Formation of Amorphous Iron-Calcium Phosphate with High Stability

Song Chen,* Dachuan Liu, Le Fu, Bing Ni, Zongkun Chen, Jennifer Knaus, Elena V. Sturm, Bohan Wang, Håvard Jostein Haugen, Hongji Yan, Helmut Cölfen,* and Bin Li*

Amorphous iron-calcium phosphate (Fe-ACP) plays a vital role in the mechanical properties of teeth of some rodents, which are very hard, but its formation process and synthetic route remain unknown. Here, the synthesis and characterization of an iron-bearing amorphous calcium phosphate in the presence of ammonium iron citrate (AIC) are reported. The iron is distributed homogeneously on the nanometer scale in the resulting particles. The prepared Fe-ACP particles can be highly stable in aqueous media, including water, simulated body fluid, and acetate buffer solution (pH 4). In vitro study demonstrates that these particles have good biocompatibility and osteogenic properties. Subsequently, Spark Plasma Sintering (SPS) is utilized to consolidate the initial Fe-ACP powders. The results show that the hardness of the ceramics increases with the increase of iron content, but an excess of iron leads to a rapid decline in hardness. Calcium iron phosphate ceramics with a hardness of 4 GPa can be achieved, which is higher than that of human enamel. Furthermore, the ceramics composed of iron-calcium phosphates show enhanced acid resistance. This study provides a novel route to prepare Fe-ACP, and presents the potential role of Fe-ACP in biomineralization and as starting material to fabricate acid-resistant high-performance bioceramics.

1. Introduction

Calcium phosphates (CaPs) are the main inorganic components of vertebrate hard tissues. Amorphous calcium phosphate (ACP), prevalent in biological organisms, represents a unique class of calcium phosphates.[1] It has no translational and orientational long-range order of the atomic positions, showing essentially glass-like physical properties.[2] Previous studies revealed that intracellular ACP precursors reside in the mitochondria of mineralizing cells, and they are transferred from mitochondria via the lysosomal pathway.[3] Recent studies have suggested that ACP might be the precursor of many biominerals, and it plays a vital role in the functions of these structures.[4] As a transitory phase, ACP is highly unstable in an aqueous medium, readily transforming to crystalline calcium phosphates, such as hydroxyapatite.[5] Some trace elements,
such as magnesium and strontium have been found in natural hard tissues, and these divalent ions are able to stabilize ACP by either substituting the calcium or adsorbing on the surface of ACP to disrupt the crystallization process.[6] Recently, the function of iron-based phases in the biomineralization process has attracted much attention. Iron has been found to exist in hard tissues of organisms in various forms, such as ferrhydrate, magnetite, and goethite.[7] The function of iron oxide in teeth hardening has been demonstrated in limpets, chitons and chichlid fishes.[8] Another study shows that the iron-rich phases strengthen the incisor of feral copper. The iron-rich enamel shows a higher mechanical strength (Hardness \( \approx 4.6 \) GPa) than the non-pigmented enamel (Hardness \( \approx 3.5 \) GPa).[9] Moreover, a recent investigation on rodent teeth shows that amorphous intergranular phases control the mechanical properties of enamel. The mixture of ferrhydrte and amorphous iron-calcium phosphate in the intergranular phases makes the enamel harder and more resistant to acid attack.[10] An important question related to iron-hardened minerals is how iron-rich phases, such as amorphous iron-calcium phosphate (Fe-ACP) are formed. Previous studies have shown that the presence of iron affects the crystallinity and solubility of hydroxypatite and octacalcium phosphate.[11] However, there is still no evidence revealing the mechanisms of formation of the Fe-ACP in aqueous media, whether or not such an iron-bearing amorphous phase is a transient precursor remains unclear. Therefore, the synthesis of Fe-ACP and the unrevealing of its formation process are essential for understanding biomineralization of iron-rich calcium phosphate-based biomimetics.

