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A New Approach for Hydroxyapatite Coating on Polymeric Materials Using Laser-Induced Precursor Formation and Subsequent Aging

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ABSTRACT A new process, laser-induced precursor formation and subsequent aging in a supersaturated calcium phosphate aqueous solution (CP solution), was applied for coating a hydroxyapatite (HAP) film on a polymeric material, ethylene−vinyl alcohol copolymer (EVOH). Laser irradiation onto EVOH immersed in the CP solution induced the formation of CP precursors, and an HAP film composed of a submicrometer-scale cavernous structure was formed by subsequent aging in a CP solution without laser irradiation. The resulting HAP film coated on EVOH demonstrated excellent structural and chemical uniformity and cell adhesion with the CHO-K1 and BHK-21 cells. This process provides a practical technique for coating HAP onto polymeric materials.

KEYWORDS: hydroxyapatite • ethylene−vinyl alcohol copolymer • laser technique • CHO-K1 cell • BHK-21 cell

INTRODUCTION

Hydroxyapatite (HAP; $Ca_{10}(PO_4)_6(OH)_2$, $P6_3/m$) is the principal inorganic component of human hard tissues such as cortical bone and teeth (1) and hence has a high affinity to living tissues, especially to hard tissues (2, 3). HAP also has a high ability to adsorb biopolymers such as protein (4). Therefore, HAP has been investigated with great interest, not only as a coating of orthopedic and dental implants (3, 5) but also for fabrication of a biopolymer−HAP composite film utilizing the high affinity of HAP to biopolymers (6−8). Various physical coating processes, such as plasma spray coating, sputtering, and pulsed laser deposition, have been used for the deposition of HAP films on artificial materials (5). Although the deposition rates of these physical processes are relatively high, it is difficult to tune the composition and crystal structure of the products and to obtain a uniform HAP film because of the thermally unstable character of HAP. For example, products deposited by the plasma spray coating tend to be amorphous or decomposed to calcium oxide, tricalcium phosphate, or tetracalcium phosphate. These byproducts are metastable in physiological fluids and thereby are detrimental to the chemical stability of the coating (9).

A chemical coating using an aqueous solution supersaturated with respect to HAP (10−14) has also been applied to form a uniform HAP thin film on artificial materials at relatively low temperature. The composition and structure of HAP deposited by this process can be tuned by controlling various solution conditions, such as pH, reagent concentration, and temperature (15, 16). This process consists of two steps, the surface modification of materials for initial HAP nucleation and HAP crystal growth in the coating solution. However, the initial nucleation rate is relatively low because of the mild chemical conditions. In addition, the procedure includes a complicated and long-term surface-modification process for HAP nucleation. A recent improvement to provide a simple and quick surface-modification process has been achieved by HAP precursor deposition onto a polymer surface using a simplified alternate dipping treatment (13, 14), although it still requires a complicated procedure. Therefore, there still remains room for further improvement to simplify the surface-modification process.

Recently, a laser-irradiation process onto an immersed solid target or powder dispersed in a liquid environment has been intensively investigated to prepare various kinds of nanomaterials (17, 18). This process provides a plasma state generated by the transient temperature increase induced by optical absorption of laser light by the matter placed in liquid and therefore can be a combination of a physical process induced by laser and a subsequent chemical process in liquid. For laser irradiation onto a substrate placed in an aqueous solution supersaturated with respect to HAP, such a transient process may induce the segregation of the HAP precursor onto the hot laser-irradiated area. A few attempts have been reported for HAP formation but only at a relatively high laser fluence exceeding 10 W/mm^2, which may damage polymeric substrates (19).

