droplet reactors gives rise to a high porosity and surface area. For an inner solution with 1 M calcium and 0.6 M phosphorus precursor concentrations, the BET surface area of the powder is 162.8 m\textsuperscript{2}/g; by comparison, powders obtained from bulk reaction typically have a BET surface area of 26.0 m\textsuperscript{2}/g. In the absence of any micellar templates or catalysts, our powder achieves a higher surface area than powders prepared with a hydrothermal method (31-43 m\textsuperscript{2}/g)\textsuperscript{17}, or a reverse micelle synthesis (52 m\textsuperscript{2}/g)\textsuperscript{20} and a surface area comparable with powders prepared with a catalyzed process (173 m\textsuperscript{2}/g).\textsuperscript{43} The nitrogen sorption isotherms for both powders exhibit hysteresis loops, which are characteristic of mesoporous type IV isotherms, as shown in Fig. 6a. However, the volume adsorbed is consistently higher at all relative pressure for the powder prepared in the double emulsion droplet reactors, in agreement with its higher surface area. The total pore volume is 0.499 ml/g for the powder prepared in double-emulsion-droplet reactors, as compared to 0.133 ml/g for the powder prepared in bulk. Apart from having more pore volume, the powder formed in droplets also has a different pore size distribution, as shown in Fig. 6b. While both powders have a large proportion of pores with diameters between 20 nm and 80 nm, the powder prepared in droplets also has about 20\% of its pores with diameters under 6 nm. While most previous studies focus on the engineering of pores in the microscale,\textsuperscript{11, 44-46} our technique offers a novel route to
synthesis HAp powders with nanosized pores. Introduction of nanosized pores significantly enhances the total surface area of the powder. It will also promote integration of the powder with other materials for improved mechanical properties. Nanostructuring can also potentially improve the mechanical properties of hydroxyapatite after pressureless sintering$^{27}$ while microporosity in the hydroxyapatite can facilitate the adsorption of proteins and further the adhesion and proliferation of human bone cells$^{47}$. Biomolecules and drugs can also adsorb onto the powders and interface with each other on the nanometer-sized scale to increase bioactivity.

Phase analysis: For phase and compositional analysis, the powder is heat-treated or calcined at various temperatures. From thermogravimetric analysis (TGA), the powder formed in the droplet reactors first experiences a steady decrease in weight until about 200 °C due to the evaporation of adsorbed water and other low boiling point contaminants, if any, as shown in Fig. 7. At ~ 250 °C, the weight drops at a higher rate until ~ 300 °C. The slope of the curve remains almost the same until about 820 °C where another dip occurs. The weight loss until 820 °C is attributed to carbon loss from the system, due to the high heating rate of 5 °C /min. The weight of the sample then starts to level off at about 86.5% above 850 °C. The sudden sharp drop in slope at 500 °C is due to the 10-minute holding time at the temperature and does not correspond to any change in phase. To study the phase transformations behavior, room temperature XRD has been used to analyze the powder after calcinations at various temperatures. The XRD patterns for powders before and after calcinations at 600 °C appear very similar to those that show signature peaks for HAp (JCPDS # 09-0432), as shown in the curves labeled a and b in Fig. 8. The peaks are wide indicating that the powder is still partially amorphous even
after calcination at 600 °C for 1h. The peaks obtained from the powder after calcination at 800 °C for 1h become significantly sharper, revealing pure crystalline HAp phase, as shown in the curve labeled c in Fig. 8. Interestingly, after the powder has been calcined at 1000 °C for 1h, more peaks appeared in the powder XRD pattern, as shown in the curve labeled d in Fig. 8. A combination of peaks primarily from both HAp and β-TCP (Ca₃(PO₄)₂) (JCPDS # 09-0169) phases are observed with a small amount of α-TCP (JCPDS # 09-0348). If the powder is left for calcination at a slower rate of 5°C/min, instead of 30°C/min, the powder is completely converted to β-TCP and CaO (JCPDS # 82-1691), as indicated by the curve labeled e in Fig. 8. Similar conversion to β-TCP has also been observed during high temperature sintering of porous HAp in air. 48

In summary, the powder formed in our droplet microreactors is predominantly amorphous hydroxyapatite, which can be transformed to crystalline HAp, and other phases of calcium phosphates by calcination. The precise compositions of the final powder are likely influenced by the reaction conditions in the droplet reactors. A better understanding of the influence may enable the manipulation of the compositions of the powder.