Except for the important role of biomineralization, iron-based materials show great potential in the biomedical field. As an essential component for cell metabolism and biochemical reaction, iron plays a crucial role in some body functions, such as oxygen transport, DNA synthesis, and cooperates with many enzymes. Iron-containing particles have been successfully applied in photothermal and photodynamic therapies for the treatment of cancer.[12] More recently, the function of iron-based bioceramics in tissue regeneration has been extensively explored. It is found that iron-containing bioceramics can promote angiogenesis and osteogenesis by regulating the expression of vascular endothelial growth factor and HIF-1\( \alpha \) in endothelial cells.[13] Our recent research has shown the great osteogenic property of an iron-bearing calcium phosphate cement in vivo.[14] The use of amorphous calcium phosphate in the biomedical field has been reported, including implant coatings,[15] drug delivery vehicles,[16] and reinforcing agents for self-setting cements.[17] Therefore, it is our interest to synthesize the iron-containing ACP particles and explore their potential use in the dental and orthopedic fields.

Many attempts have been made to produce ACP using wet route syntheses or dry route syntheses.[18] The wet synthesis route usually involves the rapid mixing of calcium salt with phosphate salt to prevent phase transformation during the preparation. Organic molecules, such as polycrylic acid (PAA),[19] polyaspartic acid (PASp),[20] polyethylene glycol,[18a] poly(allylamine) hydrochloride,[21] and triethylamine[22] have been applied to slow down the conversion rate. Citrate ions, which account for 1–2 wt.% of natural bone, significantly affect the stability of calcium phosphates and regulate their crystal growth.[21] It is worth noting that most reported ACP are prepared under neutral or basic conditions, and the study by Posner has shown that increasing pH can greatly slow down the conversion rate of ACP.[24] At a more acidic pH, dicalcium phosphate dihydrides and octacalcium phosphates are the most common phases. In the presence of magnesium and citrate ions, which are known as crystallization inhibitor of apatite, acidic ACP can be prepared at solution pH of 6.0–6.5.[25] Although an acidic disordered form of calcium phosphate is detected in the bones of zebrafish,[26] to the best of our knowledge, no ACP has been prepared in even more acidic aqueous solutions. In the oral environment, the acidic ACP-containing materials can take advantage of enhanced acid-resistance, which can potentially be applied as dental repair materials. Moreover, when preparing the iron-bearing calcium phosphates, the acidic reaction medium can minimize the precipitation of iron hydroxide, which might facilitate the formation of Fe-ACP.

In the present work, we have explored the synthesis routes to prepare an iron-bearing amorphous calcium phosphate in aqueous media at ambient temperature. Ammonium iron citrate (AIC), which contains both iron and citrates, is selected as the iron source for the preparation. The amorphous iron-calcium phosphate can be prepared at pH values as low as 4 prepared in the presence of AIC. The resultant particles kept their amorphous feature in water and SBF solution when prepared under high concentration of AIC. The iron-containing calcium phosphate particles showed good biocompatibility and osteogenic properties in vitro. Subsequently, the synthesized Fe-ACP was applied as starting material to fabricate acid-resistant high-performance bioceramics. Together, the study reveals clues about the biomineralization process of Fe-ACP and provides new insight into the stability of the amorphous phase. The as-synthesized particles might play a key role in the biomineralization process of iron-rich hard tissues, and possess potential as starting materials to fabricate acid-resistant high-performance bioceramics.

2. Results and Discussion

2.1. Preparation and Characterization of Fe-ACP

Typical Fe-ACP particles which were synthesized with 0.3 \( \text{m} \) \( \text{PO}_4^{3-} \), 0.5 \( \text{m} \) \( \text{Ca}^{2+} \), and 0.04 \( \text{m} \) ammonium iron (III) citrate (the pH after reaction is \( \approx 5 \) without adjustment) is shown in Figure 1. The diameters of the particles are between 50 and 200 nm.
Figure 1. Typical amorphous iron-calcium phosphate prepared in the presence of 0.04 M ammonium iron (III) citrate. a) SEM image (Magnification x50,000). b) TEM micrograph (Magnification x40,000). c) SAED pattern of the particles. d) The XPS spectra over a wide range. e) The XPS spectra of the Fe $2p_{3/2}$ peak.

