

**How to synthesize
apatitic calcium
phosphate (*Ap-CaP*)
on, or from,
different starting
materials
(*i.e.*, ceramics, biometals and
biopolymers)?**

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This document summarizes the peer-reviewed publications of my previous research groups (consisting of my collaborators, my graduate students and my undergraduate students, whenever applicable) only on the specific topic described in the Title.

I will provide the addresses of those publications and provide primarily the SEM (scanning electron microscope) or TEM (transmission electron microscope) images. I think even a simple viewing of self-descriptive, self-speaking images might kindle the interest of general audiences with no special background whatsoever in the materials chemistry of biomaterials used as synthetic bone-substitutes/bone grafts.

My R&D work shared here was based on aqueous chemical synthesis routes practiced at “temperatures $\leq 90^{\circ}\text{C}$ ” so that one could form apatitic calcium phosphate (*Ap-CaP*) nanoneedles even in simple, sealed bottles (glass, HDPE or polycarbonate) without a need for using expensive autoclaves.

- *The hyperlinks throughout the text allow access to the publications.*

Biography

May '93	Ph.D. in Materials Sci. and Eng., Major: ceramic engineering (<i>Iowa State University, Iowa, USA</i>)
Jun '93 - Nov '97	Assistant Professor (<i>METU, Ankara, Turkey</i>)
Nov '97 - Jan '02	Associate Professor (<i>METU</i>)
Feb '99 - Feb '01	Visiting Professor (<i>Max-Planck-Institut für Metallforschung, Stuttgart, Germany</i>) – now defunct
Sep '01 - May '03	Senior Staff Scientist (<i>Merck Biomaterials GmbH, Darmstadt, Germany</i>) – now defunct
May '03 - Apr '06	Research Associate Professor (<i>Clemson University, South Carolina, USA</i>)
May '06 - Nov '06	Professor (<i>Mersin University, Mersin, Turkey</i>)
Nov '06 - Sep '10	Professor (<i>Yeditepe University, Istanbul, Turkey</i>)
Aug '07 - Jul '08	Visiting Prof. (<i>New York University, College of Dentistry, New York City, USA</i>)
Sep '10 - Oct '11	Visiting Prof. (<i>University of Oklahoma, College of Dentistry, Oklahoma City, USA</i>)
Apr '12 - Jun '15	Research Scholar (<i>University of Illinois at Urbana-Champaign, Illinois, USA</i>)
Jul '15 - Dec '22	Senior Principal Scientist (<i>Solidia Technologies, New Jersey, USA</i>) – now defunct
Jan '23 - Dec '23	Principal Scientist (<i>Queens Carbon, New Jersey, USA</i>)
Start date: Mar '24	Principal Materials Scientist (<i>Carbon Limit, Florida, USA</i>)

Metrics

Peer-reviewed Journal publications:	71
Google Scholar metrics:	>7300 citations h-index: 43 i ₁₀ -index: 79
Symposium/Conference presentations:	127
Book chapters:	34
ICDD X-ray diffraction patterns:	18
Phase diagrams:	2
Patents (<i>issued</i>):	31

Academician: from 5/1993 to 9/2001 and from 6/2003 to 6/2015, total of 20 years
Industrial scientist (*CO₂ mineralization*): since 7/2015, >10 years

Considering the hard tissue mineral as a stoichiometric compound calcium hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ is a misleading oversimplification of that mineral. Actually, it is a non-stoichiometric, low crystallinity, and complex substance (doped with several mono- or di-valent cations (such as, Na, K, Mg, Zn, Fe, *etc.*) as well as with carbonate ions) with a generic formula of $\text{Ca}_{8.3}(\text{PO}_4)_{4.3}(\text{HPO}_4, \text{CO}_3)_{1.7}(\text{OH}, \text{CO}_3)_{0.3}$. The hard tissue mineral is not Ca-hydroxyapatite, but it is an apatitic calcium phosphate (Ap-CaP) with its A and B crystallographic sites partially doped with HPO_4^{2-} and CO_3^{2-} ions [1].

The hard tissue mineral itself does not dissolve in physiological solutions (this is why our bones remain intact in blood during our lifetimes). Bone is resorbed only by osteoclast cells, with new bone forming under the combined action of osteocytes and osteoblasts. This process of biomineralization takes place on a daily basis in healthy bones. This process of resorption+new bone formation is also named as bone remodeling [1].

When one implants a human-made material (to serve as a synthetic bone graft or bone defect/void filling agent) such as high crystallinity and high alkalinity Ca-hydroxyapatite (with a Ca/P molar ratio of 1.67), regardless of its porosity (to allow the invasion of the implanted material by the bone cells), then that hydroxyapatite will not be resorbed by osteoclasts but the osteoblasts will deposit quite a thin layer of new bone on the surfaces of hydroxyapatite. As a result, stoichiometric hydroxyapatite only displays osteoconductivity. We do not want any synthetic bone substitute material that does not take part in bone remodeling [1].

So, there arises the need to synthesize Ap-CaP which is (simultaneously) non-stoichiometric, low crystallinity, of a Ca/P molar ratio of 1.4 to 1.55, and has its lattice sites doped with hydrogen phosphate, carbonate and physiologically important elements such as Zn, Mg, Fe and Cu (see Table 2 of [Ref. 20](#) of [1]).

[1] A. C. Tas, "Participation of calcium phosphate bone substitutes in the bone remodeling process," *Key Engineering Materials*, Vols. 264-268, pp. 1969-1972 ([2004](#)).

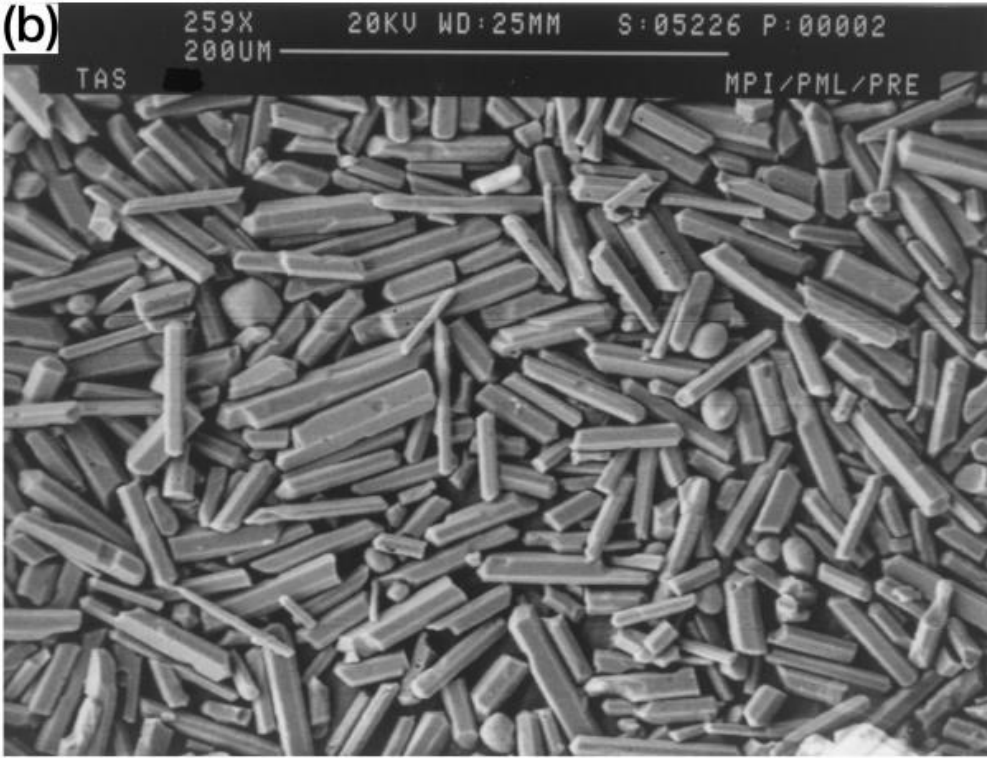
In order to name a calcium phosphate-based material as “calcium hydroxyapatite,” with a formula of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, its FTIR scan must exhibit a sharp IR band at 3571 cm^{-1} . This specific band is caused by the $(\text{OH})_2$ of the above formula.

To observe a broad band over the range of 4000 to 3000 cm^{-1} , which shows the adsorbed H_2O , is not enough to label the material as “hydroxyapatite.”

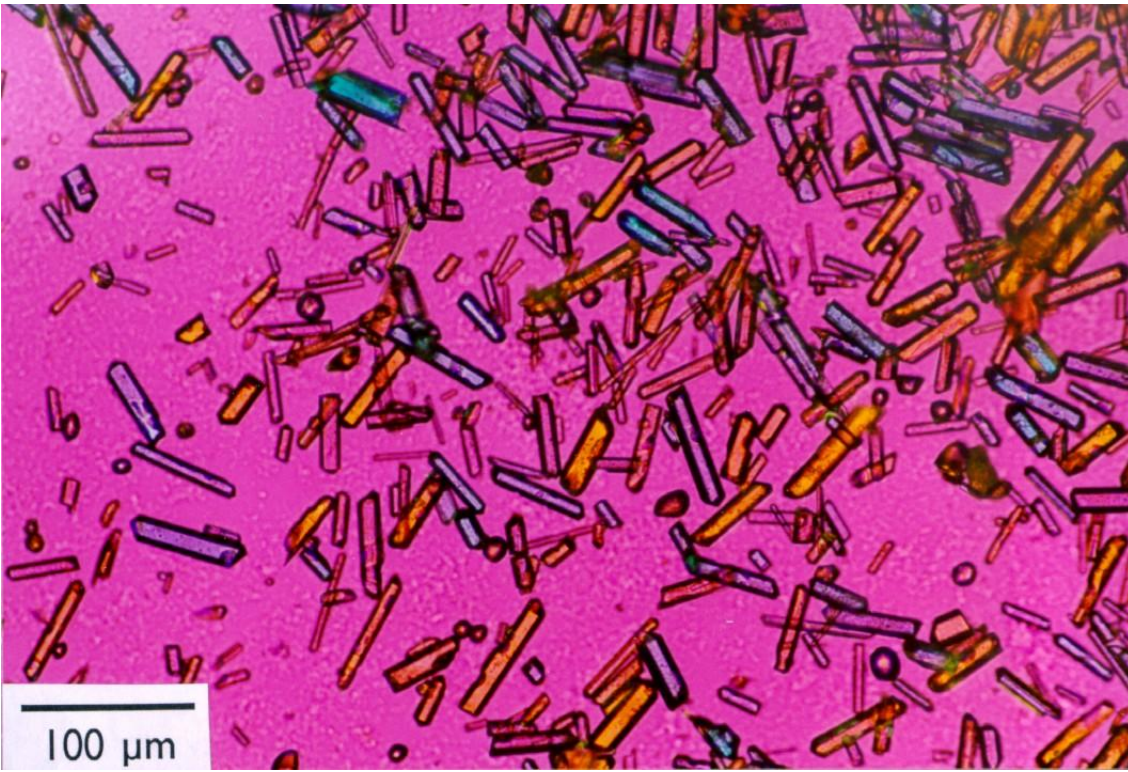
If the FTIR data of a material does not display that sharp band at 3571 cm^{-1} , then that material can only be named as “apatitic calcium phosphate, *Ap-CaP*.”

To begin with, my first significant contribution to the bioceramics/biomaterials literature on the “needle/whisker” synthesis appeared in the *Journal of The American Ceramic Society* [2] in 2001. Its manuscript was submitted in the year 2000. This was a study on the molten salt synthesis of calcium hydroxyapatite needles/whiskers. These needles/whiskers were not submicron though. I also determined the standardized XRD pattern of these whiskers which is available in the [ICDD-PDF](#) database under the card file number of [00-055-0592](#).

1



SEM image [2]



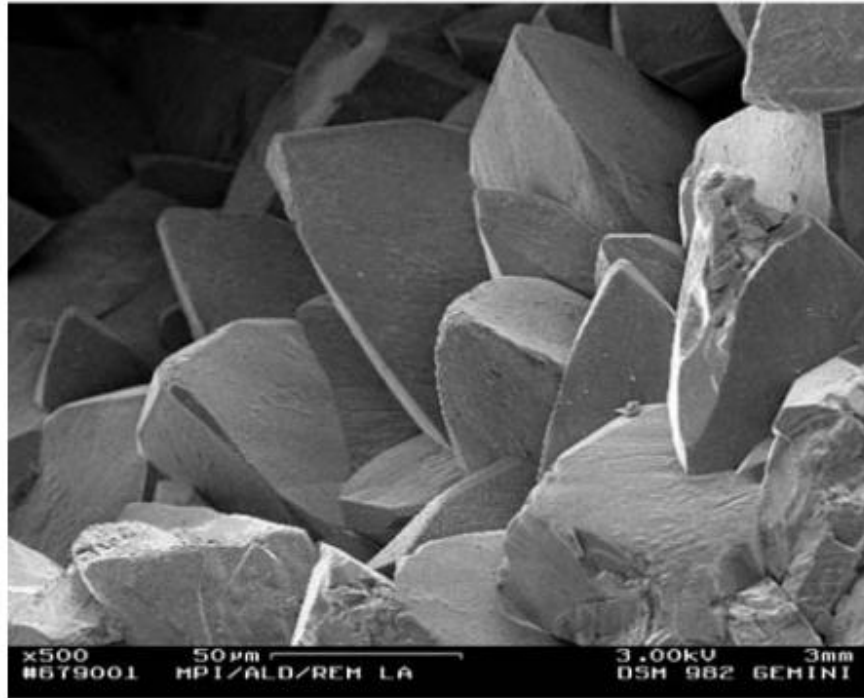
polarizing optical microscope image [3]

[2] A. C. Tas, “Molten salt synthesis of calcium hydroxyapatite whiskers,” *Journal of The American Ceramic Society*, 84, 295-300 (2001).

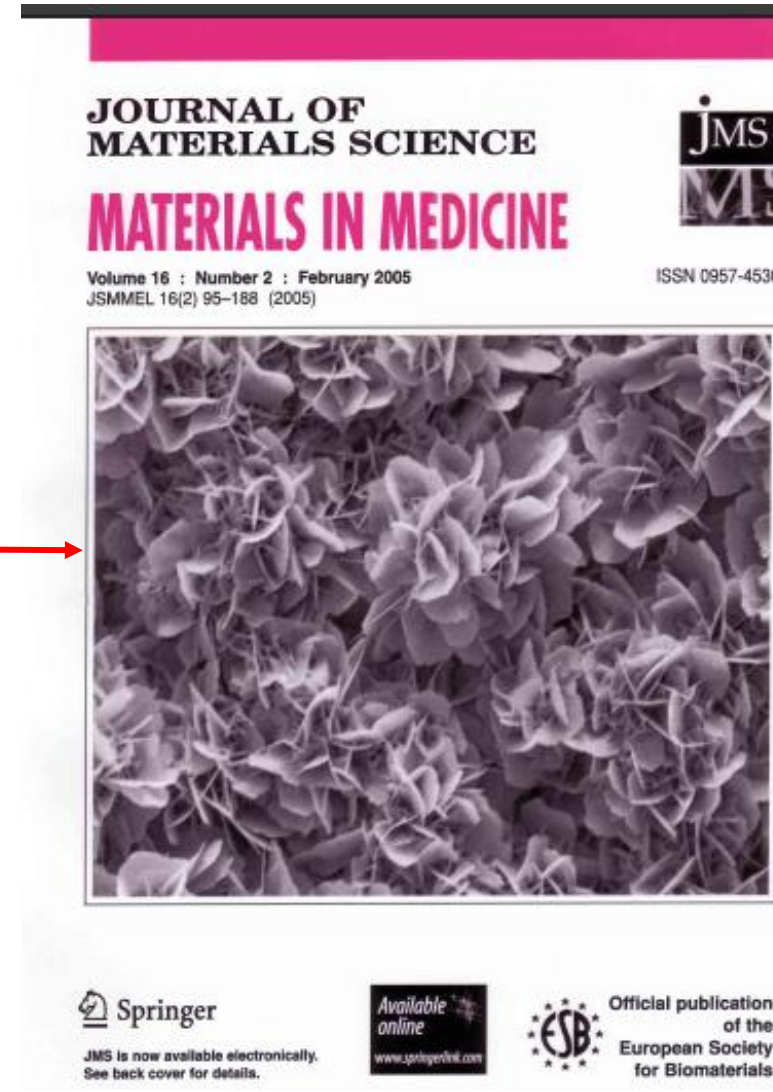
[3] A. C. Tas, “X-ray diffraction data for flux-grown calcium hydroxyapatite whiskers,” *Powder Diffraction (ICDD)*, 16, 102-106 (2001).