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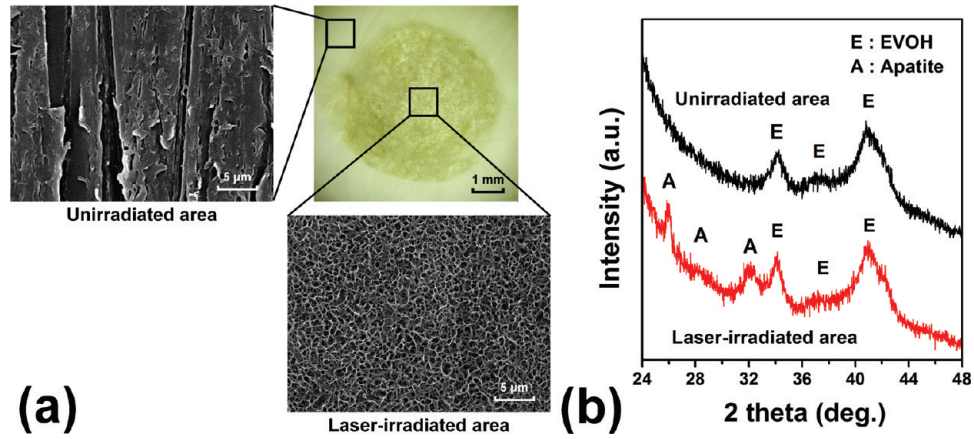

FIGURE 1. (a) Morphologies of EVOH surfaces after laser irradiation for 3 h and subsequent immersion in a CP solution for 24 h observed by optical microscopy (upper right) and SEM (upper left and lower right). (b) XRD patterns of the laser-irradiated and unirradiated areas of the EVOH surface.

Here we propose a novel HAP coating process utilizing laser-induced precursor formation. A weak pulsed laser beam was irradiated to segregate precursors onto a substrate immersed in a supersaturated calcium phosphate solution (CP solution 7, 8) and subsequently immersed for coating of the HAP film. In this paper, we demonstrate the feasibility of our HAP coating process on polymeric materials at room temperature and ambient pressure, as well as the biocompatibility evaluation of the coated HAP by a cell adhesion assay.

MATERIALS AND METHODS

Preparation of the Ethylene−Vinyl Alcohol Copolymer (EVOH) Plates. An EVOH substrate, with high mechanical strength and good biocompatibility, was used for an HAP coating experiment. Substrates (10 × 10 × 1 mm^3) were obtained from EVOH pellets with a quoted ethylene content of 32 mol % (Kuraray Co. Ltd., Tokyo, Japan) by hot-pressing at 210 °C. The EVOH substrate was polished with SiC paper (average grain size = 7.6 μm) and then washed with acetone and ethanol. The EVOH plate was dried at 100 °C under vacuum for 24 h.

Preparation of the Metastable CP solutions (7, 8). A CP solution was prepared by dissolving NaCl (142 mM), $K_2HPO_4 \cdot 3H_2O$ (1.50 mM), and $CaCl_2$ (3.75 mM) (Nacalai Tesque Inc., Kyoto, Japan) in ultrapure water and buffering the solution to pH $^1/_4$ 7.4 at 25 °C using tris(hydroxymethyl)aminomethane (50 mM) and 1 M aqueous HCl (Nacalai Tesque Inc., Kyoto, Japan).

Laser Coating. Laser irradiation was performed on an EVOH substrate immersed in 10 mL of the CP solution for 0.5 or 3 h with an output of the third harmonic (355 nm) of a Nd:YAG laser operated at 10 Hz with a maximum output of 50 mJ/pulse and a pulse duration of 7−8 ns. The power density per pulse of laser irradiation was estimated to be about 0.64 mJ/mm^2. The laser beam was irradiated through a circular laser mask (5 mm diameter) without focusing onto the EVOH substrate surface to clearly distinguish irradiated and unirradiated areas. Subsequently, the laser-irradiated EVOH specimen was immersed in the CP solution (20 mL) for 24 h.

Cell Adhesion Assay. Chinese hamster ovary (CHO-K1) and baby hamster kidney (BHK-21) cells, cell lines supplied by the RIKEN Cell Bank (Ibaraki, Japan), were used to evaluate the cell adhesion property of the coated HAP. CHO-K1 and BHK-21 cells were suspended at a concentration of 1 × 10^5 mL^{-1} in a RPMI 1640 medium including 10% fetal bovine serum (FBS) and in Dulbecco's modified eagle medium including 10% FBS, respectively. These cells were seeded with 0.5 mL of the cell suspensions on the EVOH specimen and cultured for 24 h at 37 °C in a 5% CO_2 incubator. The specimens were washed with phosphate-buffered saline to remove nonadhering cells. The cells remaining adhered to the specimens were fixed with a 10% formaldehyde neutral buffer solution and stained with crystal violet for optical microscope observation.