Overall, our results offer direct visualization of HAp formation from its precursors in microreactors. With our droplet microreactors, the evolution of the macroscale morphology can be monitored in isolated droplets. Since the shells of the double emulsion droplets have roughly the same thickness, each droplet can be treated as identical reactors, making it possible to monitor large number of droplets simultaneously. This is difficult to achieve with other methods. Visualization of HAp formation helps us to understand different stages of formation as well as influence of precursor concentration on the morphology of as formed mesoporous HAp powders. Lower precursor
concentration in the microreactor helps form first a highly porous networked nano-structured HAp; then, with time, some consolidation takes place in the as-formed powders within the microreactor (See Movie S1 in supporting materials). With increasing precursor concentration within the same size of double-emulsion-droplet microreactors, the as-formed HAp morphology changes from an open ended network mesoporous structure to a dense mesoporous structure. The total pore volume, 0.499 ml/g, is significantly higher for the powder prepared in double emulsion droplet reactors as compared to that prepared in bulk, 0.133 ml/g. While most of the pores are between 20 nm and 80 nm in diameter for powders synthesized in bulk and in microreactors, the HAp powder prepared in droplet microreactor also has about 20% of pores with diameters under 6 nm. The presence of nanoscale porosity significantly increases the specific average surface area of the as-formed powders to 162.8 m²/g, as compared to 26.0 m²/g for powders obtained from bulk reaction. Calcination of as-formed powder up to 800 °C results in the formation of a pure HAp phase.

**Conclusions**

We present a novel method for synthesizing mesoporous hydroxyapatite (HAp) powders using double emulsion droplets as microreactors. Double emulsion droplets are ideal microreactors as they afford excellent encapsulation of the starting reactants and allow on-demand addition of additional reactants. Our results suggest that the calcium and phosphorus precursors are well encapsulated inside the inner drops of the double emulsion reactors while alkali introduced in the continuous phase effectively adjusts the pH and triggers the CaP formation in the inner drops. The porosity of the HAp aggregates
synthesized can be tuned by adjusting the reactant concentration in the inner drop. Aggregate networks of different degrees of compactness have been synthesized by varying the concentrations of the calcium precursor from 0.1M to 1M and the phosphorus precursor from 0.06M to 0.6M. Powder formed with our technique demonstrates remarkable microstructures and significant increase in the total surface area and porosity. The surface area and the total pore volume of the powders prepared are as high as 162.8 m²/g and 0.499 mL/g, respectively. From our thermal and phase analyses, we confirm that the amorphous CaPs formed is first transformed to crystalline HAp and then at a higher temperature to β-TCP. Our technique represents a novel way of using double emulsion droplets as microreactors for the synthesis of inorganic particles as well as imaging of the synthesis process. It would be advantageous to also monitor other aspects, such as the chemical compositions and microstructures, during the formation of the hydroxyapatite. If this can be done, we believe that our droplet microreactors will lead to a comprehensive understanding of the formation mechanism. The novel synthesis approach will not only improve the performance of calcium phosphate-based materials, but will also enable the control of nanoscale porosity and morphology of inorganic particles.

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**Supporting Information Available.** Movie showing the evolution of the droplet reactor during formation of hydroxyapatite. This material is available free of charge via the Internet at [http://pubs.acs.org](http://pubs.acs.org).