(Figure 1a). TEM and SAED pattern confirmed that the prepared particles were non-crystalline nanoparticles (Figure 1b,c). The specimen exhibited peaks of Fe 2p, O 1s, Ca 2p, C 1s, and P 2p over a wide binding energy region (Figure 1d). The binding energy of the Fe 2$p_{3/2}$ peak was 710 eV (Figure 1e), which can be assigned to Fe$^{3+}$ bonded to phosphate groups. [27]

We have shown that the concentration of ammonium iron citrate is essential for forming Fe-ACP. (Figure 2). The preparation of amorphous calcium phosphate by precipitation is usually conducted in alkaline solutions. [28] Under acidic conditions, the final products are normally brushite (dicalcium phosphate dihydrate, CaHPO$_4$·2H$_2$O, and DCPD) or monetite (dibasic calcium phosphate anhydrate, CaHPO$_4$, and DCPA) with high crystallinity. [29] Although ACP has been prepared at pH 6 in the presence of magnesium and citrate ions, the synthesis of ACP at pH lower than 5 has, to our knowledge, never been reported. Our previous research showed that cetyltrimethylammonium bromide and ammonium chloride effectively regulate the crystal growth of CaPs, forming DCPD or DCPA particles with various morphologies and hierarchical structures. [30] In this study, we have shown that AIC is a strong crystallization inhibitor of acidic calcium phosphates, with which ACP under pH 5 was synthesized. As shown in Figure 2, the inhibition effect of AIC is concentration-dependent. With a low concentration of AIC (0.007 and 0.02 M), the final phase was still brushite (Figure 2a), but the morphology of the powders changed from micro-sized platelets to block-like particles (Figure 2c). Nano-sized Fe-ACP can be obtained by increasing the AIC concentration up to 0.04 M. Further increasing the AIC concentration to 0.1 and 0.2 M had no influence on the morphology and final phase of the particles. We further investigated the inhibition effects of citrate and iron ions, respectively, to better understand the formation process of Fe-ACP. The presence of iron ions alone was not effective in forming the Fe-ACP (Figure S1, Supporting Information). With 0.04 or 0.10 M iron nitrate, the crystalline phase was still plate-like brushite. Previous studies revealed that citrate regulates the crystal growth of hydroxyapatite by strongly binding to the hydroxyapatite surface. [31] More recently, it is found that the citrates can facilitate the intrafibrillar formation of hydroxyapatite to produce an inorganic–organic composite by reducing the interfacial energy between the biological matrix and the amorphous calcium phosphate precursor. [32] In accordance with these reports, hydroxyapatite nanocrystals formed in the presence of citrate (Figure S1a,b, Supporting Information). Therefore, the formation of Fe-ACP is regulated by the synergistic effect of both iron ions and citrates.
Figure 2. Effect of AIC concentration on the preparation of the particles. a) XRD of the powders prepared at different concentrations of AIC. The patterns are matched with the standard diffraction pattern of brushite (PDF 01-072-0713). b) FTIR of the powder. c) SEM of the particles (Magnifications ×5000 and ×20 000).

The structural differences of the obtained particles were confirmed by infrared spectra (Figure 2b). The spectrum showed sharp bands of $\nu_1\text{PO}_4^{3-}$ ($\approx 985$ cm$^{-1}$), $\nu_2\text{PO}_4^{3-}$ ($\approx 1056$ and $\approx 1132$ cm$^{-1}$), and $\nu_3\text{PO}_4^{3-}$ ($\approx 524$ cm$^{-1}$). In contrast, the spectra of AIC004, AIC010, and AIC020 had rounded absorption bands $\approx 556$ and $1074$ cm$^{-1}$, confirming the amorphous features of these samples.\cite{28,33} It is worth noting that particles prepared in the presence of AIC show additional bands $\approx 1400$ and $1610$ cm$^{-1}$, which can be attributed to vibrations of the carboxyl group for the associated citrate, suggesting a relevant amount of citrate remaining in the resultant particles.\cite{31c,34}