Surface Analysis of the Patterning Samples. The microstructures of constituents for the patterned HAP were characterized by a field-emission scanning electron microscope (Hitachi S-4800), operating at an accelerating voltage of 15 kV, and a transmission electron microscope (JEOL 2000 FXII), operating at 200 kV. The surface composition and chemical states of the HAP films were examined by X-ray photoelectron spectroscopy (XPS; PHI-5600ci). A standard Al Kα excitation source ($h\nu$ = 1486.6 eV) was employed. The binding energy (BE) scale was calibrated by measuring the reference peak of C 1s (BE = 284.5 eV) from the surface contamination. Identification of the crystal structure for the synthesized HAP was analyzed by thin-film X-ray diffraction (TF-XRD) employing Cu Kα X-rays and selected-area electron diffraction (SAED) with transmission electron microscopy (TEM).

RESULTS AND DISCUSSION

Figure 1a depicts the EVOH surface after laser irradiation for 3 h and subsequent immersion in a CP solution for 24 h. The optical microscope image clearly demonstrates that the laser-irradiated area of the EVOH substrate is changed to a dark color with the mask shape (Figure 1a, upper right). A low-magnification scanning electron microscopy (SEM) image of the dark, laser-irradiated area revealed a cavernous structure (Figure 1a, lower right). In contrast, the unirradiated area (Figure 1a, upper left) revealed a structure equivalent to that of the original EVOH substrate, with many scratches generated by mechanical polishing of EVOH and no porous structure.

Nanocrystalline HAP formation was confirmed only on the irradiated area from TF-XRD patterns (Figure 1b). Broad diffraction peaks at 25.9° and 32.0° were detected in the TF-XRD pattern from the irradiated area. The former peak can be ascribed to (002) and the latter peak to (211), (112), and (300) of the nanocrystalline HAP phase. No HAP formation was observed on the unirradiated area, indicating that