Formation of apatitic calcium phosphate submicron needles on the surface of natural white marble blocks [4] (*selected as the Journal issue cover image; performed during my tenure as visiting Professor at the Max-Planck-Institut für Metallforschung, Stuttgart, from 2/1999 to 2/2001*)

2



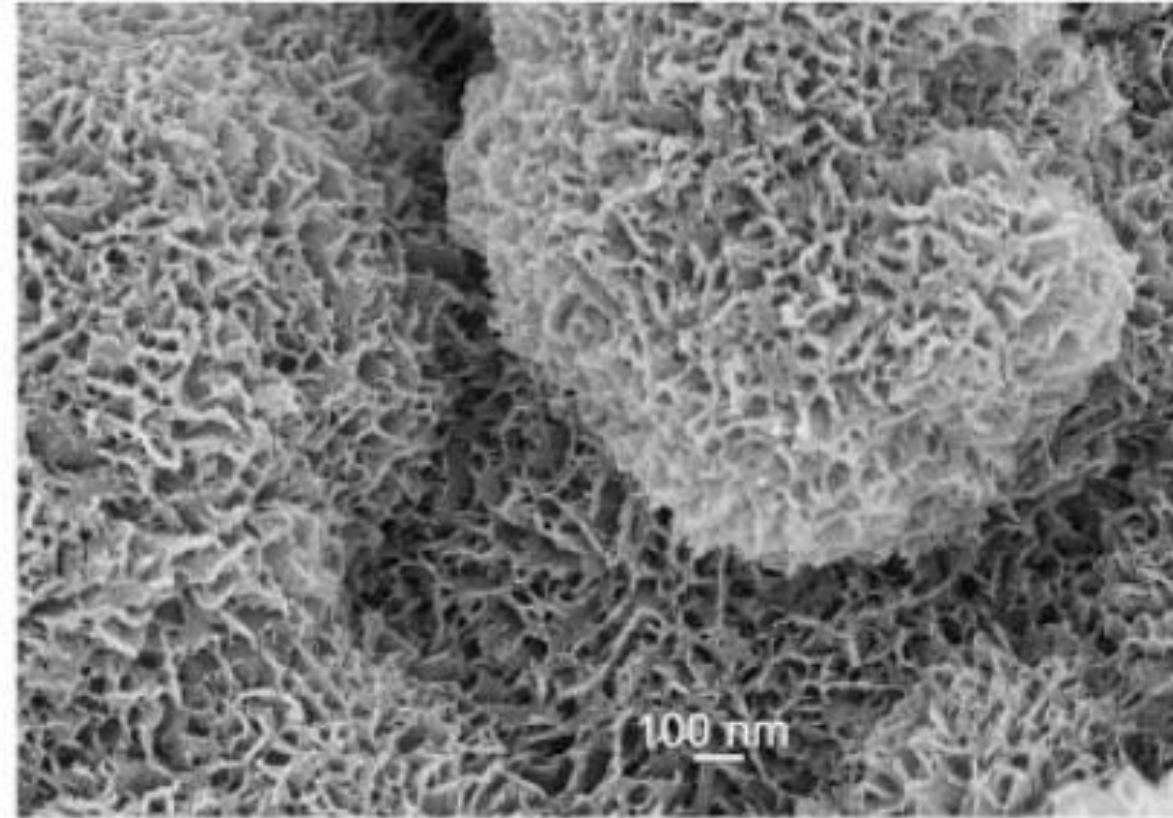
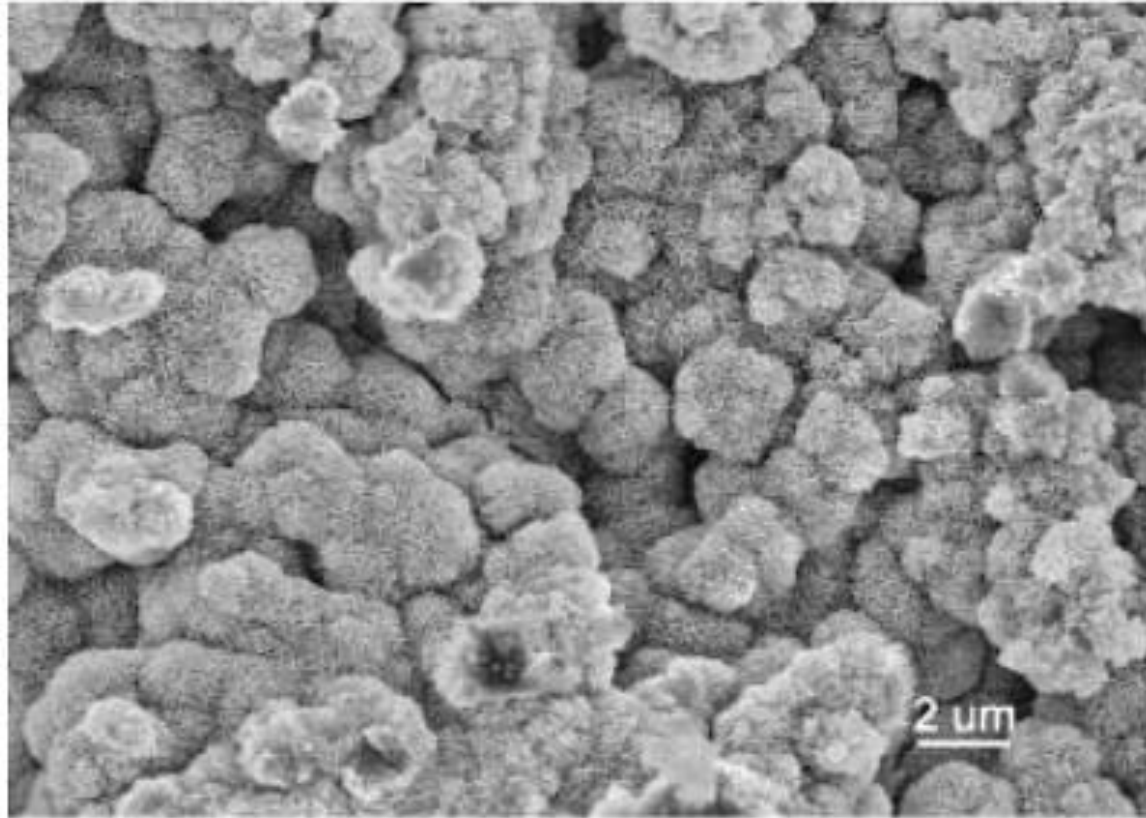
SEM image of the starting natural marble (calcite) surface



[4] A. C. Tas and F. Aldinger, "Formation of apatitic calcium phosphates in a Na-K-phosphate solution of pH 7.4," *Journal of Materials Science: Materials in Medicine*, 16, 167-174 (2005).

Formation of apatitic calcium phosphate globules/spherules comprised of nanoneedles of apatitic calcium phosphate on Ti-6Al-4V surfaces pretreated with 5 M KOH by using my own 10X SBF solution [5] (without having any TRIS or HEPES) to shorten the apatitic calcium phosphate formation time (at RT not at 37°C) from several weeks to 6 hours

3



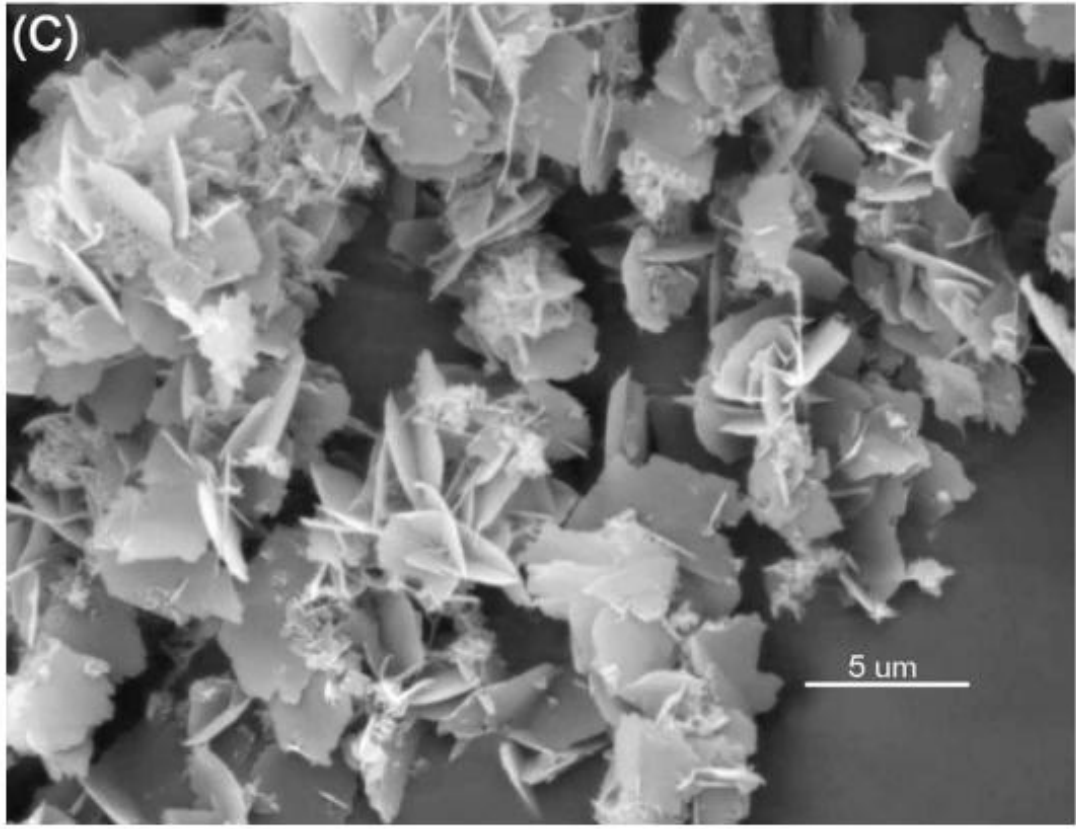
[5] A. C. Tas *et al.* "Rapid coating of Ti6Al4V at room temperature with a calcium phosphate solution similar to 10X simulated body fluid," *Journal of Materials Research*, 19, 2742-2749 ([2004](#)).

Conversion of DCPD (dicalcium phosphate dihydrate, brushite, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) to apatitic calcium phosphate [6] when soaked in [Tas-SBF](#) (having TRIS and 27 mM HCO_3^- concentration) at 37°C in 1.5 to 7 days

4



SEM image of the DCPD crystals studied (the cover image of the Dec 2004 issue)

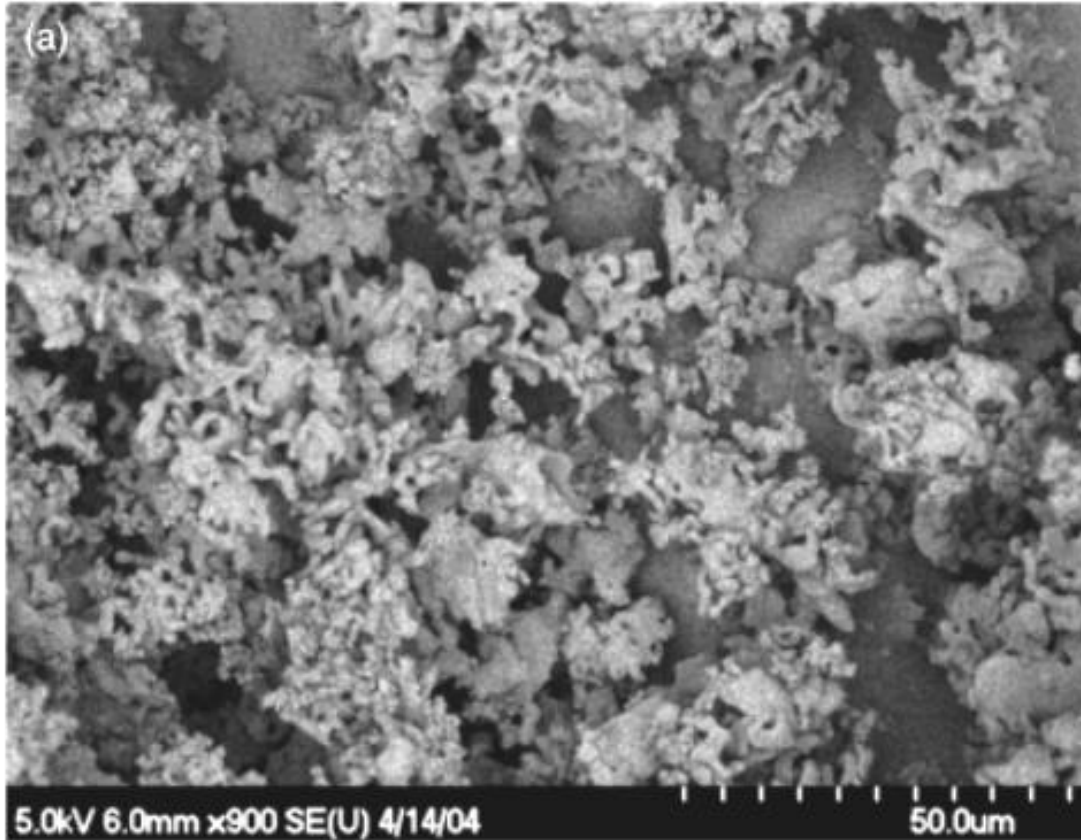


DCPD crystals started their conversion to apatitic calcium phosphate only after 1.5 days of immersion in [Tas-SBF](#) at 37°C

[6] A. C. Tas *et al.* "Chemical processing of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$," *Journal of The American Ceramic Society*, 87, 2195-2200 (2004). As a side note, I am proud to mention that I initiated the Ap-CaP-based bioceramic research at the Dept. of Materials Sci. & Eng. of Clemson University in 6/2003 (till the end of 2006) to build upon my 10 years-long previous experience in the field at METU, Max-Planck-Institut and Merck Biomaterials GmbH.

Transformation of TTCP (tetracalcium phosphate), produced by a novel chemical synthesis method (followed by calcination of the TTCP-precursors) developed in this work, to apatitic calcium phosphate [7] in a [Tas-SBF](#) solution at 37°C

5



SEM image of the TTCP powders studied



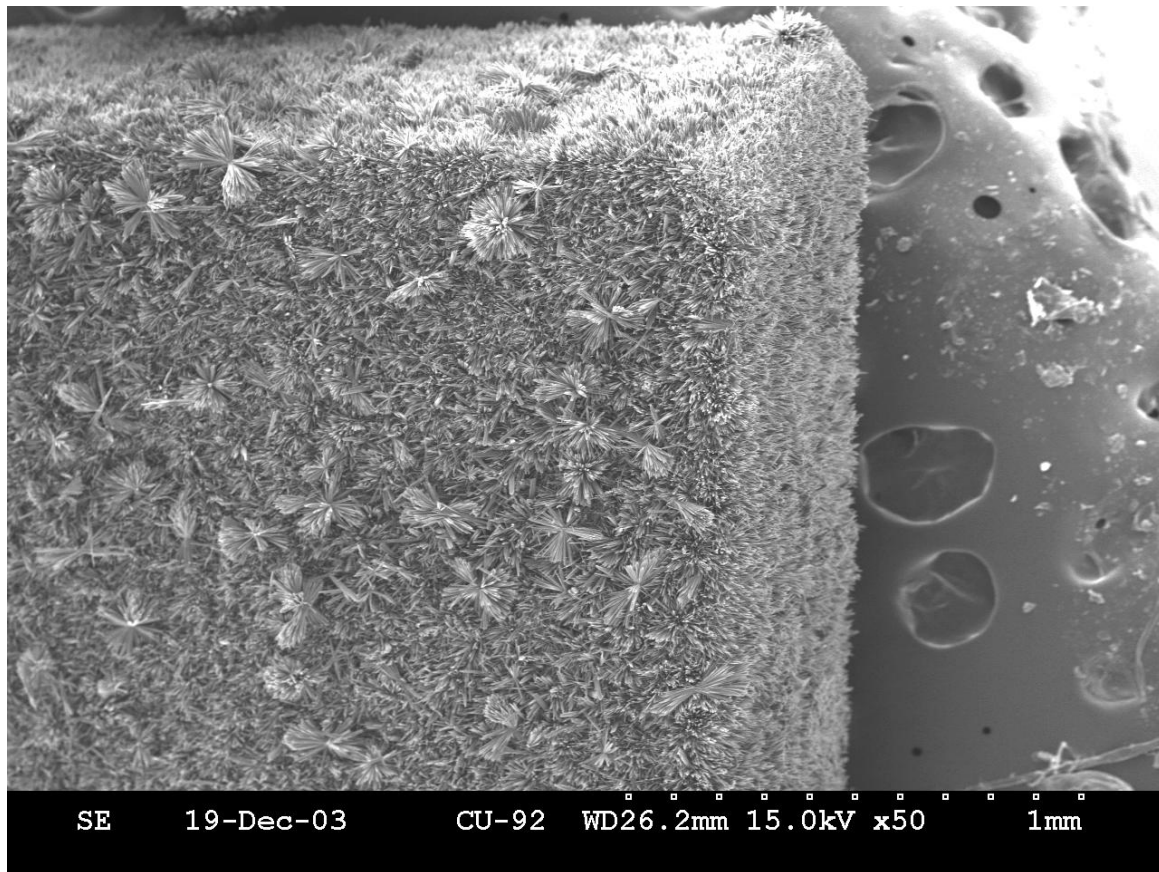
TTCP particles started their conversion to apatitic calcium phosphate only after 3 days of immersion in [Tas-SBF](#) at 37°C

[7] A. C. Tas *et al.* "Synthesis of HA-seeded TTCP ($\text{Ca}_4(\text{PO}_4)_2\text{O}$) powders at 1230°C from $\text{Ca}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ and $\text{NH}_4\text{H}_2\text{PO}_4$," *Journal of The American Ceramic Society*, 88, 3353-3360 ([2005](#)).

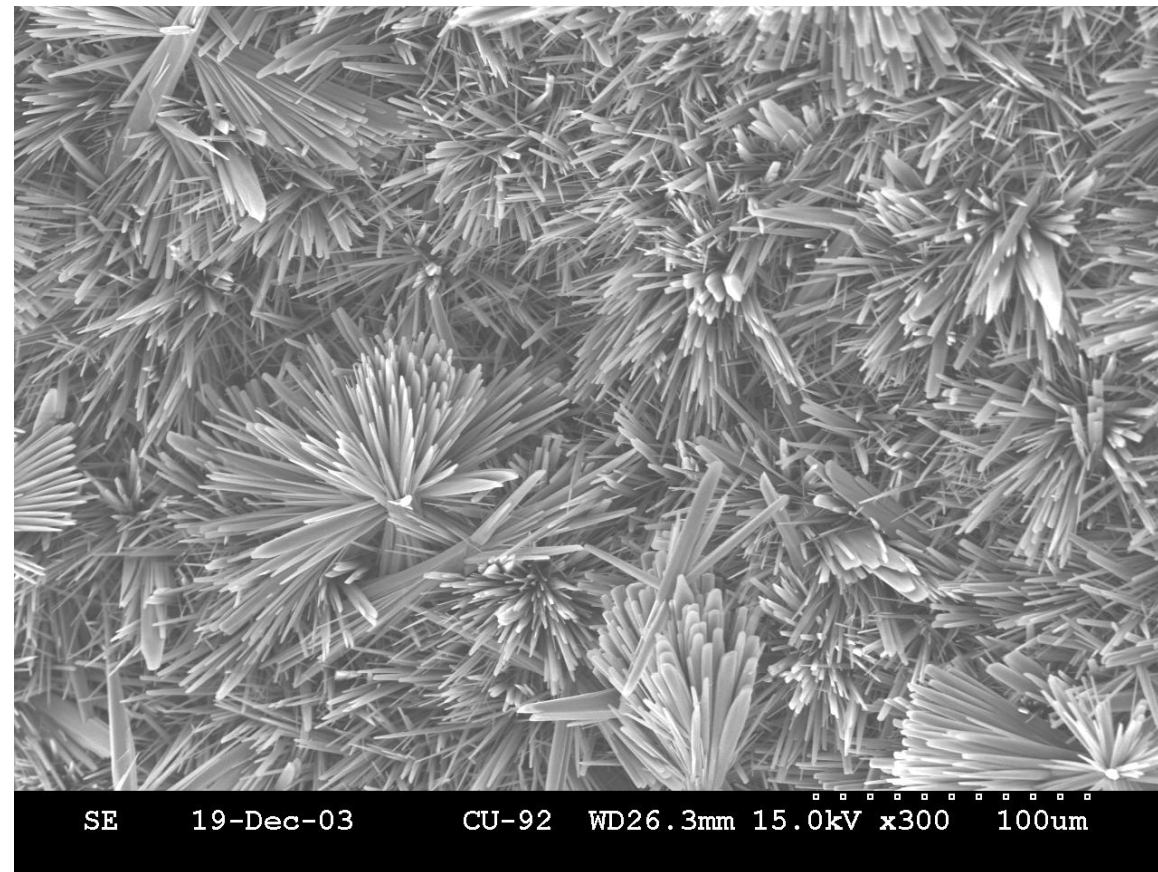
Novel solutions for electroless deposition [8] of DCPD (brushite, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) on cm-sized Ti-6Al-4V coupons (pretreated in 5 M KOH solution) at RT

The brushite-coated coupons were not immersed in a Tas-SBF solution to transform the brushite crystals to apatitic calcium phosphate since it was needless (to do that) under the light of the 4th example (in red)given above.