The pH changes during the synthesis of AIC000, AIC004, AIC010, and AIC020 are shown in Figure S2 (Supporting Information). The final pH dropped with the addition of 0.04 mol L$^{-1}$ AIC, but continuously increasing the AIC concentration in the solution to 0.10 and 0.20 mol L$^{-1}$ resulted in a slight increase in the pH. The chemical composition of Fe-ACP is reported in Table S1 (Supporting Information). TGA-DTG analysis revealed that all the samples present a small amount of carbonate and citrate ions (Figure S3, Supporting Information). In general, the amount of iron, as well as citrate and carbonate in the particles, increased with the AIC concentration in solution. The detected Fe/(Ca+Fe) ratio was 0.11 when the concentration of AIC was 0.07 mol L$^{-1}$, and it increased to 0.29 and 0.35 when 0.10 and 0.20 mol L$^{-1}$ AIC were added. Simultaneously, the amount of phosphate decreased with increasing AIC concentration. Possibly, it was replaced by carbonate and citrate. The structural water content for AIC 0007 was $\approx 5.0\%$ and it increased to $8.7\%$ for AIC020 samples. The percentage of adsorbed water varied in the range of 16–21%. It is worth noting that Fe-ACP can be prepared
even at pH 4 (adjust with hydrochloric acid) at the presence of 0.10 mol L\(^{-1}\) AIC (Figure S4, Supporting Information). The resulting particles were spherical, with diameters \(\approx 300\) nm. The Fe/Ca ratio of the particles was higher than that of the particles prepared at pH 5. TEM images showed that some kind of layered structure existed within the AIC004 particles (Figure 3a), which was not observed in AIC010 particles (Figure S5a, Supporting Information). The element mapping showed that the iron was uniformly distributed among the particles (Figure 3b,c; Figure S5b,c, Supporting Information).

### 2.2. Stability of Fe-ACP

In the bone mineralization process, ACP has been proposed as the precursor and transition phase of crystalline apatite.\(^{[4c,5b,35]}\) Several studies have investigated the phase transformation process of ACP to crystalline calcium phosphates in solution.\(^{[36]}\) In an aqueous solution, the amorphous phase can only exist for several hours.\(^{[24]}\) The conversion rate can be slowed down by adding stabilizers, such as polyethylene glycol\(^{[37]}\) or adenosine triphosphate.\(^{[38]}\) Some ions, such as magnesium and strontium are demonstrated to be efficient in stabilizing the ACP as well.\(^{[40,39]}\) In this study, the stability of Fe-ACP was investigated by immersing the particles in water and simulate body fluid (SBF, the composition is shown in the Experimental Section). It is interesting to find that the stability of these particles is highly related to the concentration of AIC used during preparation. AIC004 (0.04 mol L\(^{-1}\) AIC) particles transformed to hydroxyapatite after immersion in water and SBF for 7 days (Figure 4a,b). The particles after conversion showed irregular plate-like morphology (Figure S6, Supporting Information). When the concentration of AIC was 0.10 mol L\(^{-1}\) (AIC010), no conversion was observed, even after 31 days of immersion in water and SBF (Figure 4a,b). This was further confirmed by TEM micrographs and SAED patterns of AIC010 particles, which have shown amorphous features after immersion in SBF for 7 and 31 days (Figure S7, Supporting Information). It is similar for AIC020 particles, which showed no phase transformation after storage for 45 days in water and SBF (Figure S8, Supporting Information). This can likely be attributed to the inhibiting action of citrate, which is known to stabilize ACP\(^{[40]}\) and even prenucleation clusters, as well as liquid precursor phases.\(^{[41]}\) That way, crystallization can be effectively inhibited. We further investigated the thermal stability of the AIC particles by TGA/XRD. As shown in Figure 4c, continuous weight loss is observed on heating Fe-ACP. The water molecules loosely absorbed on the surface of Fe-ACP were removed between 25 and 200 °C. The weight loss in the range of 200–400 °C was attributed to the loss of strongly bound water molecules. The total mass loss up to 800 °C was \(\approx 35\%\). The XRD patterns showed that the particles kept their amorphous feature when heating up to 500 °C, without any diffraction peaks. At 800 °C, the amorphous phase converted to calcium iron phosphate (Ca\(_9\)Fe(PO\(_4\))\(_7\)) (Figure 4d). Dissolution experiments were carried out in acetate buffer solution (pH 4, the composition is shown in the Experimental Section) up to 15 and 60 min (Figure 4e,f; Figure S9, Supporting Information).
2.3. Biological Performance of Fe-ACP