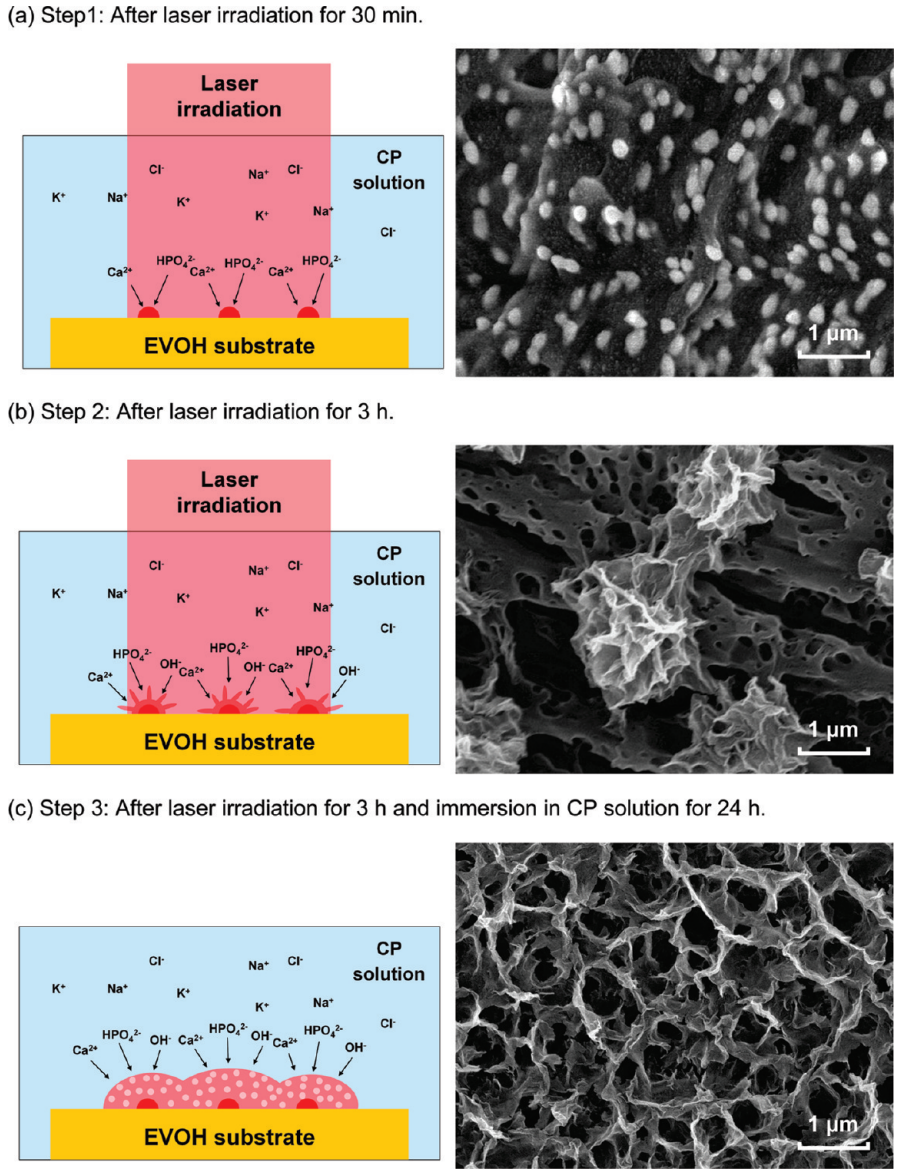

FIGURE 2. Schematic illustrations (left) and corresponding high-magnification SEM images (right) of HAP formation processes on EVOH by laser irradiation and subsequent aging in a CP solution. (a) Step 1: after laser irradiation for 30 min. (b) Step 2: after laser irradiation for 3 h. (c) Step 3: after laser irradiation for 3 h and immersion in a CP solution for 24 h.

the initial laser irradiation is a crucial process for the successful coating of HAP. A uniform HAP thin film can thus be formed on an EVOH substrate by the two-step process of laser irradiation and subsequent aging in a CP solution at room temperature and ambient pressure. XPS peaks from Ca and P were only detected from the laser-irradiated area, which also supported the above observations.

Figure 2 depicts stepwise morphological changes observed by high-magnification field-emission SEM (FE-SEM) to clarify the process of HAP film formation. First, 200 nm ellipsoidal particles were created on EVOH by laser irradiation for 30 min in a CP solution, although they were undetectable by XRD (Figure 2, step 1). The nanostructured particles consisted of CP with a Ca/P atomic ratio of 1.79 (±0.49) by XPS measurements.

As the laser-irradiation time increased to 3 h, the CP particles grew to form a flowerlike structure (Figure 2, step 2). HAP growth was possibly initiated on the CP particles produced during the initial stage of laser irradiation and advanced by consumption of HAP components from the CP solution, although the underlying EVOH surface was still observable from FE-SEM and XPS data.

In the aging by immersion in a CP solution for 24 h, the HAP agglomerates grew further, merged, and finally covered the whole surface by forming an HAP film (Figure 2, step 3). As the film thickened during the aging process, the microscale bumpy surface gradually became flat, while leaving a nanoscale cavernous structure typical of that of HAP formed in an aqueous solution supersaturated with respect to HAP (7, 8). The Ca/P atomic ratio of the coated HAP in this stage was 1.66 (±0.23), which well matched that of stoichiometric HAP, 1.67. We believe that the CP particles produced during the initial stage of laser irradiation acted as HAP precursors and induced the continuous HAP film formation during the aging process. Needlelike HAP nanocrystal aggregates were observed by TEM imaging in Figure

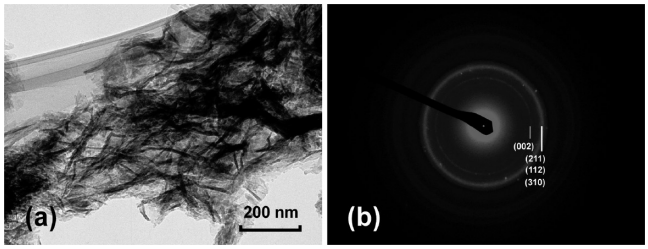

FIGURE 3. TEM micrographs of (a) the HAP obtained under the same conditions as those in Figure 1 and (b) the corresponding SAED pattern.

3a. The SAED pattern taken from these aggregates (Figure 3b) was identified as the crystalline HAP phase. The XRD and TEM results did not suggest the formation of any CP species other than HAP. Thus, the coated nanocrystalline HAP had good compositional homogeneity and phase purity and was well stabilized against decomposition in a CP solution. The different phase from HAP presented during the laser irradiation is most likely to be transformed into HAP during the aging process because HAP is the most thermodynamically stable phase among all of the CPs in an aqueous solution with a neutral pH (20).