6



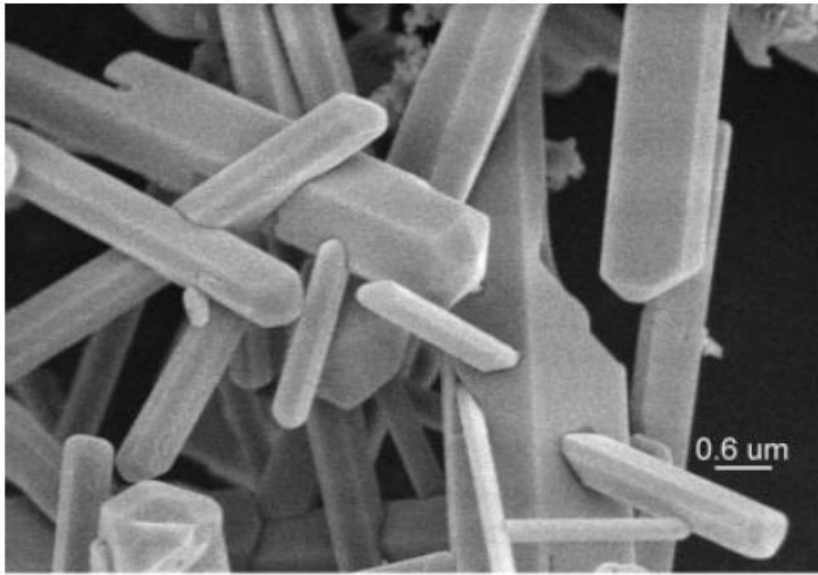
SEM image of brushite-coated Ti-6Al-4V (low mag) stuck to adhesive C tape



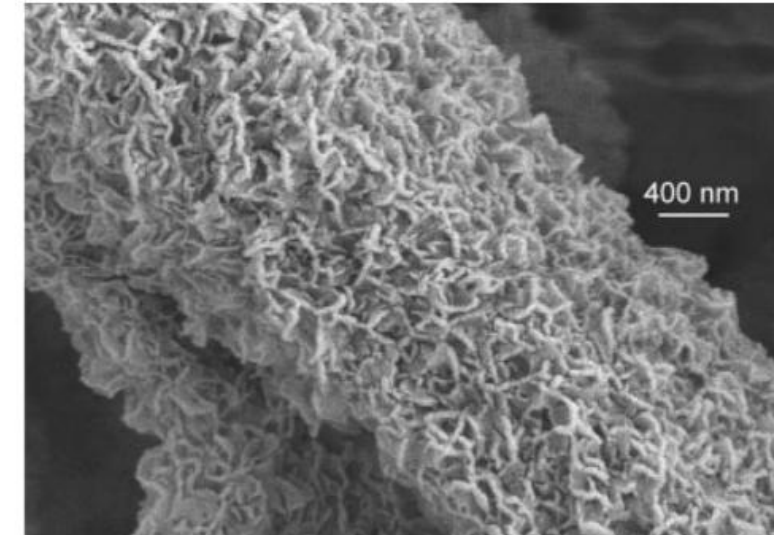
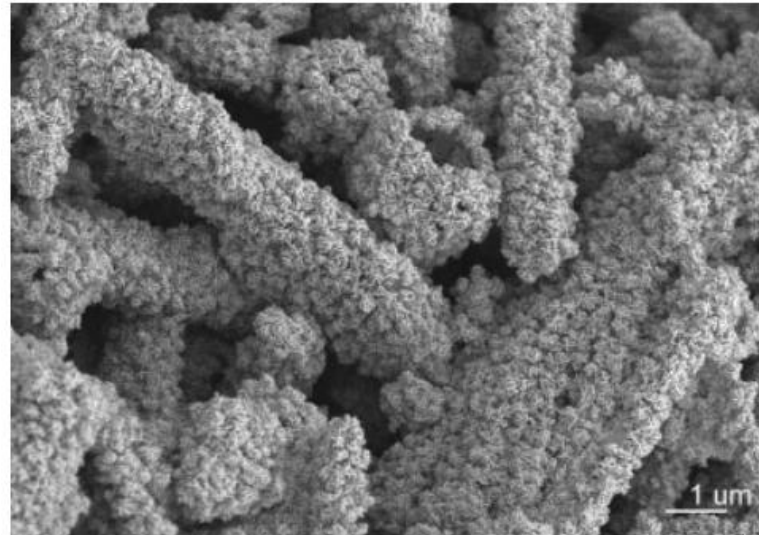
SEM image of brushite-coated Ti-6Al-4V (higher mag)

[8] A. C. Tas, "Electroless deposition of brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) crystals on Ti-6Al-4V at room temperature," *International Journal of Materials Research (formerly Zeitschrift für Metallkunde)*, 97, 639-644 (2006).

Conversion of HA, β -TCP or biphasic HA+ β -TCP whiskers to apatitic calcium phosphate (Ap-CaP) whiskers in [Tas-SBF](#) [9]



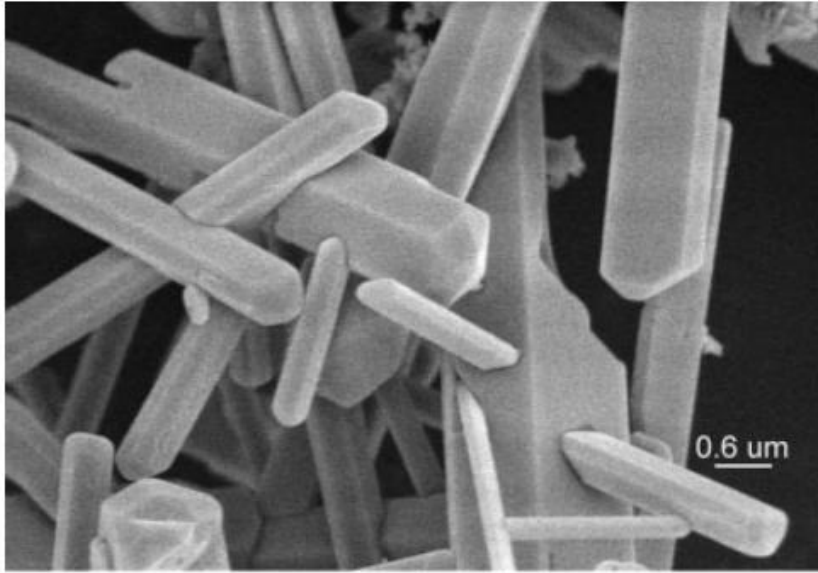
SEM image of starting β -TCP whiskers (as an example; where HA and biphasic HA+ β -TCP whiskers had the same morphology) [9]



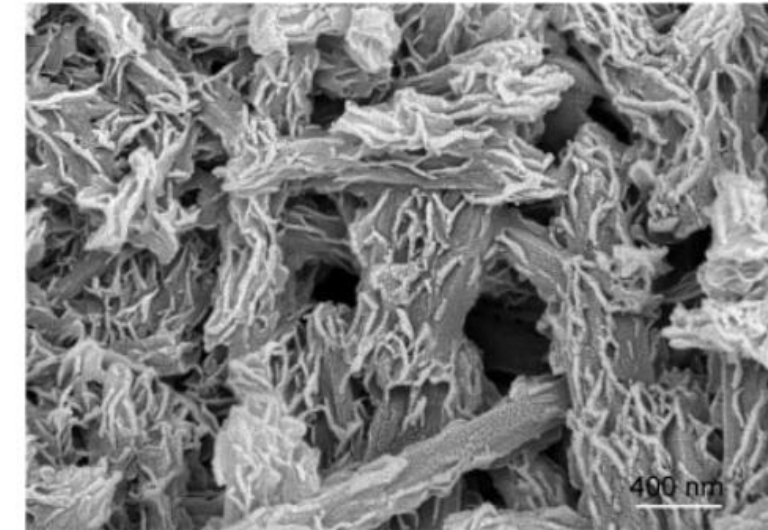
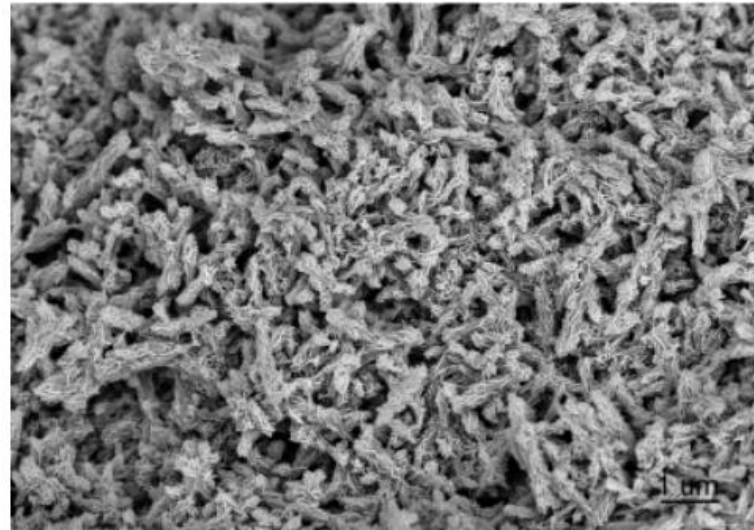
SEM images of starting β -TCP whiskers turned into apatitic CaP whiskers after 1 week, at 37°C, in Tas-SBF [9]

[9] A. C. Tas *et al.* "In vitro testing of calcium phosphate (HA, TCP and biphasic HA-TCP) whiskers," *Journal of Biomedical Materials Research Part A*, 78A, 481-490 ([2006](#)).

Conversion of HA, β -TCP or biphasic HA+ β -TCP whiskers to apatitic calcium phosphate (Ap-CaP) whiskers in [Tas-SBF](#) [9]



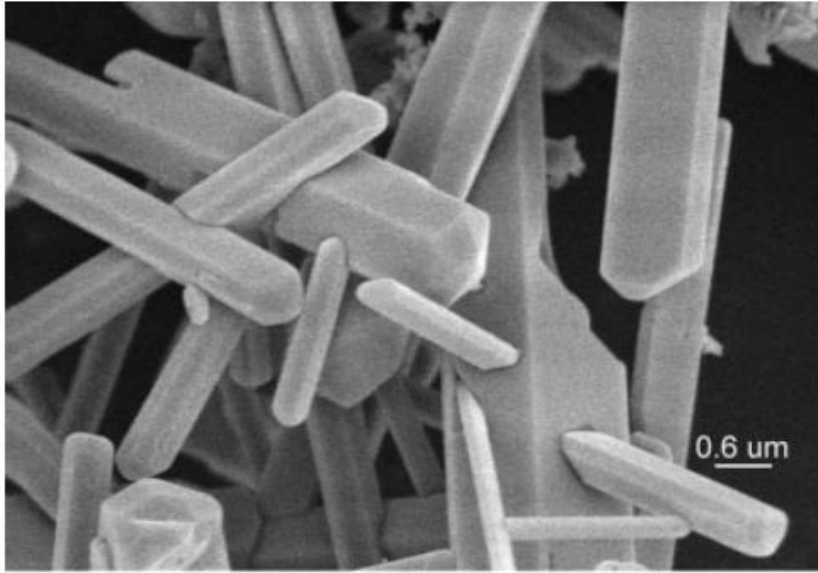
SEM image of starting β -TCP whiskers (as an example; where HA and biphasic HA+ β -TCP whiskers had the same morphology) [9]



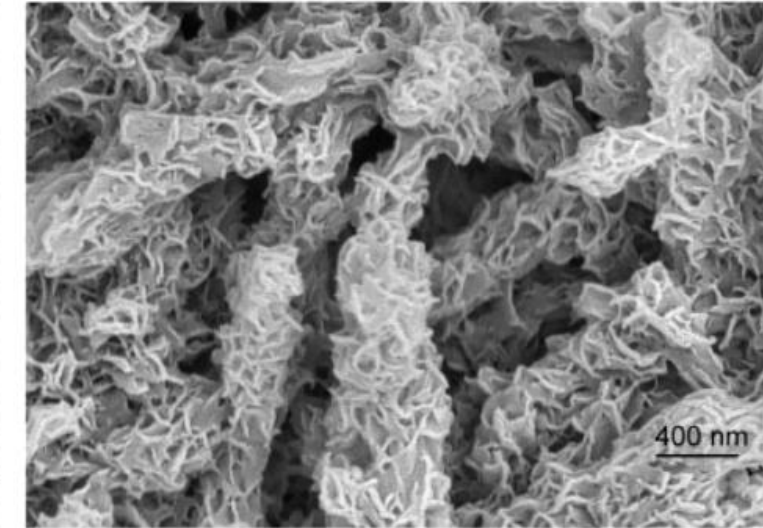
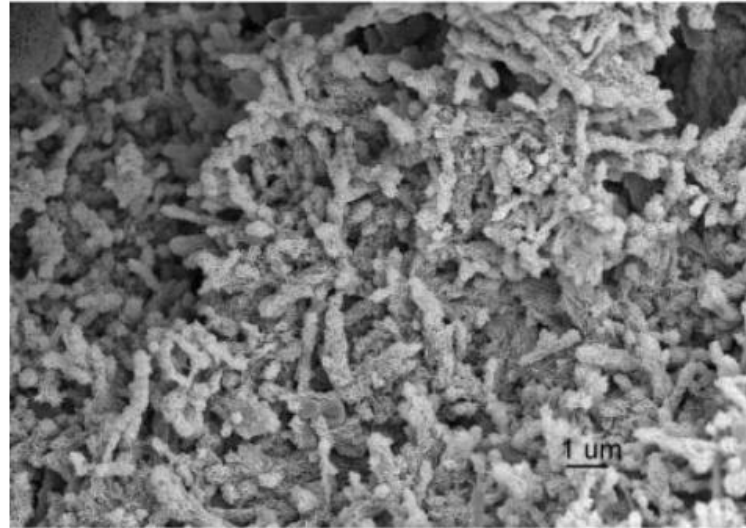
SEM images of starting **HA** whiskers turned into apatitic CaP whiskers after 1 week, at 37°C, in Tas-SBF [9]

[9] A. C. Tas *et al.* "In vitro testing of calcium phosphate (HA, TCP and biphasic HA-TCP) whiskers," *Journal of Biomedical Materials Research Part A*, 78A, 481-490 ([2006](#)).

Conversion of HA, β -TCP or biphasic HA+ β -TCP whiskers to apatitic calcium phosphate (Ap-CaP) whiskers in [Tas-SBF](#) [9]

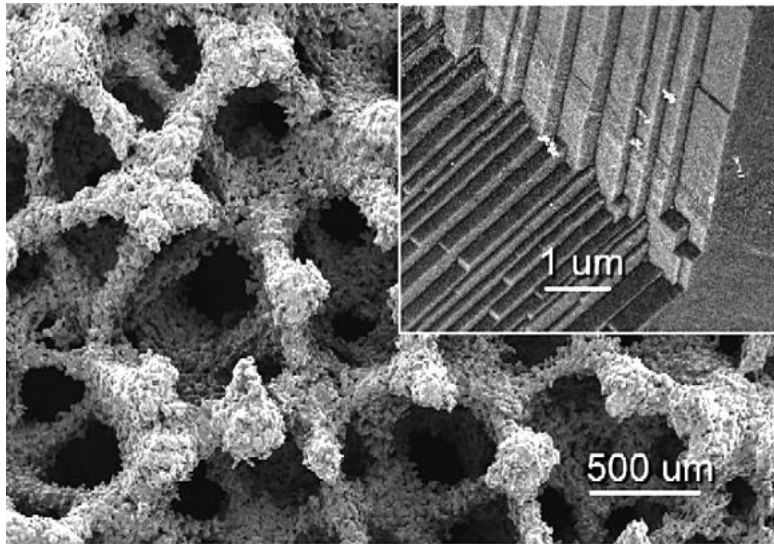


SEM image of starting β -TCP whiskers (as an example; where HA and biphasic HA+ β -TCP whiskers had the same morphology) [9]

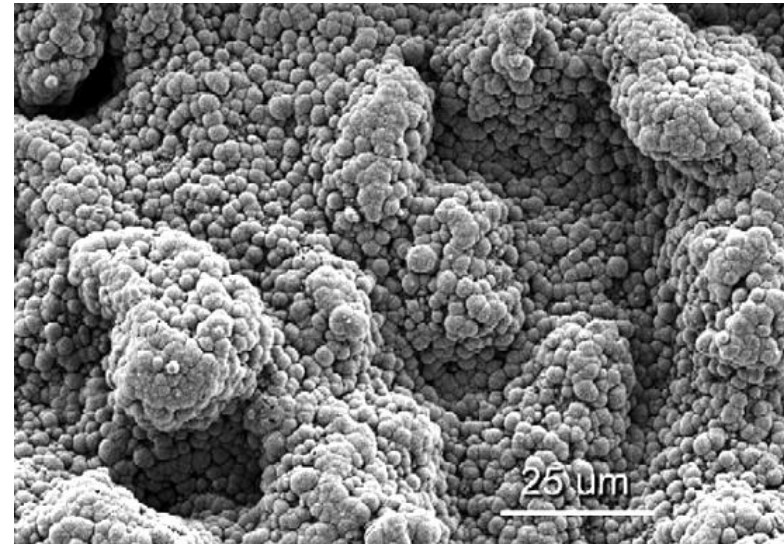


SEM images of starting **biphasic HA+ β -TCP** whiskers turned into apatitic CaP whiskers after 1 week, at 37°C, in Tas-SBF [9]