Calcium phosphate-based materials show good biocompatibility and bioactivity, having broad applications in dental and orthopedic fields.\(^\text{42}\) As one of the trace elements in the human body, an iron ion is non-toxic in a physiological range.\(^\text{43}\) In order to verify the biocompatibility of Fe-ACP nanoparticles, the effects of Fe-ACP nanoparticles with different concentrations and iron contents on cell proliferation activity were investigated. Fe-ACP nanoparticles had no inhibitory effect on cell proliferation at the concentrations of 50, 100, and 200 μg mL\(^{-1}\) (Figure 5a–c). With higher concentrations of Fe-ACP particles (100 and 200 μg mL\(^{-1}\)), the cell proliferations were slightly promoted. Alkaline phosphatase staining (ALP) and alizarin red staining (ARS) were applied to evaluate the osteogenic properties of AIC010 nanoparticles (Figure 5d,e). The in vitro study revealed that
Figure 5. The proliferation of BMSCs cultured with different concentrations of Fe-ACP nanoparticles. a) 50 $\mu$g mL$^{-1}$, b) 100 $\mu$g mL$^{-1}$, and c) 200 $\mu$g mL$^{-1}$ was detected by CCK-8 assay at days 1, 3, and 5 ($n = 6$). d) ALP staining images of BMSCs cultured for 7 days with different AlCo10 particles. e) Alizarin red staining images of mineralized BMSCs cultured with AlCo10 particles for 14 days.

the Fe-ACP particles had good biocompatibility and osteogenic properties, having the potential to be applied in the biomedical field.

2.4. Spark Plasma Sintered Bioceramics

One potential application of the Fe-ACP particles is as starting materials for high-performance bioceramics. In this study, Spark Plasma Sintering (SPS) was used to sinter calcium iron phosphate ceramics. Our results have clearly shown that the presence of iron has a great impact on hardness of sintered bioceramics. The ceramics without iron showed a hardness of 3.0 GPa, and it reached 4.0 GPa for the AlCo10 sample, which is higher than that of human enamel (≈2.7–3.7 GPa).\cite{44} Further increasing the iron content (AlCo20) resulted in a decrease in hardness (2.3 GPa) (Figure 6a). This is in accordance with the findings in rodent teeth, showing that the iron-containing enamel is harder than that without iron.\cite{10} XRD patterns of the sintered ceramics are shown in Figure 6b. The final phase of the ceramics was $\text{Ca}_2\text{Fe}($PO$_4$)$_3$, with the increase of the iron content. The compositions and unit cell parameters were calculated by Rietveld refinement of XRD data and are shown in Table S2 (Supporting Information). The fracture surfaces of the ceramics were examined through SEM (Figure 6c–f). The ceramics with AlCo000 and AlCo004 as the starting materials showed many micro and nano pores on the surface (Figure 6c,d), while ceramics using AlCo10 and AlCo20 as starting materials showed more dense fracture surfaces (Figure 6e,f). The acid resistance of the ceramics was evaluated (Figures S10 and S11, Supporting Information). The surface morphologies after the acid attack are shown in Figure S10 (Supporting Information). For the samples without or with low amount of iron, the surfaces were severely damaged (Figure S10a,b, Supporting Information). Meanwhile, for the samples with high iron contents, the surfaces were much smoother (Figure S10c,d, Supporting Information). The acid resistance of the ceramics was further estimated by the amounts of Ca and P dissolved from the samples. The AlCo10 and AlCo20 ceramic samples showed much lower amounts of dissolved Ca and P, in comparison with AlCo00 and AlCo04 ceramic samples, indicating improved acid resistance of the ceramics in the
presence of iron. Overall, the ceramics with higher amount of iron exhibited superior acid resistance, showing its great potential for dental applications.