Next, we evaluated the biocompatibility of the coated HAP by assaying the adhesion property of CHO-K1 and BHK-21 cells. Figure 4 presents optical micrographs of laser-irradiated and unirradiated areas on the specimen after the cell adhesion assay. Dark dots in Figure 4 are the stained nuclei of the viable cells. The number densities of the cells adhering on the laser-irradiated area were apparently higher than those adhering on the unirradiated area on the specimen for both CHO-K1 and BHK-21 cells. This was quantitatively confirmed by the graph in Figure 4. These results suggest that the coated HAP has a better cell adhesion property than the naked EVOH.

Chemical coating using an aqueous solution supersaturated with respect to HAP (10−14) has been well-known to be useful for the preparation of uniform HAP coatings on any material. The rate of HAP growth, however, cannot be greatly increased by varying the controllable parameters, such as the temperature and pH of the coating solution because the degree of supersaturation of the solution should be kept within a certain range for inducing heterogeneous HAP deposition on a material's surface rather than homogeneous HAP precipitation in the solution. Initial surface modification for providing the material's surface with nucleating agents for HAP is also a complicated and/or time-consuming process.

In this paper, we developed a simple surface-modification process of a polymer to deposit HAP precursor (nucleating agent for HAP) by laser irradiation at relatively weak laser fluences. This new process is carried out using only one solution by one step, whereas our previous process (13, 14) requires three solutions and multiple steps to deposit the HAP precursor on a polymeric substrate. In our new process, laser irradiation induced the formation of HAP precursors on the polymeric substrate immersed in a CP solution.

Because a CP solution does not have any optical absorbance at 355 nm, almost all energy input by laser irradiation is absorbed by the EVOH substrate. The energy absorbed through electronic excitation was transformed into thermal energy during nanosecond laser irradiation and accumulated on a thin surface layer because of the low thermal conductivity of the polymeric material. Thus, this situation is completely different from the case of laser ablation in liquid for nanoparticle fabrication. On the interface between the substrate and the aqueous solution, the thermodynamic state may induce local segregation and reactions of solute elements to form precursors. In fact, the precursors at the early stage of laser irradiation are round, thermodynamic nucleation of nanoprecursor upon laser irradiation during the process. Nucleation of solute element species could have possibly occurred because the thermodynamic state with high temperature and high pressure of laser irradiation provides a good opportunity for chemical reactions between molecules of the supersaturated solution, as observed in step 1 in Figure 2.

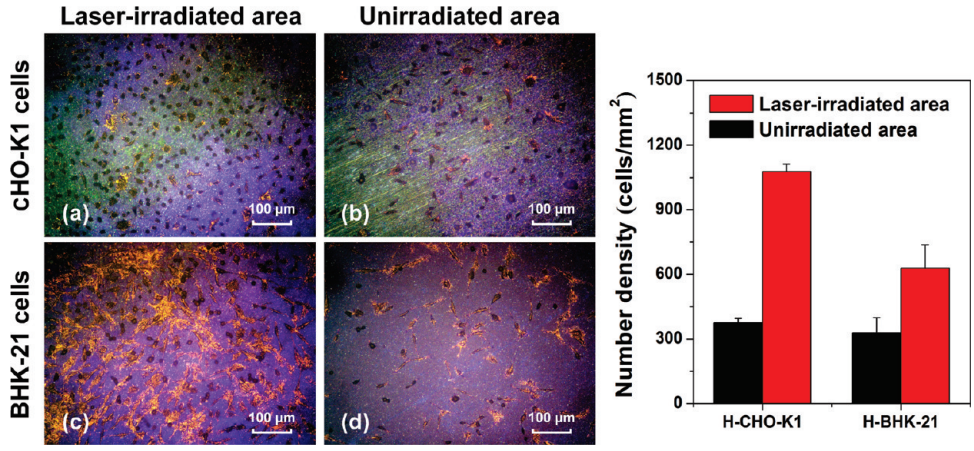

FIGURE 4. Optical micrographs of stained cells after a cell adhesion assay on the specimen obtained under the same conditions as those in Figure 1. (a) Laser-irradiated and (b) unirradiated areas for CHO-K1 cells and (c) laser-irradiated and (d) unirradiated areas for BHK-21 cells. The graph summarizes the number density of CHO-K1 and BHK-21 cells adhering on the laser-irradiated and unirradiated areas on the specimen.