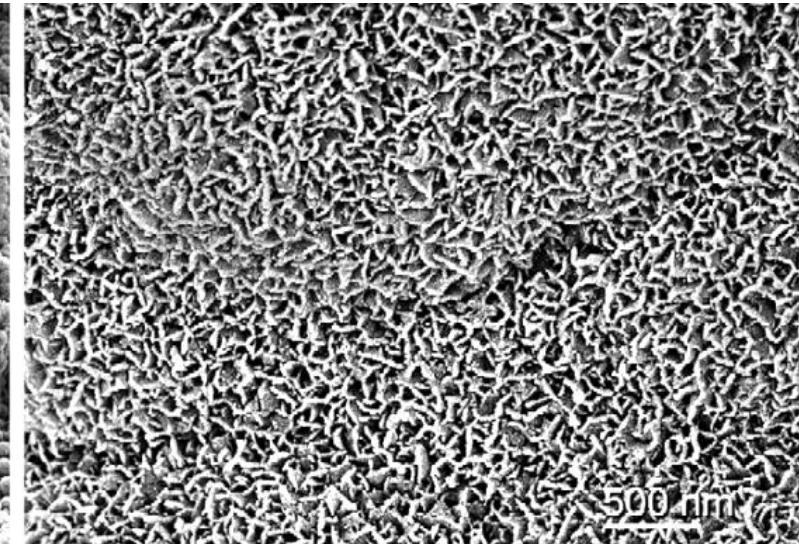
Formation of Ap-CaP on Ti-6Al-4V foams and Ti-6Al-4V wire springs by using 1.5X Tas-SBF at 37°C [10]



starting Ti-6Al-4V foam

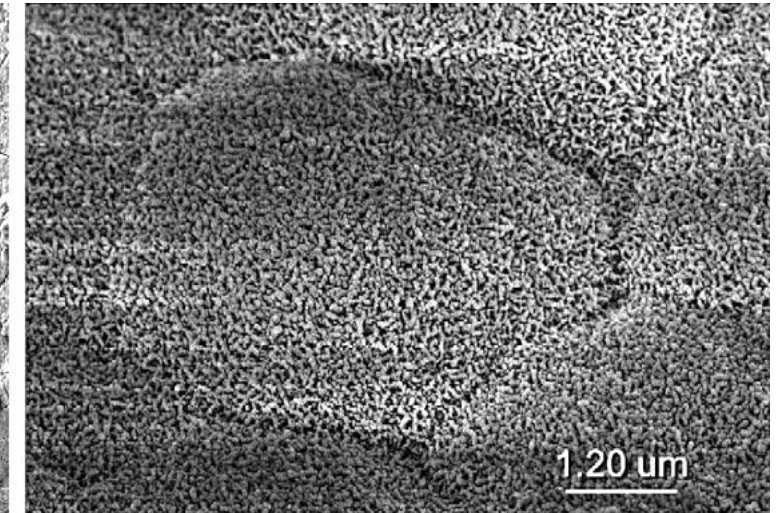
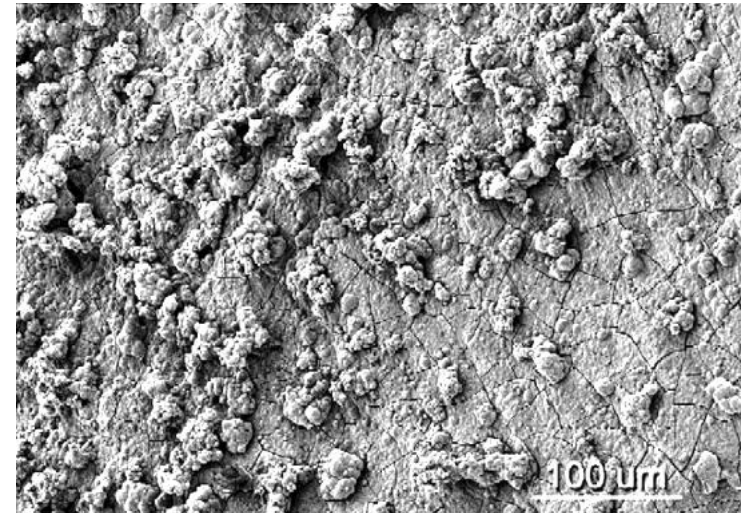
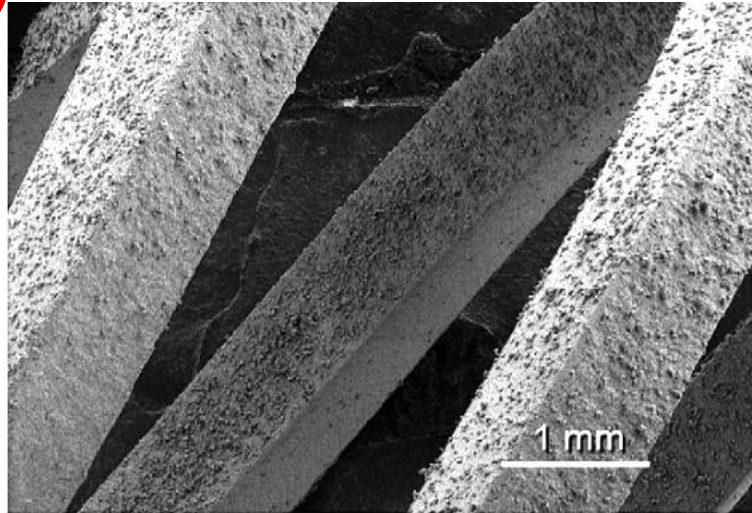


Ap-Cap on the foam (low mag)



Ap-Cap nanoneedles on the foam (high mag)

10

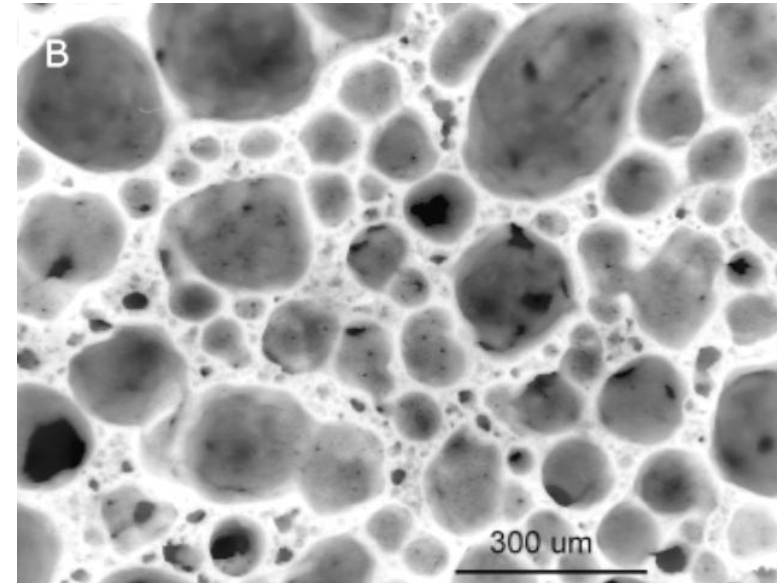
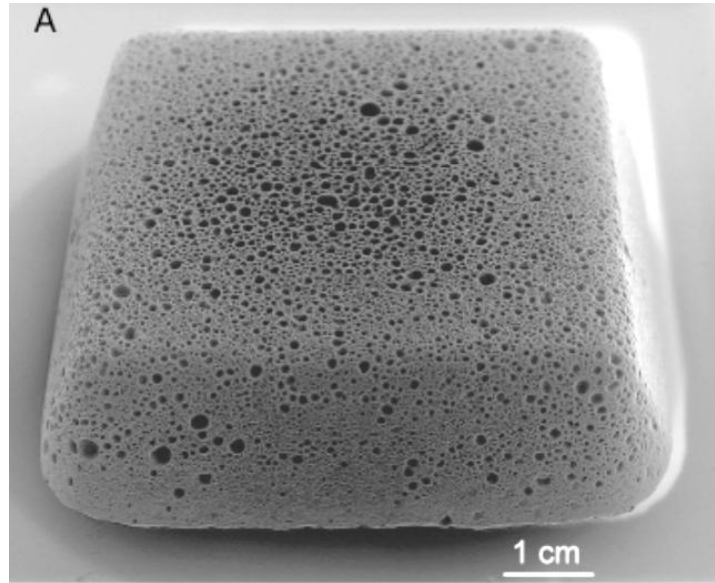


Ap-Cap on the T-6Al-4V wire springs (increasing magnifications from left to right)

[10] A. C. Tas *et al.* "Osteoblast proliferation on neat and apatite-like calcium phosphate-coated titanium foam scaffolds," *Materials Science and Engineering C*, 27, 432-440 ([2007](#)).

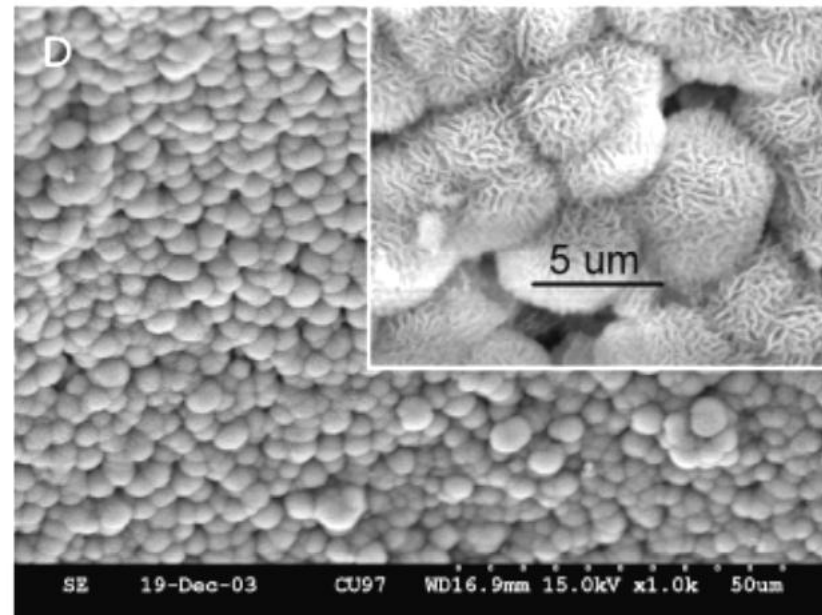
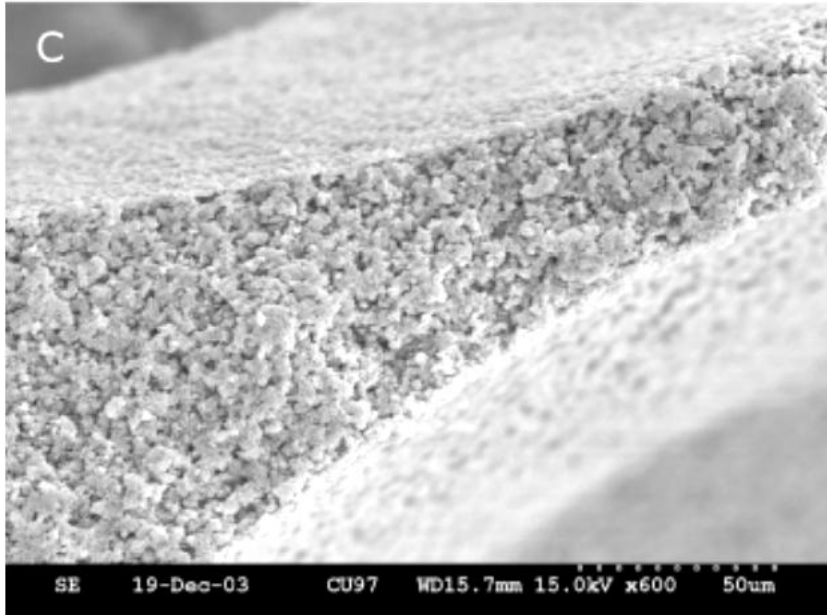
Calcite-based macro- and micro-porous cement scaffolds / conversion to Ap-CaP using 0.5 M phosphate buffer [11]

optical camera image of green, porous scaffold



SEM image of green, porous scaffold

11

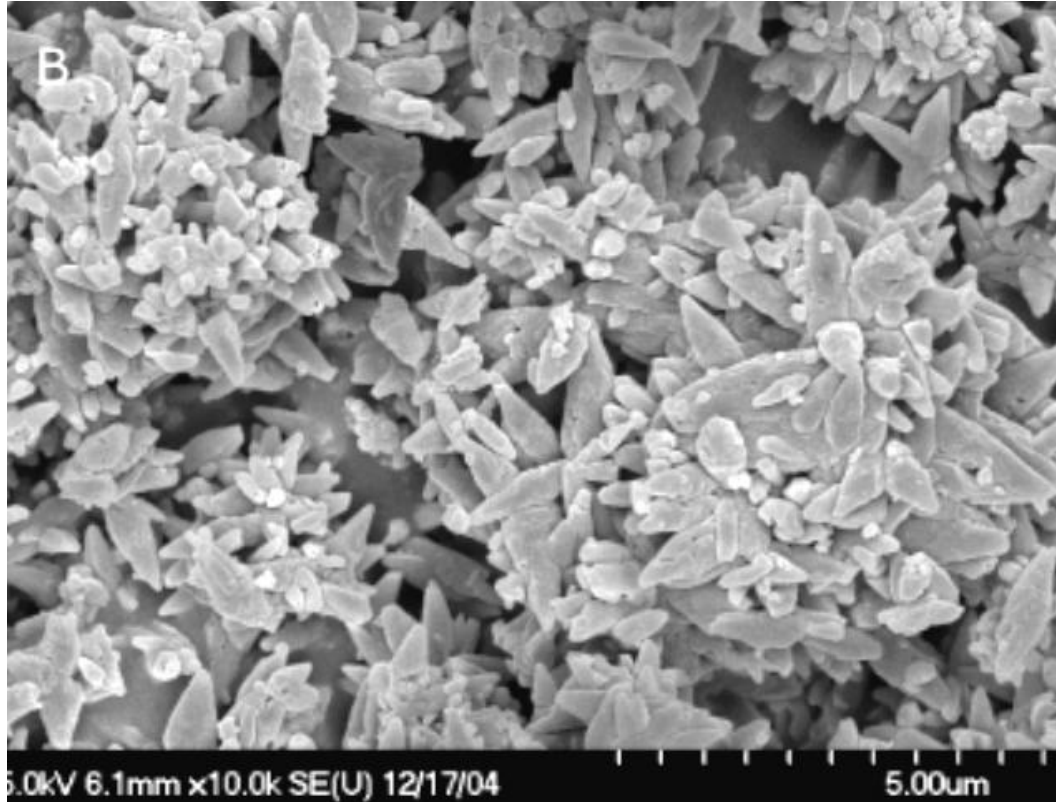


SEM images (at two different magnifications) of porous scaffolds soaked (in glass bottles) in 0.5 M phosphate buffer for 36 h at 80°C

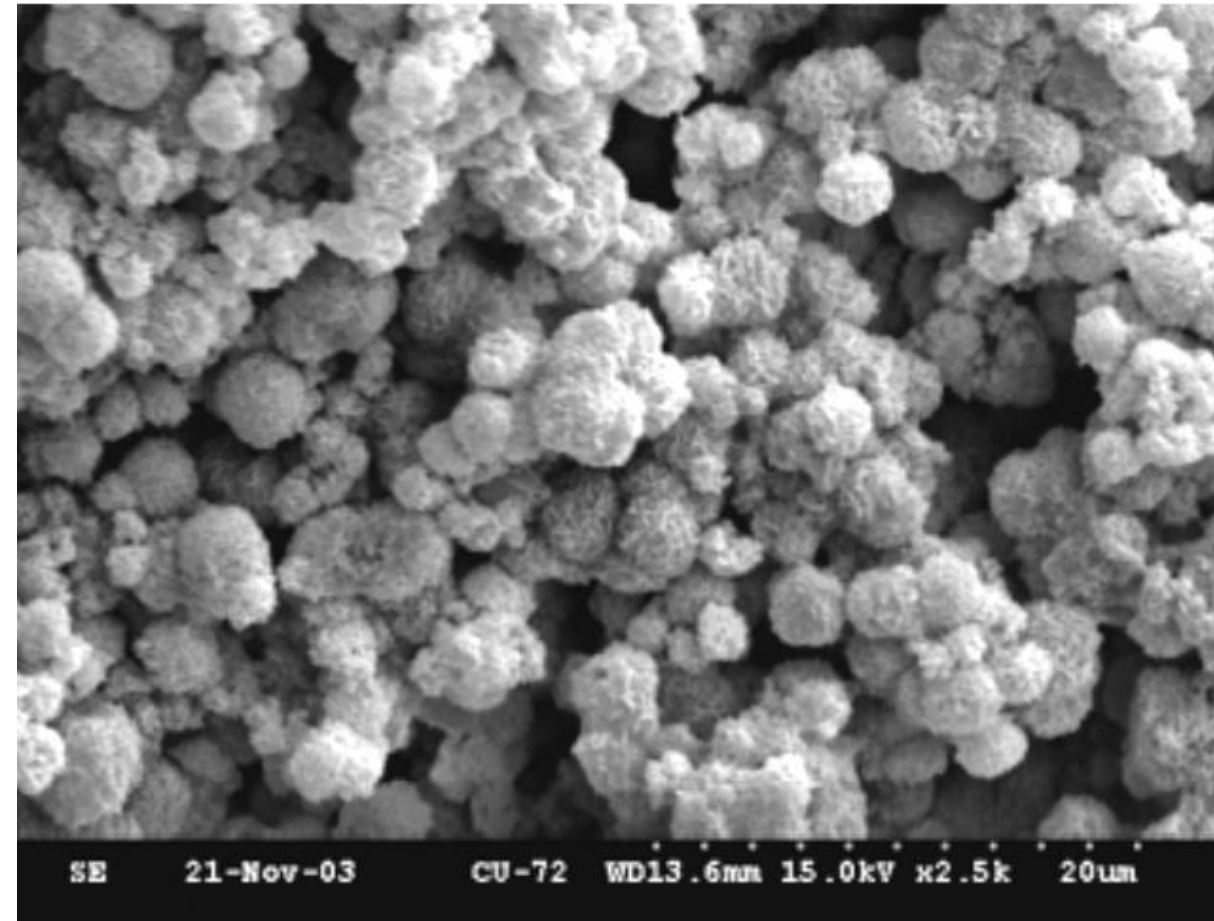
Ap-CaP nanoneedles formed as globules/spherules

[11] A. C. Tas, "Porous, biphasic CaCO₃-calcium phosphate biomedical cement scaffolds from calcite (CaCO₃) powder," *International Journal of Applied Ceramic Technology*, 4, 152-163 (2007).