3. Conclusion

Fe-ACP particles with variable content of iron are prepared in the presence of ammonium iron citrate. The Fe-ACP particles are highly stable in aqueous media, such as water, SBF and acetate buffer solutions, and their stability in water and in SBF solution depends on the amount of ammonium iron citrate. The Fe-ACP particles show good biocompatibility and osteogenic properties in vitro, and can be applied as starting materials to fabricate calcium phosphate ceramics. With the presence of a proper amount of ammonium iron citrate, ceramics with a hardness higher than human tooth enamel and superior acid resistance can be prepared. The work highlights a novel route to prepare Fe-ACP particles, and presents the potential role of Fe-ACP in biomineralization and as starting material to fabricate acid-resistant high-performance bioceramics.

4. Experimental Section

Chemicals: Ca(NO$_3$)$_2$·4H$_2$O was purchased from Chinasun Specialty Products Co., Ltd; Na$_2$HPO$_4$·12H$_2$O was obtained from Shanghai Lingfeng Chemical Reagent Co., LTD; ammonium iron (III) citrate was purchased from Sigma–Aldrich; ethanol was purchased from Sinopharm Chemical Reagent Co. All chemicals were used as received without further purification. All of the chemicals were of analytical grade. Deionized water was used in all experiments.

Preparation of Fe-ACP: In a typical experiment, solution 1 was prepared by dissolving 0.3 M Na$_2$HPO$_4$·12H$_2$O and a certain amount of ammonium iron (III) citrate (0, 0.007, 0.02, 0.04, 0.10, and 0.20 M) in deionized water. Solution 2 was prepared by dissolving 0.5 M Ca(NO$_3$)$_2$·4H$_2$O in deionized water. Solution 2 was slowly added into solution 1 under vigorous stirring at room temperature for 5 min. The pH during the synthesis was measured using a pH meter (PB-10, Sartorius). Each experiment was repeated for three times. The pH electrodes were calibrated by 50 mm C$_6$H$_2$K$_2$O$_7$ (pH = 4.01, 25°C), 25 mm NaH$_2$PO$_4$/Na$_2$HPO$_4$, standard buffer solution (pH = 6.86, 25°C), and 10 mm Na$_2$B$_4$O$_7·10$H$_2$O standard buffer solution (pH = 9.18, 25°C). When preparing Fe-ACP particles under pH 4, the pH was adjusted using hydrochloric acid. The particles were separated from the solvent by centrifugation and washed with deionized water three times. Finally, Fe-ACP was washed with ethanol and dried in a vacuum desiccator. The samples were designated as AIC000 (no AIC), AIC004, AIC010, and AIC020 as the raw materials. a) Hardness and relative density (n = 5). b) XRD pattern. The patterns are matched with the standard diffraction patterns of Ca$_3$P$_2$O$_7$ (PDF 00-003-0605) and Ca$_9$Fe(PO$_4$)$_7$ (PDF 01-089-0514). Fracture surface of the ceramics (Magnification x20 000) prepared using c) AIC000, d) AIC004, e) AIC010, and f) AIC020 as starting materials.
AIC0007 (0.007 m AIC), AIC002 (0.02 m AIC), AIC004 (0.04 m AIC), AIC010 (0.10 m AIC), and AIC020 (0.20 m AIC), according to the concentration of ammonium iron (III) citrate used for the preparation. When preparing particles without citrates, ferric nitrate (0.04 and 0.10 m) was used instead of ammonium iron (III) citrate. When preparing particles without iron ions, solution 1 was prepared by dissolving 0.3 m Na₂HPO₄·12H₂O and a certain amount of citric acid (0.04 and 0.10 m) in deionized water. The pH of solution 1 was adjusted to the same pH as when ammonium iron citrate (0.04 and 0.10 m) were added. Solution 2 was prepared by dissolving 0.5 m Ca(NO₃)₂·4H₂O in deionized water, other procedures were similar as mentioned above. When investigating the thermal stability of the particles under different temperatures, the AIC particles were calcined at a predetermined temperature using a muffle furnace (KSL-1700X, Kejing, China). The temperature of muffle furnace rose to the specified temperature at a rate of 10°C min⁻¹, then keeps the specified temperature for 3 h.