CONCLUSION

A new coating process of HAP, precursor formation by laser irradiation and aging in a CP solution, was proposed. The coated HAP possesses cavernous morphology, consisting of agglomerated nanostructured HAP. This process may further facilitate the shortening of the processing time for the initial surface-modification step for precursor formation by adjusting laser-irradiation conditions. Furthermore, this technique can be applied for a maskless patterning process for HAP and biopolymer−HAP composites by using modified CP solutions (7, 8) in the aging process, which will be reported elsewhere.

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REFERENCES AND NOTES

(1) Park, J. B.; Lakes, R. S. *Biomaterials*, 2nd ed.; Plenum Publishing Co.: New York, 1992.
(2) Jarcho, M.; Kay, J. F.; Drobeck, H. P.; Dremus, R. H. *J. Bioeng.* **1976**, *1*, 79–92.
(3) Aoki, H. *Science and Medical Applications of Hydroxyapatite*; Takayama Press: Tokyo, 1991.
(4) Tiselius, A.; Hjertén, S.; Levin, Ö. *Arch. Biochem. Biophys.* **1956**, *65*, 132–155.
(5) Yang, Y.; Kim, K.-H.; Ong, J. L. *Biomaterials* **2005**, *26*, 327–337.
(6) Liu, Y.; Layrolle, P.; de Bruijn, J.; Blitterswijk, C.; de Groot, K. *J. Biomed. Mater. Res.* **2001**, *57*, 327–335.
(7) Liu, Y.; Layrolle, P.; de Bruijn, J.; Blitterswijk, C.; de Groot, K. *J. Biomed. Mater. Res.* **2001**, *57*, 327–335.
(8) Uchida, M.; Oyane, A.; Kim, H. M.; Kokubo, T.; Ito, A. *Adv. Mater.* **2004**, *16*, 1071–1074.
(9) Cheang, P.; Khor, K. A. *Biomaterials* **1996**, *17*, 537–544.
(10) Tanahashi, M.; Yao, T.; Kokubo, T.; Minoda, M.; Miyamoto, T.; Nakamura, T.; Yamamuro, T. *J. Am. Ceram. Soc.* **1994**, *77*, 2805.
(11) Oyane, A.; Kawashita, M.; Kokubo, T.; Minoda, M.; Miyamoto, T.; Nakamura, T. *J. Ceram. Soc. Jpn.* **2002**, *110*, 248.
(12) Oyane, A.; Kawashita, M.; Nakanishi, K.; Kokubo, T.; Minoda, M.; Miyamoto, T.; Nakamura, T. *Biomaterials* **2003**, *24*, 1729.
(13) Oyane, A.; Uchida, M.; Choong, C.; Triffitt, J.; Jones, J.; Ito, A. *Biomaterials* **2005**, *26*, 2407–2413.
(14) Oyane, A.; Uchida, M.; Yokoyama, Y.; Choong, C.; Triffitt, J.; Ito, A. *J. Biomed. Mater. Res. A* **2005**, *75A*, 138.
(15) Kim, H. M.; Kishimoto, K.; Miyaji, F.; Kokubo, T.; Yao, T.; Suetsugu, Y.; Tanaka, J.; Nakamura, T. *J. Biomed. Mater. Res.* **1999**, *46*, 228–235.
(16) Oyane, A.; Kim, H. M.; Furuya, T.; Kokubo, T.; Miyazaki, T.; Nakamura, T. *J. Biomed. Mater. Res. A* **2003**, *65A*, 188–195.
(17) Yang, G. W. *Prog. Mater. Sci.* **2007**, *52*, 648–698.
(18) Sasaki, T.; Shimizu, Y.; Koshizaki, N. *J. Photochem. Photobiol. A: Chem.* **2006**, *182*, 335–341.
(19) Pecheva, E.; Petrov, T.; Lungu, C.; Montgomery, P.; Pramatarova, L. *Chem. Eng. J.* **2008**, *137*, 144–153.
(20) Elliot, J. C. *Structure and Chemistry of the Apatites and Other Calcium Phosphates*; Elsevier Science BV: Amsterdam, The Netherlands, 1994.

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