Conversion of commercially-available calcite (CaCO_3) powders into Ap-CaP using Soerensen's buffer [11]



starting CaCO_3 powder (Fisher Scientific, C63-3) with a BET surface area of 2.1 to 3.6 m^2/g

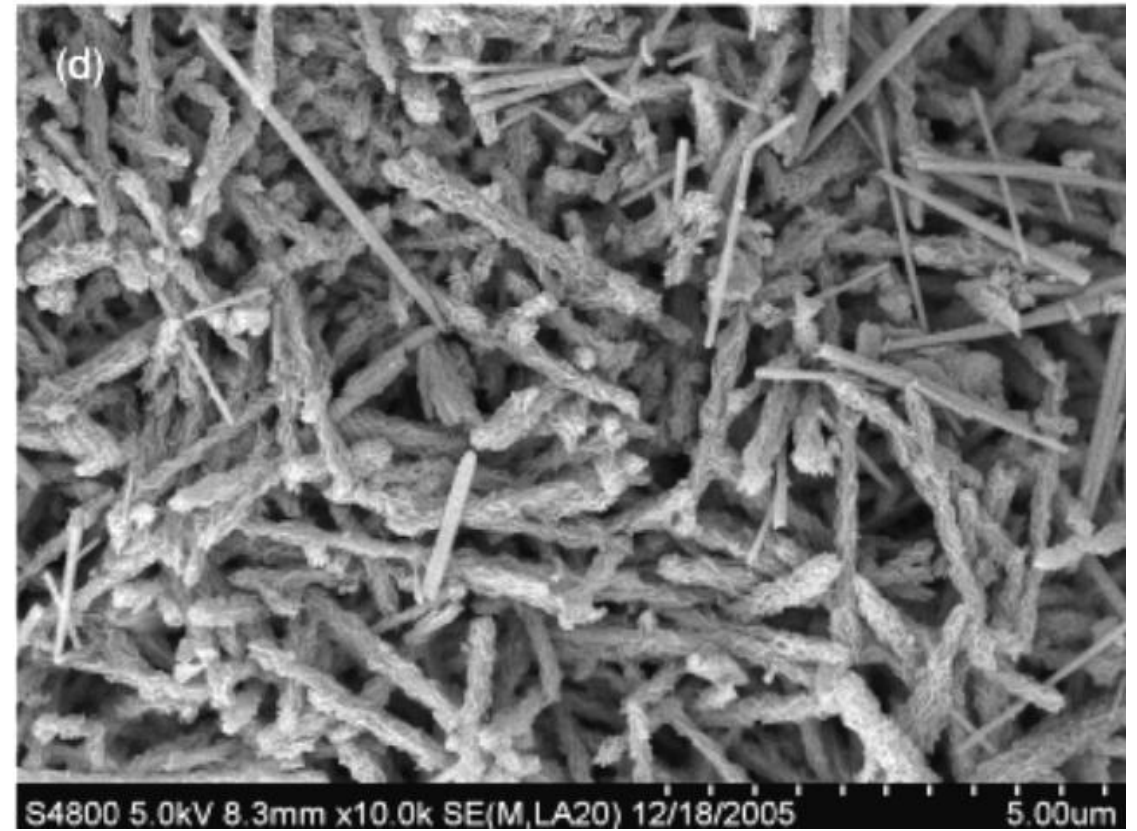
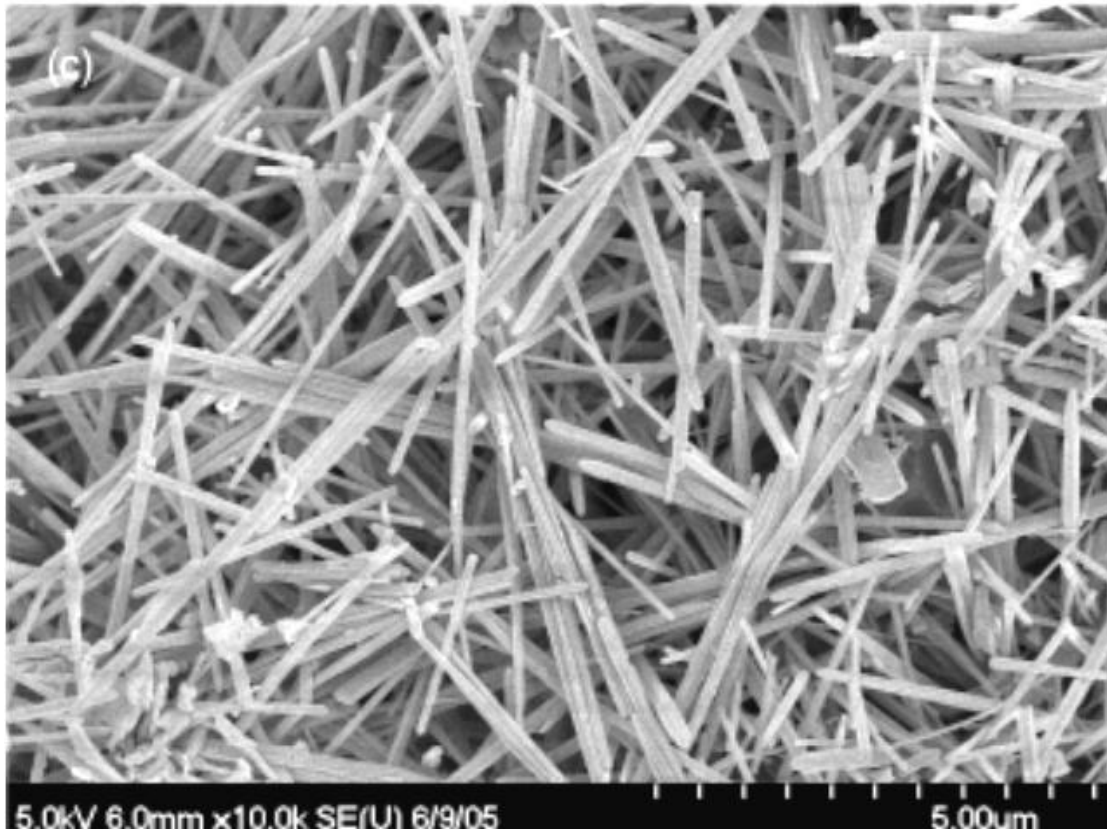


Ap-CaP powder obtained from the starting CaCO_3 powder by immersion in Soerensen's buffer at 60-80°C for 72 h (Ap-CaP globules consist of nanoneedles); BET surface area of 42 to 44 m^2/g

[11] A. C. Tas, "Porous, biphasic CaCO_3 -calcium phosphate biomedical cement scaffolds from calcite (CaCO_3) powder," *International Journal of Applied Ceramic Technology*, 4, 152-163 (2007).

A simple method to produce whiskers/needles of Ap-CaP using [Tas-SBF](#) [12]

13



SEM image of the as-synthesized "OCP (octacalcium phosphate, $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$) + Ap-CaP" biphasic whiskers

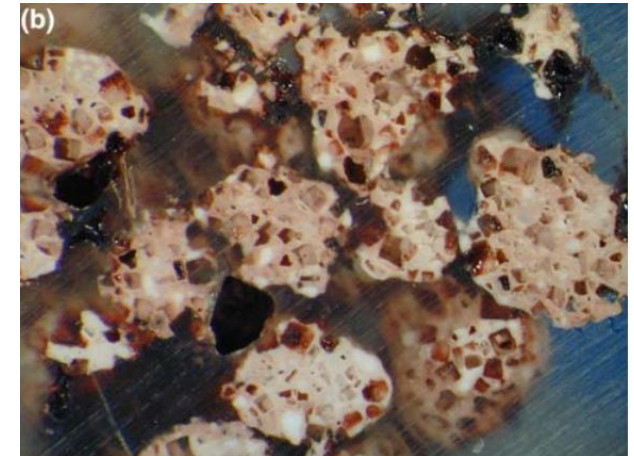
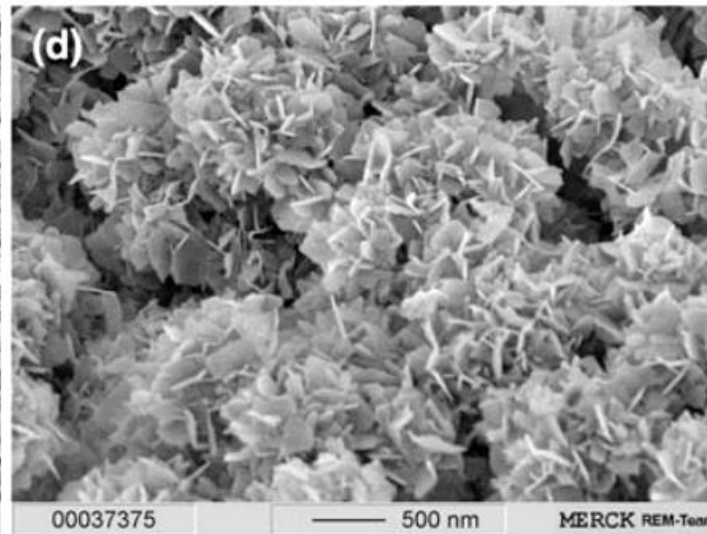
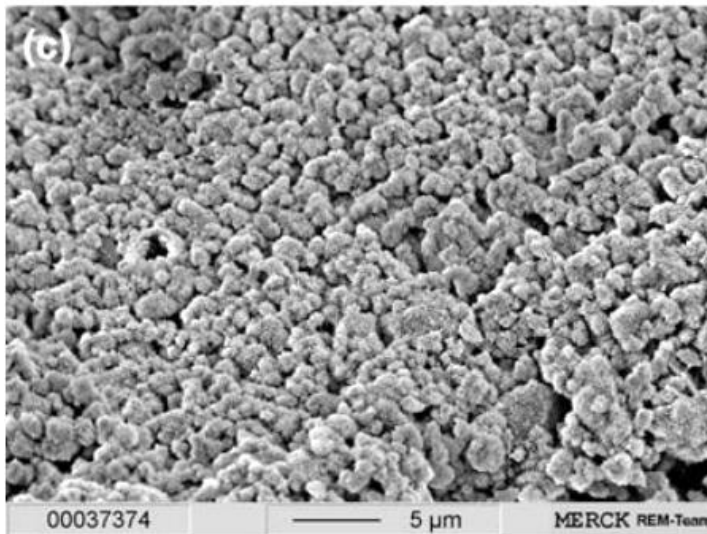
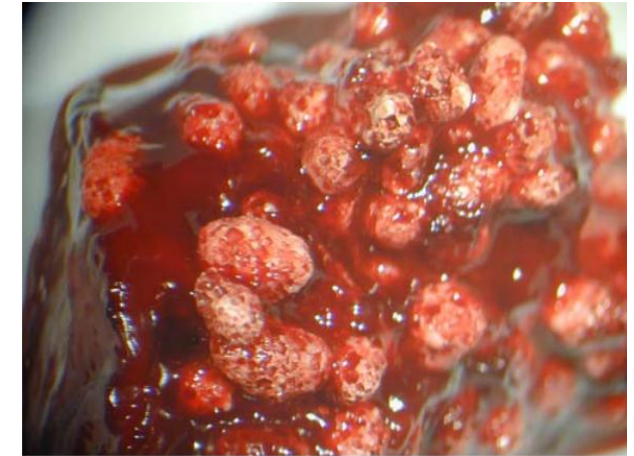
(commercially-available β -TCP powders were simply soaked in pure H_2O_2 (in bottles, w/o agitation) for 48 h at 90°C)

SEM image of the final Ap-CaP whiskers; imparted surface roughness visible

(starting whiskers were soaked in Tas-SBF for 6 days at 37°C , without any agitation)

[12] A. C. Tas, "Formation of calcium phosphate whiskers in hydrogen peroxide (H_2O_2) solutions at 90°C , *Journal of The American Ceramic Society*, 90, 2358-2362 ([2007](#)).

Preparation of macroporous apatitic granules from a calcium phosphate cement (**Calcibon® Granules**) [13, 14]



nanoneedles of Ap-Cap became visible at the highest magnification

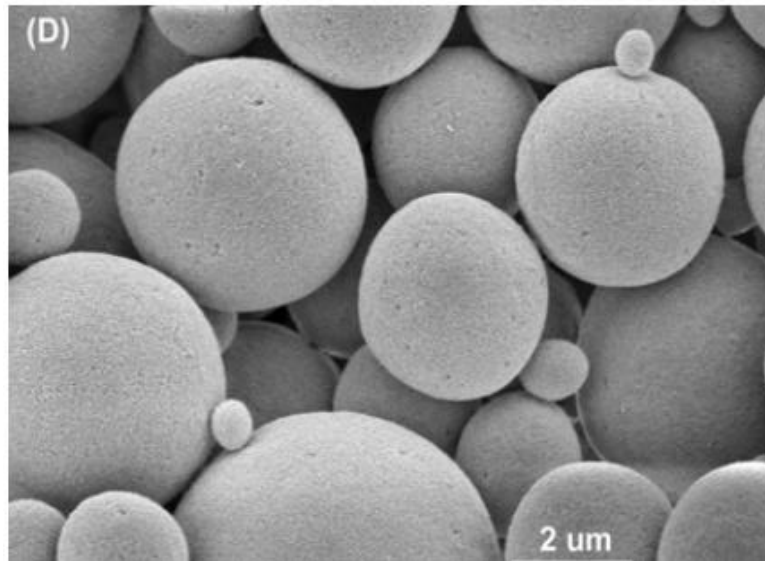
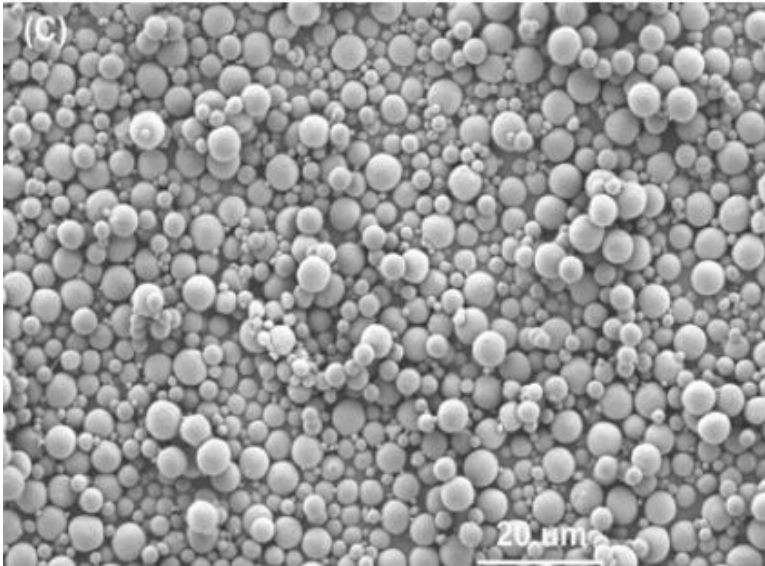
granules fixed within
clotted citrated blood

maximum granule production T = 37°C

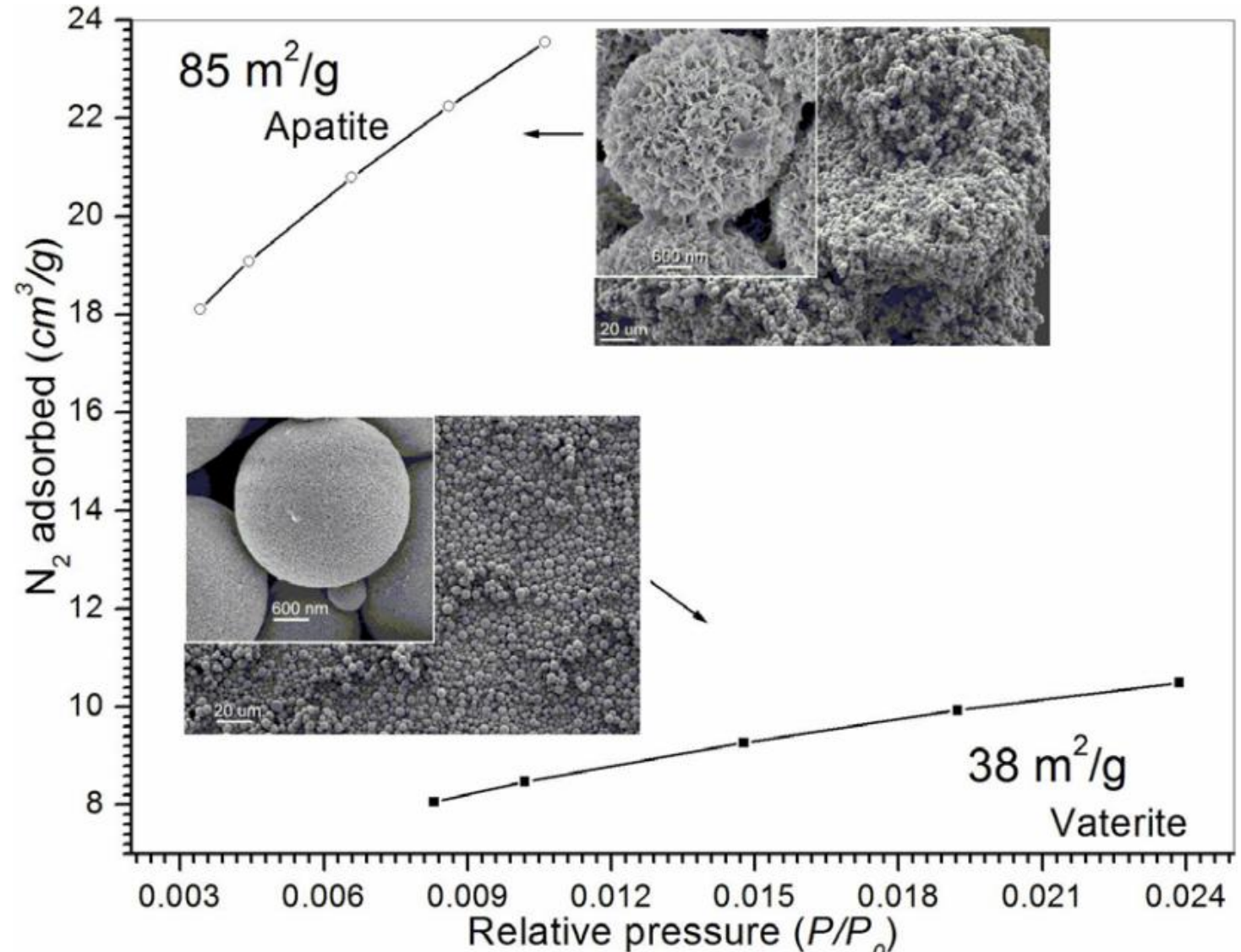
[13] A. C. Tas, "Preparation of porous apatite granules from calcium phosphate cement," *Journal of Materials Science: Materials in Medicine*, 19, 2231-2239 (2008).