Preparation of SBF and Acetate Buffer Solutions: The SBF solution was prepared by dissolving NaCl, NaHCO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, CaCl₂, and Na₂SO₄ in de-ionized water, adjusting the ion concentrations to be similar to those in human blood plasma (Table 1). The SBF was buffered at a pH value of 7.40 using (CH₃OH)₃CNH₂ and 1.0 m HCl. The acetate buffer solution was prepared by dissolving 1.86 g sodium acetate (0.02269 mol) and 4.64 g acetic acid (0.07731 mol) in 1 L deionized water (pH 4).

The Stability of Fe-ACP in Water, SBF Solution, and Acetate Buffer Solution: Stability in water and SBF solution: 100 mg freshly prepared Fe-ACP was placed in wide-mouth bottles containing 100 mL deionized water or simulated body fluid (SBF). Afterward, they were kept in an oscillating incubator at constant temperature (90 rpm, 37.5°C), respectively. After the predetermined time, the samples were isolated by centrifugation, washed with deionized water and ethanol, and dried in a vacuum desiccator. Stability in acetate buffer solution: 10 mg Fe-ACP particles were put into the beaker with 10 mL acetate buffer solution and intensively stirred. After 15 and 60 min, the solution was filtered with 0.22 µm pore-sized filter and the filtrate was used to measure the ion concentration. Each experiment was repeated for four times (n = 4).

Scanning Electron Microscopy (SEM): The morphology of dried Fe-ACP samples was observed using a scanning electron microscope (S-400, Hitachi, Japan). Specimen were sputtered with a thin Au coating for 45 s before measurement. The samples were mounted on aluminum stubs with double-sided carbon tape. The acceleration voltage was set to 15 kV.

Transmission Electron Microscopy (TEM): A JEOl 2200FS HRTEM operated at 200 kV, equipped with a JEOl EDX detector, was used to perform high-angle annular-dark-field (HAADF) scanning TEM (STEM), and the EDX element line scanning profile, as well as the mapping. When preparing samples, the particles were dispersed in ethanol and a drop of the colloidal solution was dipped on a copper grid and then air-dried. X-Ray Powder Diffraction (XRD): Dried Fe-ACP specimen were ground into fine powder and analyzed using an X-ray diffractometer (XRD, Bruker D8 Advance, Germany) equipped with a copper-source, operating at 40 kV and 40 mA. Data were collected for 2θ ranging between 10 and 80° under CuKα radiation (λ = 1.5418). The step size was 0.02° and the residence time was 0.1 s. Qualitative and quantitative phase analyses of the studied samples were conducted on an MDI Jade software. The external standard method and the whole pattern fitting refinement were utilized to obtain the lattice parameters of the studied phases.

Temperature Measurement (TEM): The JEOL 2200FS HRTEM operated at 200 kV, equipped with a JEOl EDX detector, was used to perform high-angle annular-dark-field (HAADF) scanning TEM (STEM), and the EDX element line scanning profile, as well as the mapping. When preparing samples, the particles were dispersed in ethanol and a drop of the colloidal solution was dipped on a copper grid and then air-dried.

Table 1. Ion concentrations of the simulated body fluid and human blood plasma.

<table>
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<tr>
<th>Ion</th>
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<th>Human blood plasma</th>
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atomic emission spectroscopy (SPECTRO Analytical Instrument, Agilent 7800, America), measuring atomic Ca at 393.366 nm, P at 177.495 nm, and Fe at 259.940 nm.

Statistical Analysis: Quantitative data were expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) followed by Tukey post hoc comparison (OriginLab Corporation, MA, USA) was used for statistical analysis. A value of \( p < 0.05 \) denoted a statistically significant difference.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

Author Contributions

Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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ammonium iron citrates, amorphous calcium phosphates, biomineralization, phase transformations

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