Fig. 1: Schematic illustration of the double-emulsion-templated synthesis of hydroxyapatite. The inner drops of the double emulsion consist of an aqueous solution of calcium nitrate and phosphoric acid, which are the calcium and the phosphorus precursors. The oil shells surrounding the inner drops are made up of an inert oil phase. Upon increase of pH by adding ammonium hydroxide to the continuous phase, hydroxyapatite is formed in the inner drops of the emulsion. Due to their higher osmolality, water diffuses into and swells the inner drops, leading to an increase in size of the double emulsion drops and a resulting thinning of the oil shells. Eventually, the oil shells become so thin that the double emulsions destabilize, releasing the hydroxyapatite cores.
Fig. 2: (a) Schematic of the glass microcapillary device for generating double emulsion droplets. (b-e) Optical microscope images of double emulsion drops (b) immediately and (c) 91 hours after addition of 0.1 N ammonium hydroxide solution. The change in pH triggers the formation of hydroxyapatite in the inner drops of the double emulsion. The hydroxyapatite initially spans the entire volume of the inner drops. As water diffuses into the inner drops due to a higher internal osmolality, the hydroxyapatite aggregates remain roughly the same in size. Optical microscope images of (d) a hydroxyapatite particle aggregate formed and (e) oil shells at higher magnification. The thickness of the oil shells has reduced from 18 μm to 4.8 μm after 91 hours. Scale bars are 50 μm for (b), (d) and (e), and 150 μm for (c).
Fig. 3: Optical microscope images of hydroxyapatite aggregates formed from precursor solutions with calcium nitrate and phosphoric acid concentrations of (a) 0.1 M and 0.06 M, (b) 0.5 M and 0.3 M, and (c) 1 M and 0.6 M respectively. The structure of the aggregates formed becomes more compact as the precursor concentration increases. Scale bars are 50 μm for (a) and (b), and 30 μm for (c).
Fig. 4: (a)-(c) Scanning electron microscope (SEM) images of dried hydroxyapatite powder at different magnifications. The hydroxyapatite formed is made up of smaller spherical particles of about 1 μm in size, as shown in (b). Each spherical particle has an open, feathery morphology shown in (c). The powder in the images was formed from a precursor solution of 0.5 M calcium nitrate and 0.3 M phosphoric acid. Scale bars are 20 μm for (a), 2 μm for (b), and 500 nm for (c).
Fig. 5: (a) Transmission electron microscope (TEM) image of hydroxyapatite powder; (b) magnified view of the hairs similar to the ones shown in (a). The microstructure of the powder is dominated by nanometer-sized needle-like nanoparticles shown in (b). The powder in the images was formed from a precursor solution of 1 M calcium nitrate and 0.6 M phosphoric acid. Scale bars are 300 nm for (a), and 100 nm for (b).
Fig. 6: (a) Nitrogen adsorption/desorption isotherms and (b) pore size distributions of hydroxyapatite in double emulsion droplet reactors and in bulk. The powder examined was formed from a precursor solution of 1 M calcium nitrate and 0.6 M phosphoric acid.
Fig. 7: Thermogravimetric (TG) curves of the hydroxyapatite formed. The sample heating rate was 5 °C/min and the sample was calcined at 500 °C and 1000 °C each for 10 minutes. The inset is a scanning electron microscope (SEM) image of the powder after thermogravimetric analysis. The powder examined was formed from a precursor solution of 1 M calcium nitrate and 0.6 M phosphoric acid. Scale bar in the inset is 5 μm.
Fig. 8: X-ray diffraction (XRD) patterns of hydroxyapatite powder (a) as formed, and calcined at (b) 600 °C, (c) 800 °C, and (d) 1000 °C. Samples were heated at 30 °C/min to the temperature specified and then kept at the same temperature for one hour. (e) XRD pattern of powder heated at 5 °C/min to and calcined at 1000 °C. All powders examined were formed from a precursor solution of 1 M calcium nitrate and 0.6 M phosphoric acid. The squares, stars, circles and pounds designated as β-tricalcium phosphate (TCP), α-tricalcium phosphate, hydroxyapatite and calcium oxide indicate the characteristic peaks of α-TCP (JCPDS # 09-0348), β-TCP (JCPDS file # 09-0169), hydroxyapatite (JCPDS file # 09-0432) and calcium oxide (JCPDS # 82-1691) at the marked positions respectively.
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**SYNOPSIS TOC**