[14] Granted patents / US patent [7,381,262](#), European patent [1,501,771](#), Canadian patent [2,483,859](#)

SEM / vaterite spheres we synthesized by using our novel, simple process [15]



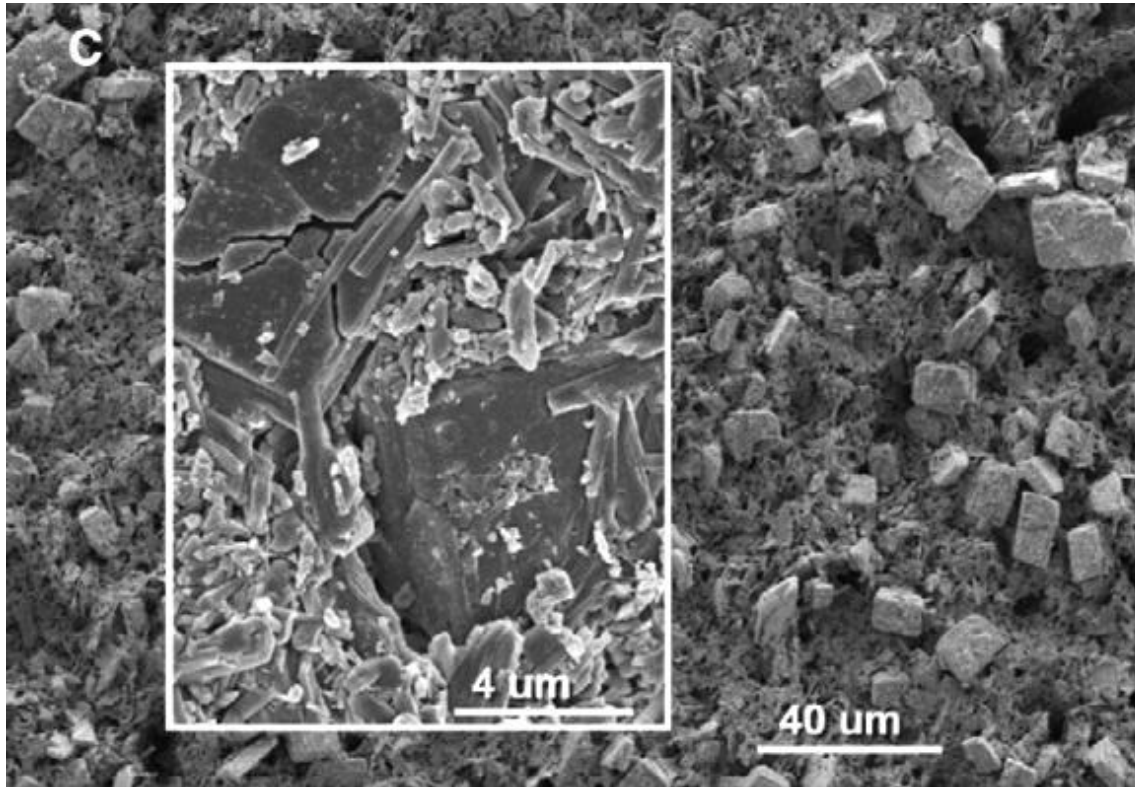
Upon soaking the vaterite spheres we synthesized in 0.5 M phosphate buffer (for 17 h at 70°C), Ap-Cap globules were obtained w/ high BET surface area [15]



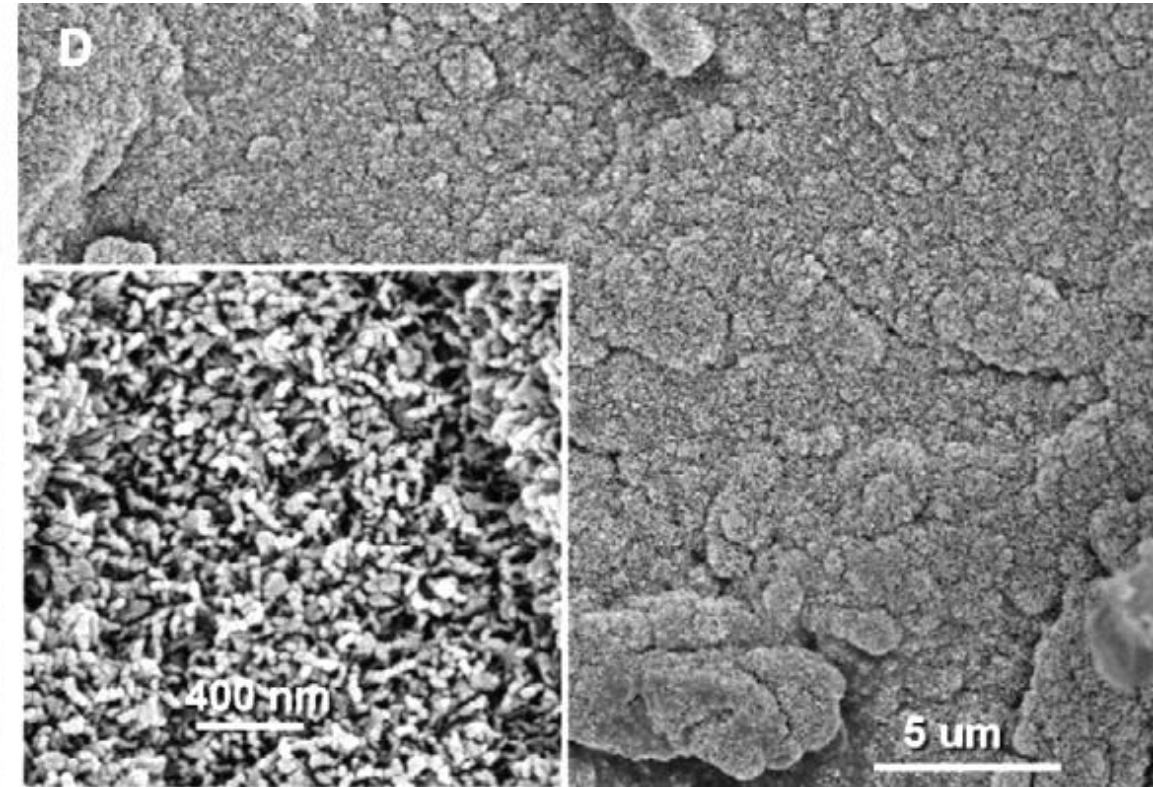
15

[15] A. C. Tas, "Use of vaterite and calcite in forming calcium phosphate cement scaffolds," *Ceramic Engineering and Science Proceedings (ACerS)*, 28(9): 135-150 (2008).

Conversion of a novel “33 wt% CaHPO_4 (=monetite) + 67 wt% plaster of Paris (=CSH= $\text{CaSO}_4 \cdot 0.5\text{H}_2\text{O}$)” self-hardening cement to Ap-CaP by soaking in [Tas-SBF](#) at 37°C for 7 days [16]



starting 33% CaHPO_4 +67% CSH cement (after it set; larger and cuboidal crystals were that of the commercial CaHPO_4 powder)



Ap-CaP nanoneedle microstructure after the starting samples stayed in the Tas-SBF for 7 days at 37°C

[16] A. C. Tas, “Using a synthetic body fluid (SBF) solution of 27 mM HCO_3^- to make bone substitutes more osteointegrative,” *Materials Science and Engineering C*, 28, 129-140 ([2008](#)).

Conversion of a commercially available collagen sponge to Ap-CaP by soaking in [Tas-SBF](#) at 37°C for 7 days [16]

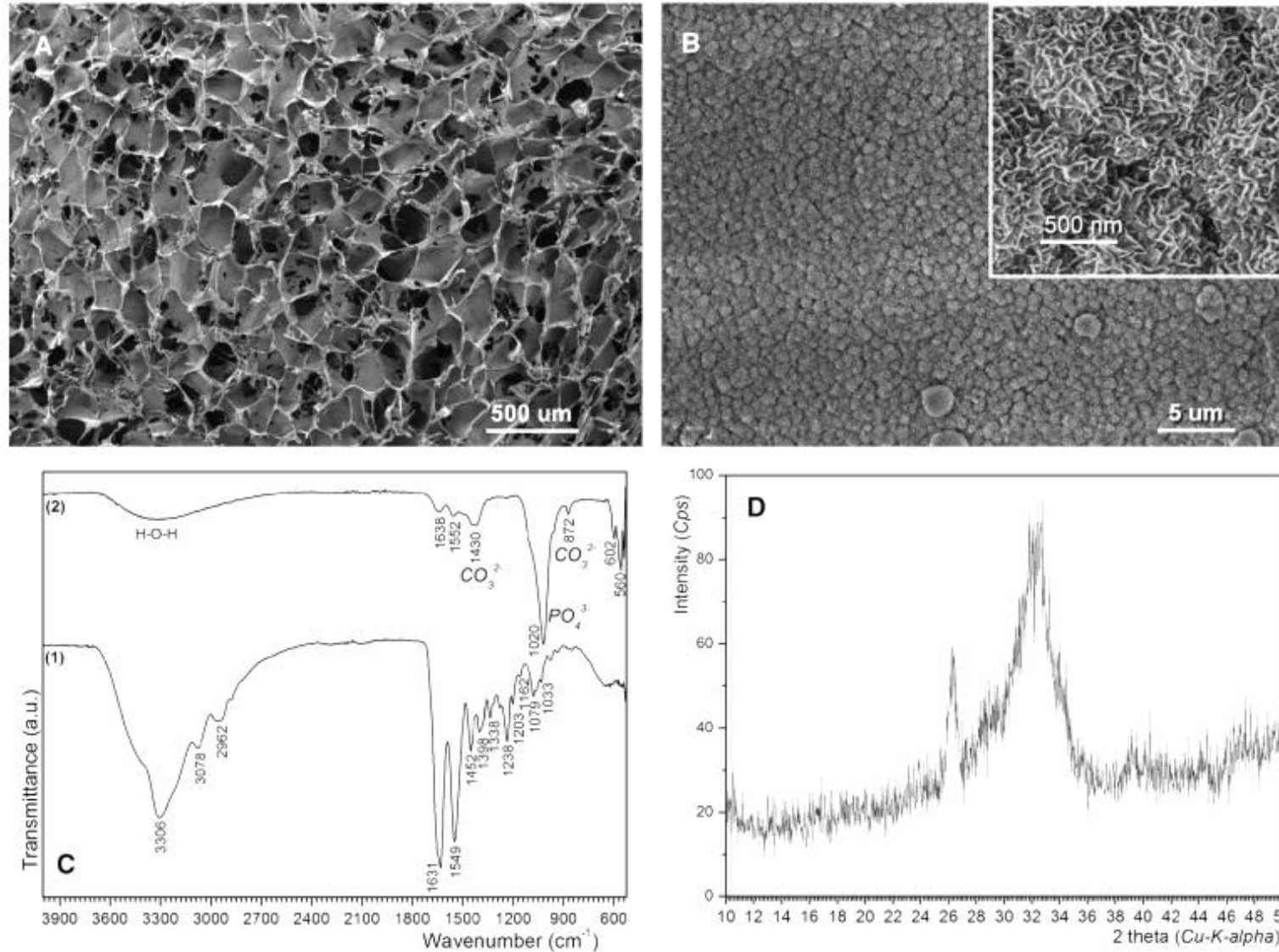
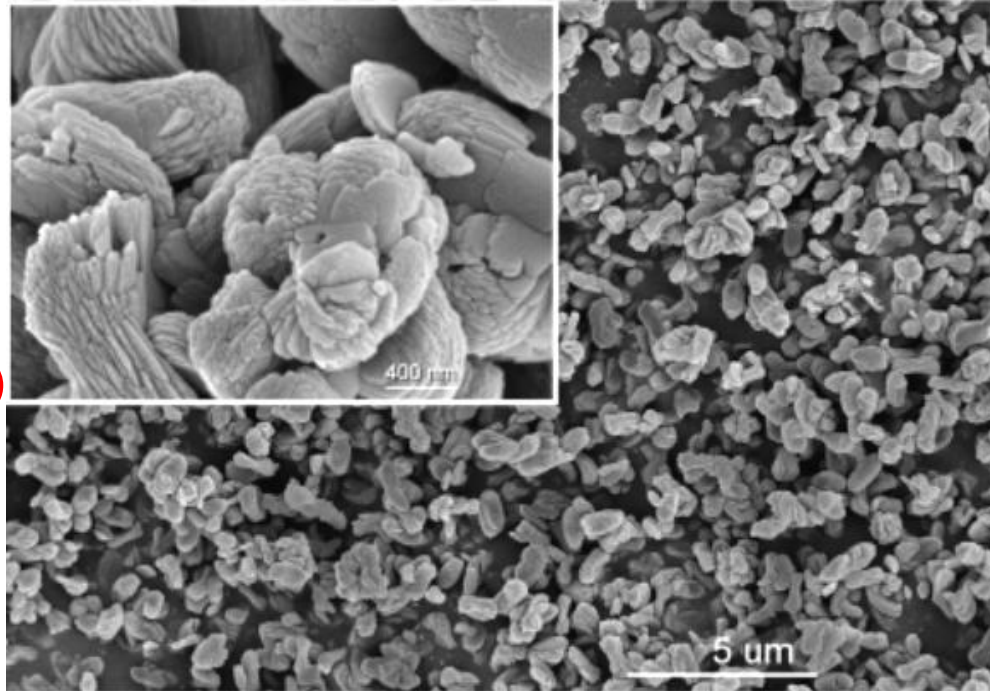


Fig. 1. (A) SEM micrograph of *as is* collagen sponge coupons; (B) collagen coupon after 1 week of soaking at 37 °C in Tris - SBF-27 mM; (C) FTIR traces of collagen coupons, (1) pristine, (2) SBF-soaked; (D) XRD trace of SBF-soaked collagen coupon.

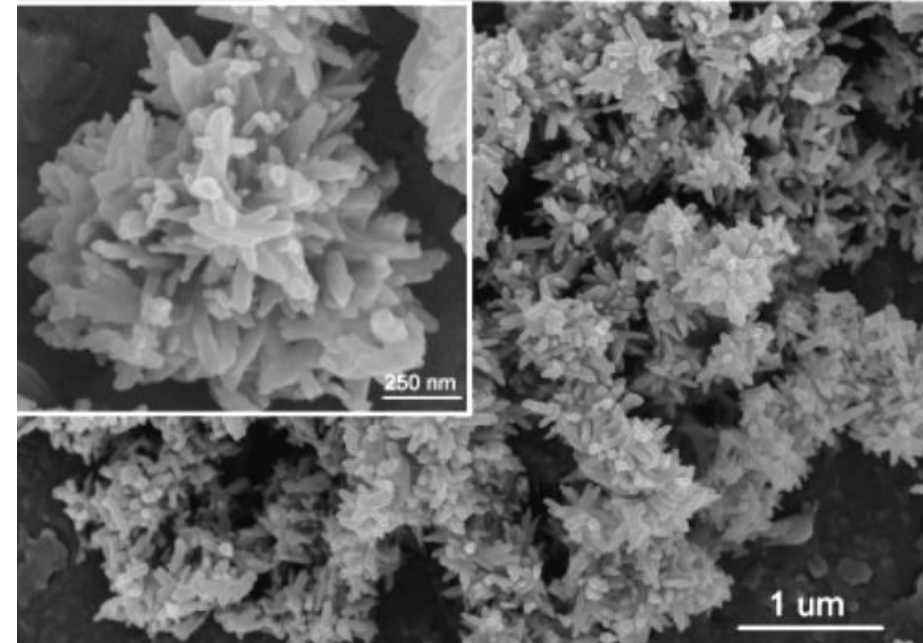
[16] A. C. Tas, “Using a synthetic body fluid (SBF) solution of 27 mM HCO₃⁻ to make bone substitutes more osteointegrative,” *Materials Science and Engineering C*, 28, 129-140 ([2008](#)).

A novel synthesis method for phase-pure CaHPO_4 (monetite) powders at RT in ethanol and their conversion to Ap-CaP by soaking in a simple Ca-containing saline solution (of only NaCl, KCl and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) at 37°C for 6 days, without any agitation of the solution [17]

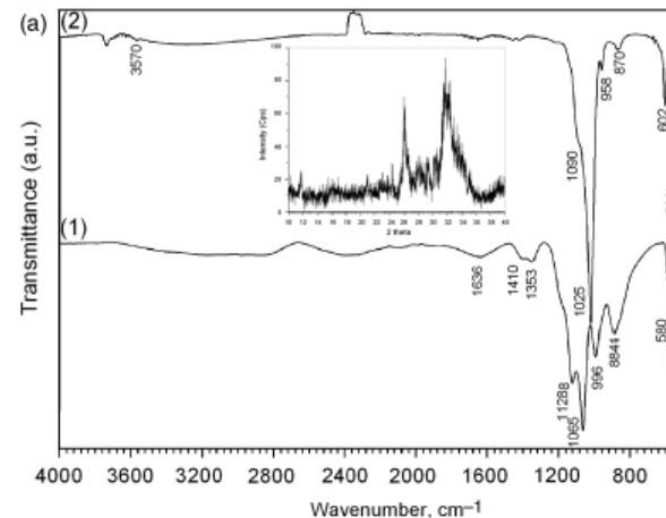
18



our own novel submicron-particulated CaHPO_4 powders



SEM image of Ap-CaP; note the nanoneedles

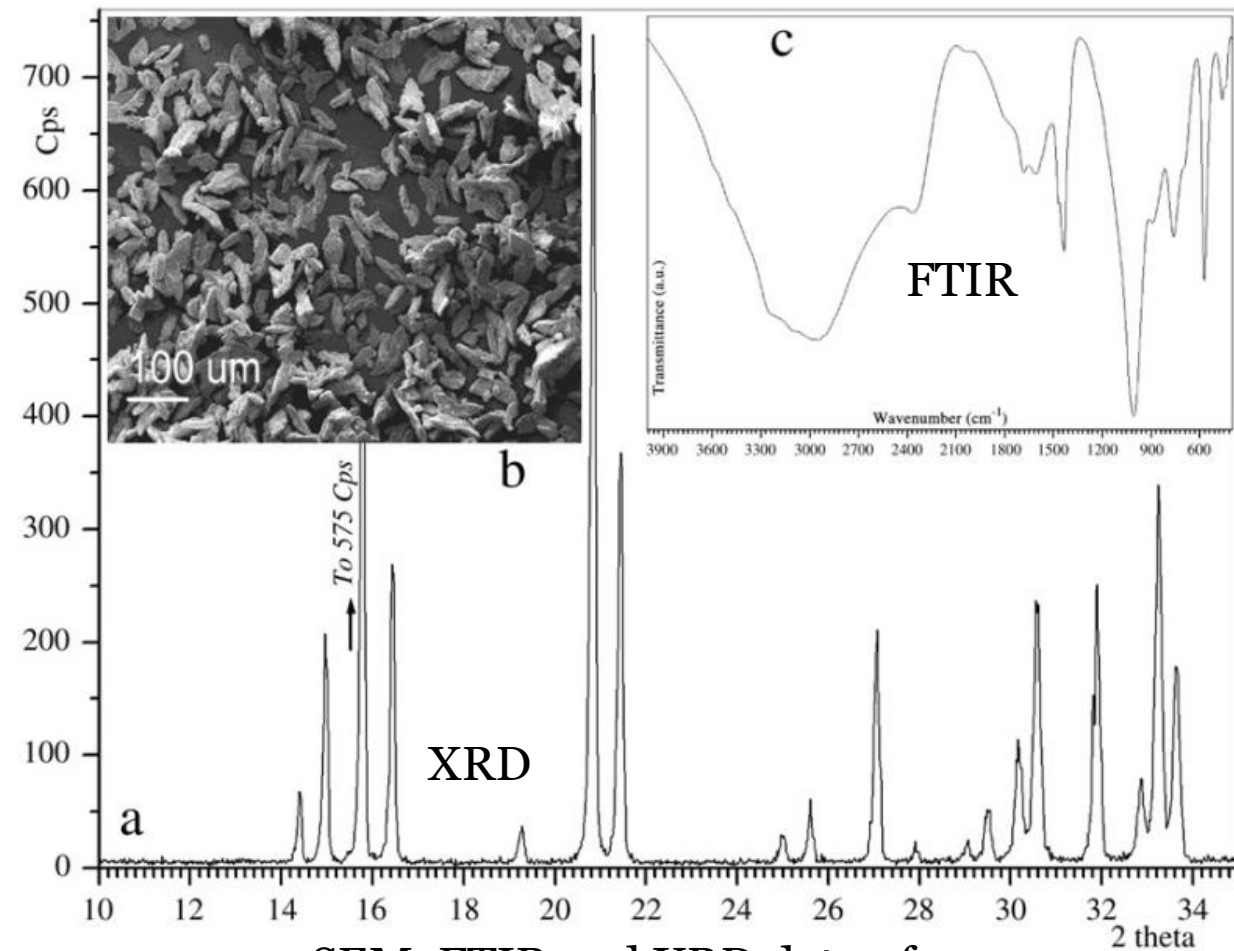


(1): FTIR / *as is* CaHPO_4
(2): FTIR / Ap-CaP produced

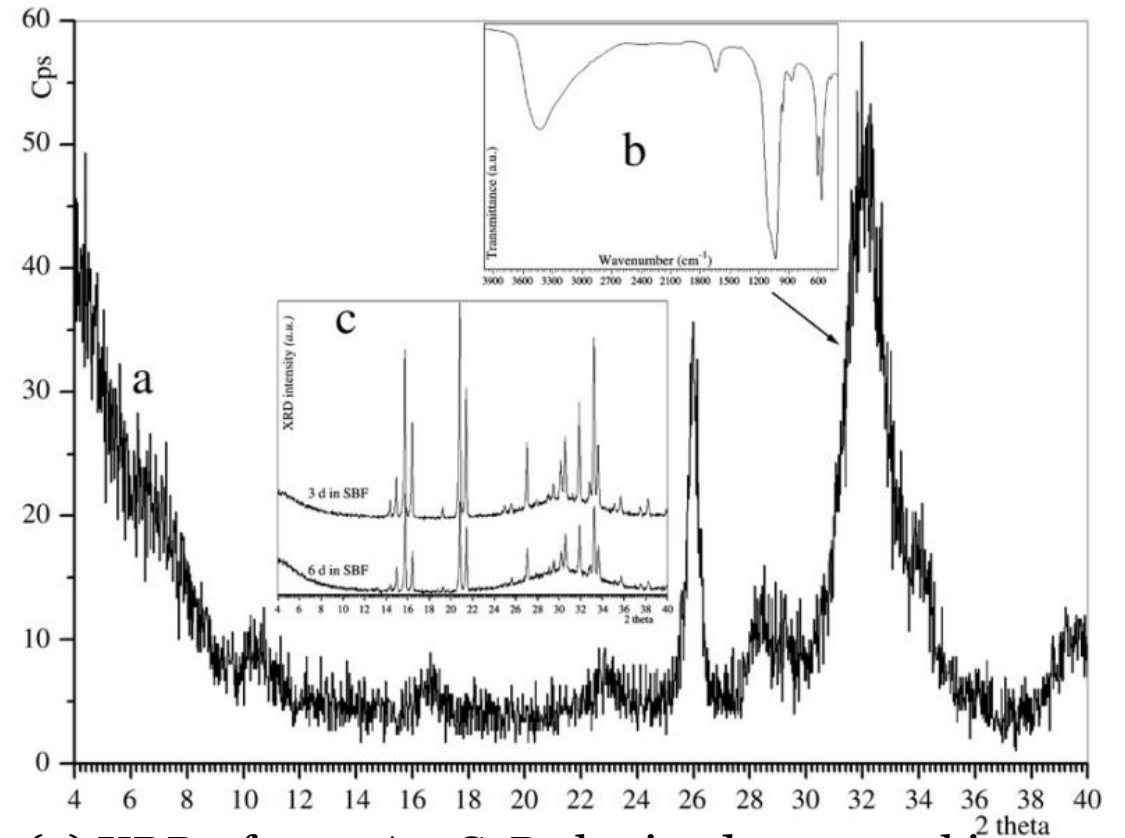
Inset: XRD of Ap-CaP

[17] A. C. Tas, “Monetite synthesis in ethanol at room temperature,” *Journal of The American Ceramic Society*, 92, 2907-2912 (2009).

Transformation of struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$, a urinary tract stone, 10% of human, 85% of feline and 70% of canine stones) powders into Ap-CaP by soaking in a simple Ca-containing saline solution (of only NaCl, KCl and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) at 37°C for 3 days, without any agitation of the solution [18]



SEM, FTIR and XRD data of phase-pure struvite we synthesized



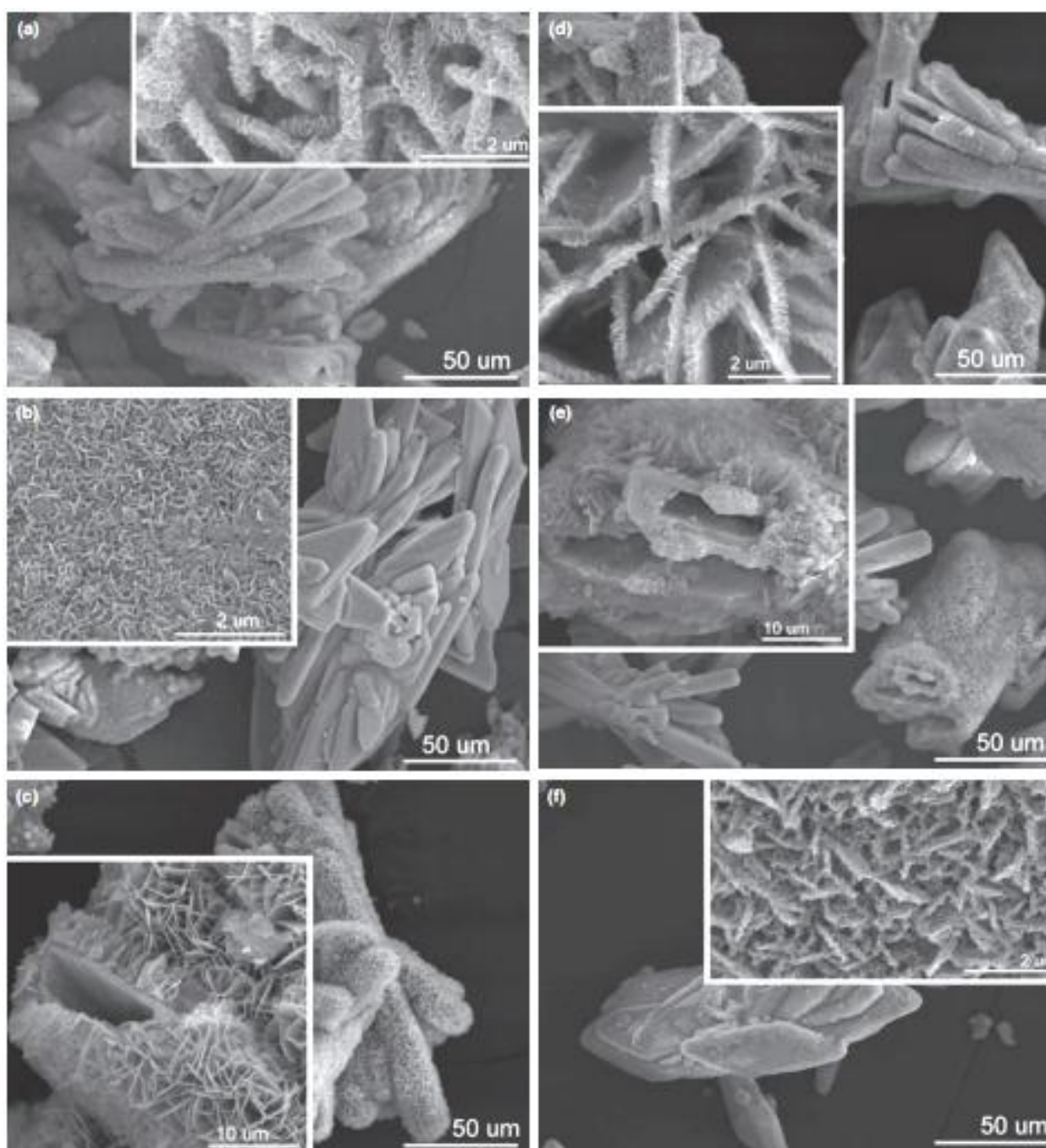
(a) XRD of pure Ap-CaP obtained upon soaking struvite in Ca-containing saline, (b) FTIR of pure Ap-CaP formed upon Ca-ctg saline soaking of struvite, (c) XRD of struvite soaked in [Tas-SBF](#) (since SBF does not have enough Ca, it is not able to rapidly convert struvite)

19

[18] A. C. Tas *et al.* "Transformations of neat and heated struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$)," *Materials Letters*, 65, 2883-2886 (2011).

Conversion of brushite (DCPD) into Ap-CaP in a number of biom mineralization solutions we developed [19]

20



7. SEM photomicrographs of sample-1 soaked at 37°C in (a) BM-7 for 2 d, (b) BM-7 for 1 week, (c) Lac-SBF for 2 d, (d) Lac-SBF for 1 week, (e) Tris-SBF for 2 d and (f) Tris-SBF for 1 week.

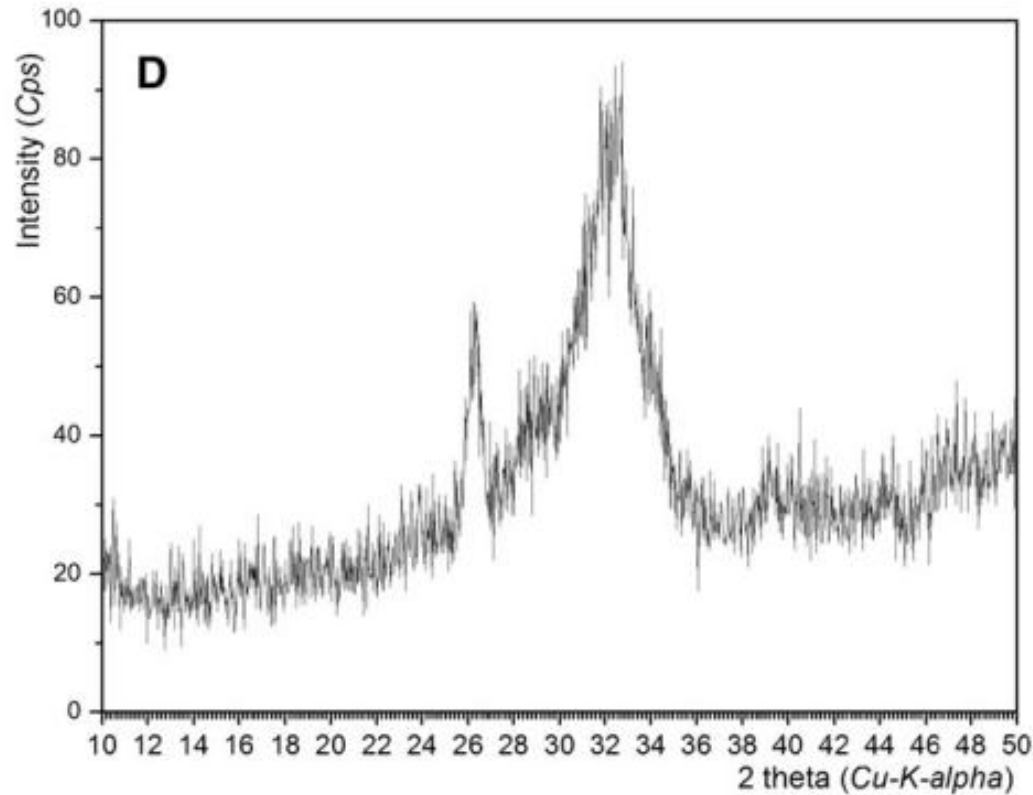
[19] A. C. Tas *et al.*, “Testing of brushite in synthetic biom mineralization solutions and *in situ* crystallization of brushite micro-granules,” *Journal of The American Ceramic Society*, 95, 2178-2188 (2012).

Lac-SBF: [20] A. C. Tas *et al.* “A new approach in biomimetic synthesis of calcium phosphate coatings using lactic acid–Na lactate buffered body fluid solution,” *Acta Biomaterialia*, 6, 2282-2288 (2010).

BM-7: [21] A. C. Tas *et al.*, “Accelerated transformation of brushite to octacalcium phosphate in new biom mineralization media between 36.5°C and 80°C,” *Materials Science and Engineering C*, 31, 1136-1143 (2011). (Check Table 1 of this article.)

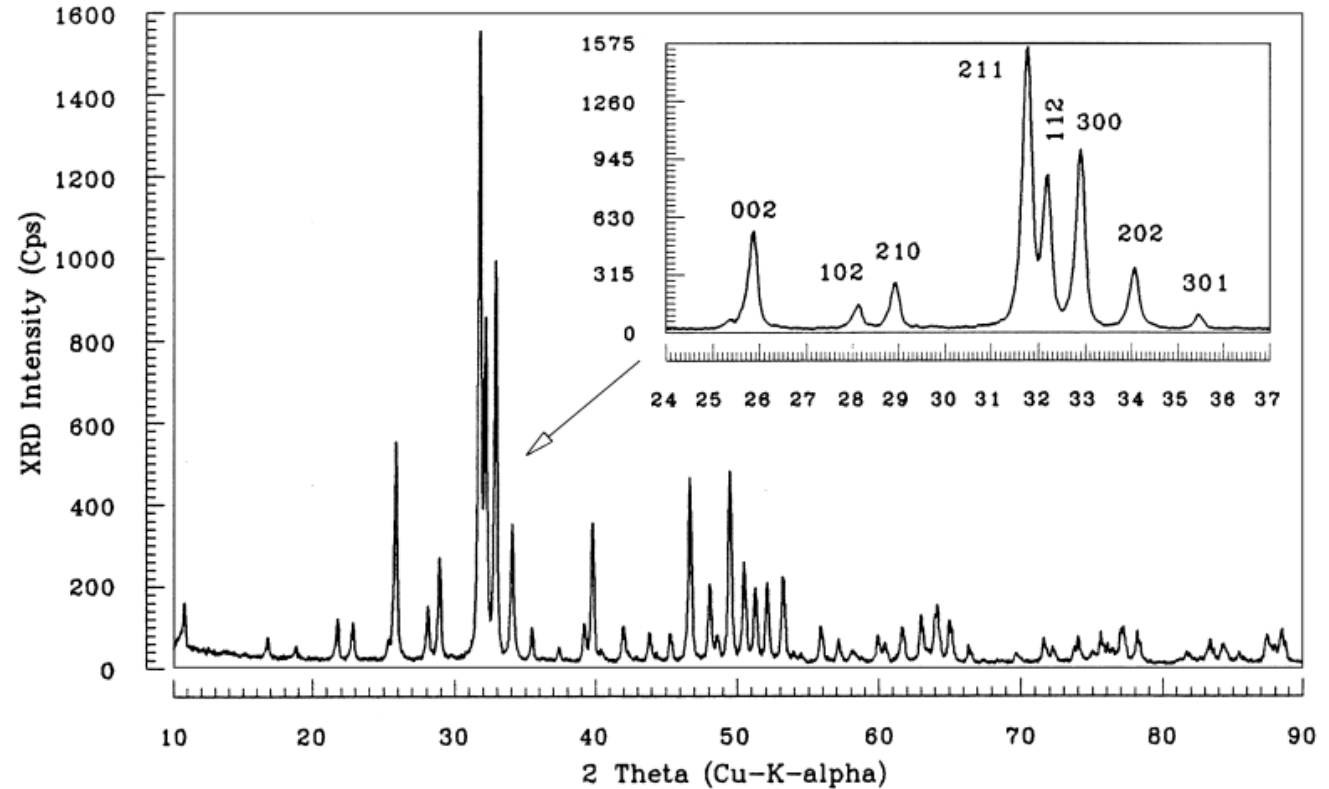
Tris-SBF appeared in the figure caption is [Tas-SBF](#).

Each and every one of the above-mentioned publications has the XRD pattern of the low crystallinity apatitic calcium phosphate (Ap-CaP). We shall now give the readers a chance to compare the XRD patterns of low crystallinity Ap-CaP and that of stoichiometric, high crystallinity calcium hydroxyapatite we synthesized.



low crystallinity Ap-CaP

[16] A. C. Tas, "Using a synthetic body fluid (SBF) solution of 27 mM HCO_3^- to make bone substitutes more osteointegrative," *Materials Science and Engineering C*, 28, 129-140 ([2008](#)).



well-crystallized stoichiometric Ca-hydroxyapatite
(sample heated at 1200°C)

[23] A. C. Tas, "Synthesis of biomimetic Ca-hydroxyapatite powders at 37°C in synthetic body fluids," *Biomaterials*, 21, 1429-1438 ([2000](#)).

What is amorphous calcium phosphate (ACP) and how can one synthesize it “without using” any organic macromolecules (which are not present in the human body) that might suppress crystallization?

A novel method for producing X-ray-amorphous calcium phosphate (ACP) powders [24]

22

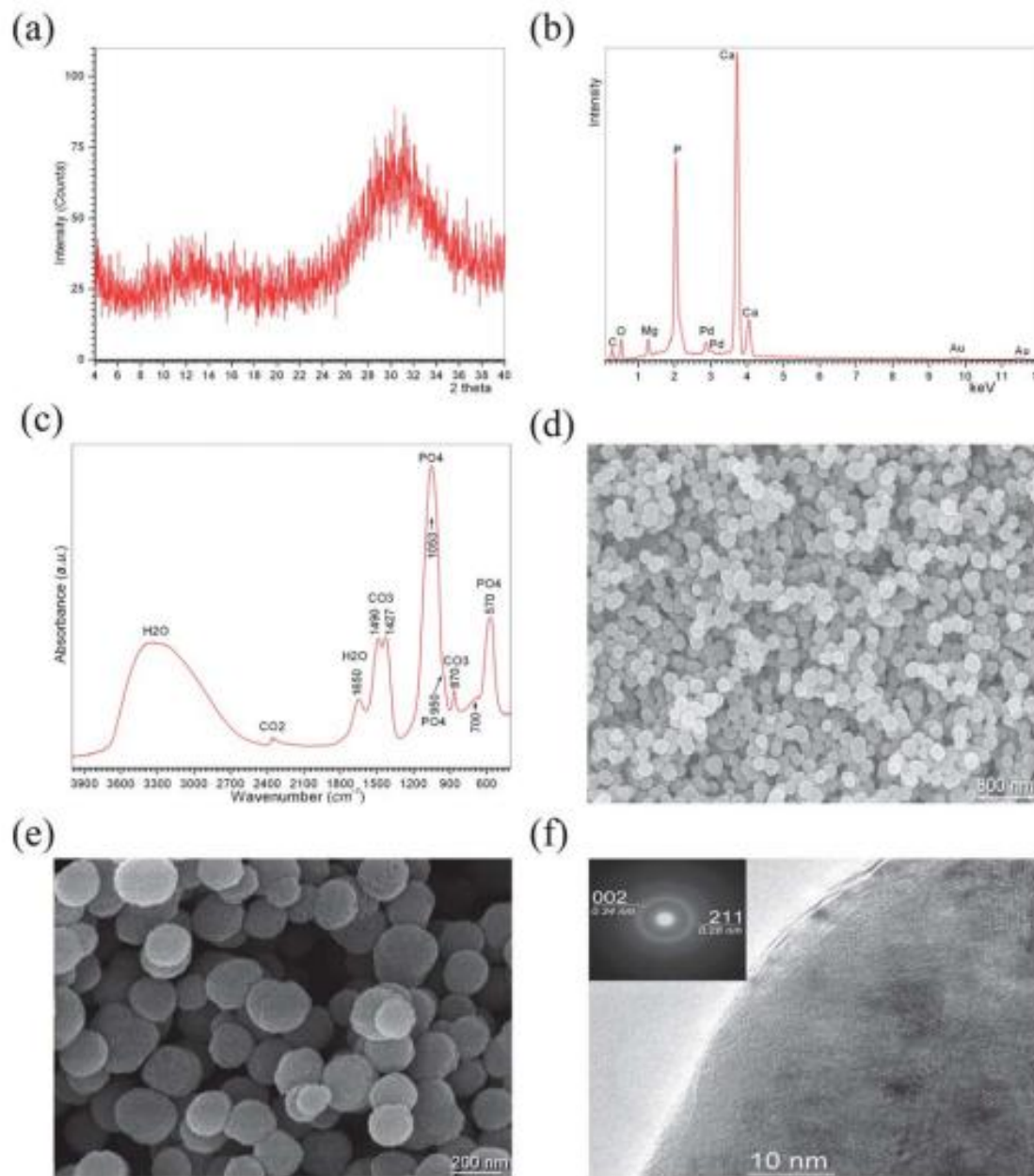


Fig. 3 ACP nanospheres synthesized in the solution of Table 1 upon heating the solution at 65 °C for 1 h; (a) XRD, (b) EDXS, (c) FTIR, (d) & (e) low and high resolution FE-SEM images, (f) TEM image and SAED pattern.

Table 1 Biom mineralization medium preparation (1 liter)

Chemical	Amount g L ⁻¹	Ion	Concentration mM
NaCl	4.7865	Na ⁺	126.86
KCl	0.3974	K ⁺	5.33
MgCl ₂ ·6H ₂ O	0.1655	Mg ²⁺	0.81
CaCl ₂ ·2H ₂ O	0.3323	HCO ₃ ⁻	44.05
NaHCO ₃	3.7005	Cl ⁻	93.37
NaH ₂ PO ₄ ·H ₂ O	0.1250	Ca ²⁺	2.26
		H ₂ PO ₄ ⁻	0.905

Synthesis procedure:

simply heat the solution at 65°C for 1 h, the process is temperature-induced homogeneous precipitation, separate the nanospheres from the suspension, wash with DI H₂O, and dry at RT

[24] A. C. Tas, “X-ray-amorphous calcium phosphate (ACP) synthesis in a simple biom mineralization medium,” *Journal of Materials Chemistry B*, 1, 4511-4520 (2013).

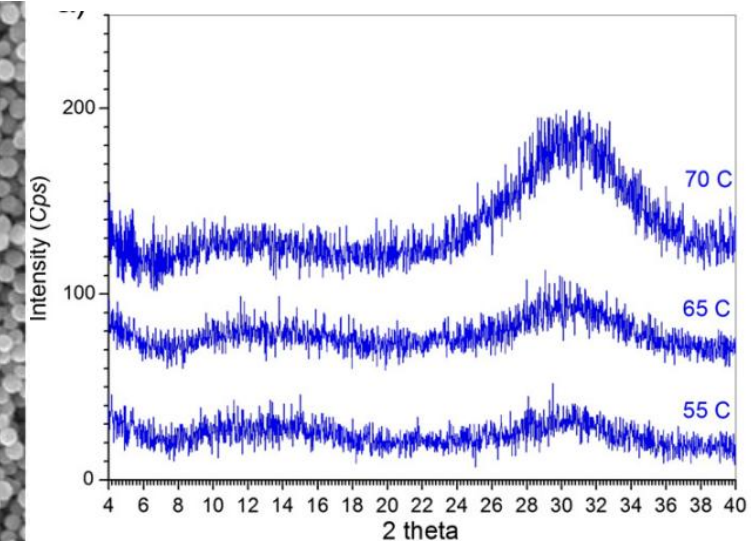
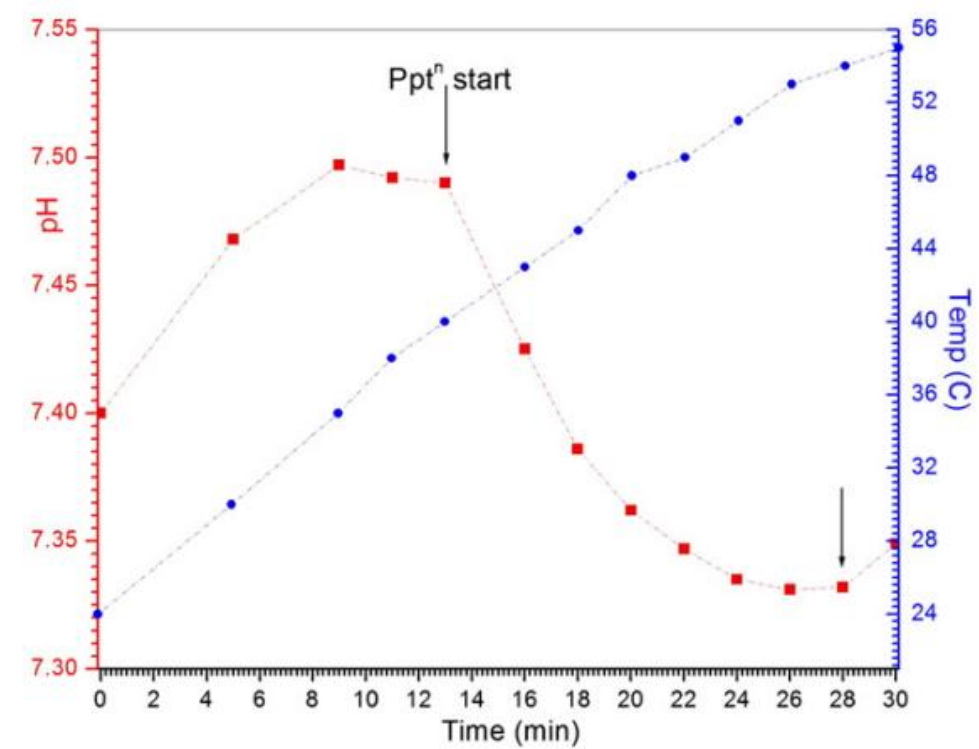
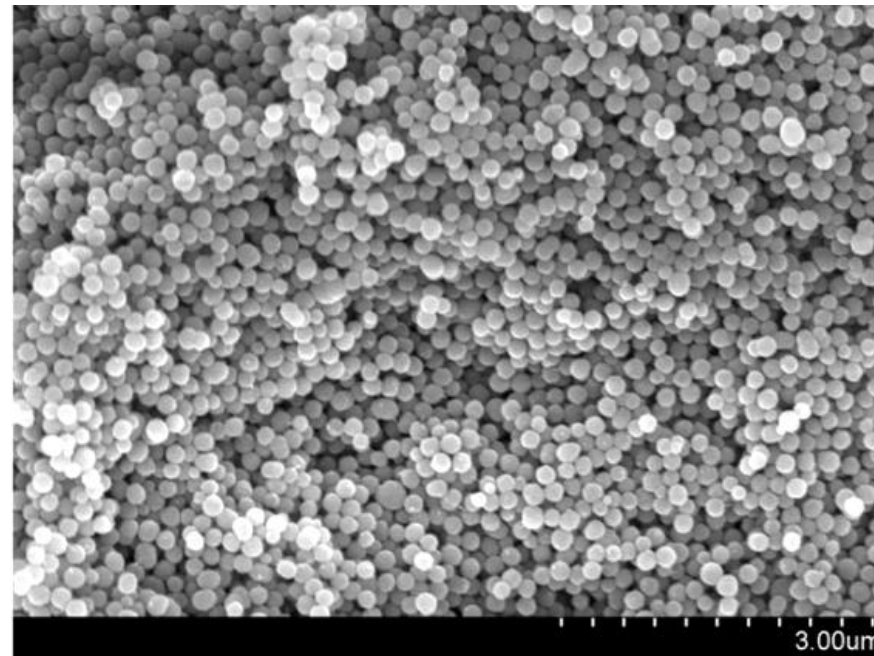
Another novel method for producing X-ray-amorphous calcium phosphate (ACP) nanospheres [25]

Table 2
Preparation of 1 L of Lac-SBF [9,10,13] solution.

Chemical	Amount (g/L)	Ion	Concentration (mM)
NaCl	5.2599	Na ⁺	142.0
NaHCO ₃	2.2682	Mg ²⁺	1.5
KCl	0.3728	K ⁺	5.0
MgCl ₂ ·6H ₂ O	0.3049	Ca ²⁺	2.5
Na ₂ SO ₄	0.0710	HPO ₄ ²⁻	1.0
CaCl ₂ ·2H ₂ O	0.3675	HCO ₃ ⁻	27.0
Na ₂ HPO ₄	0.1419	Cl ⁻	103.0
Na-lactate	2.4653	SO ₄ ²⁻	0.5
1 M lactic acid	1.6 mL	Ca/P molar ratio	2.5

Synthesis procedure:
slowly heat the [Lac-SBF](#) to 55°C and age it for 15 minutes, the process is temperature-induced homogeneous precipitation, separate the nanospheres from the suspension, wash the solids with DI H₂O, and dry at RT

23



[25] A. C. Tas, "Submicron spheres of amorphous calcium phosphate forming in a stirred SBF solution at 55°C," *Journal of Non-crystalline Solids*, 400, 27-32 (2014).

Future work: The X-ray-amorphous ACP nanospheres described in the previous couple of slides should be “osteoinductive” (test it for ectopic ossification), and *please cite my work*. You may always contact me if you encounter any issues during your reproduction and follow-up work.

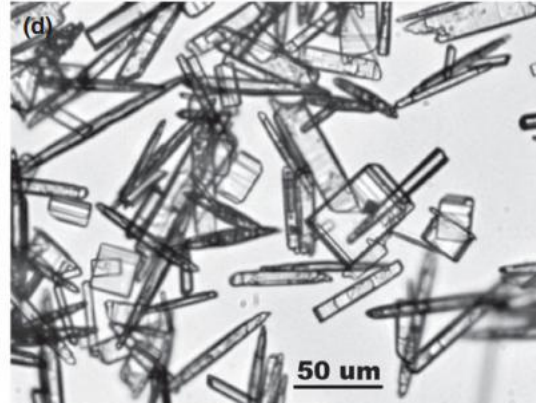
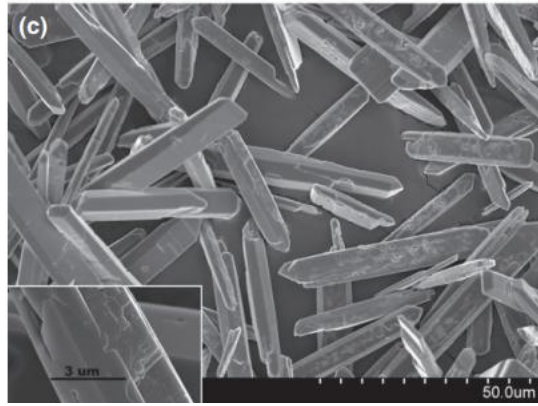
The procedure detailed in slide having the number 22 (in red) uses a completely inorganic solution whose preparation conditions is once again reproduced below. The ACP produced by using this solution [24] must give the most benign ACP for the ectopic ossification studies.

Table 1 Biomineralization medium preparation (1 liter)

Chemical	Amount g L ⁻¹	Ion	Concentration mM
NaCl	4.7865	Na ⁺	126.86
KCl	0.3974	K ⁺	5.33
MgCl ₂ ·6H ₂ O	0.1655	Mg ²⁺	0.81
CaCl ₂ ·2H ₂ O	0.3323	HCO ₃ ⁻	44.05
NaHCO ₃	3.7005	Cl ⁻	93.37
NaH ₂ PO ₄ ·H ₂ O	0.1250	Ca ²⁺	2.26
		H ₂ PO ₄ ⁻	0.905

[24] A. C. Tas, “X-ray-amorphous calcium phosphate (ACP) synthesis in a simple biomineralization medium,” *Journal of Materials Chemistry B*, 1, 4511-4520 (2013).

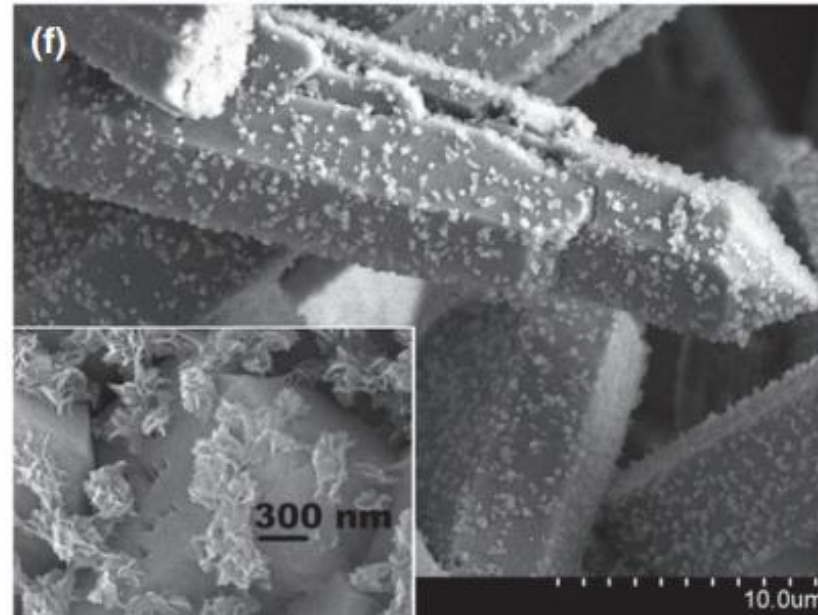
Formation of Ap-CaP on aragonite whiskers soaked in [Lac-SBF](#) [26]



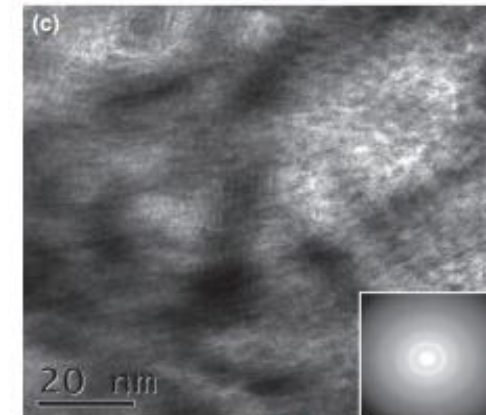
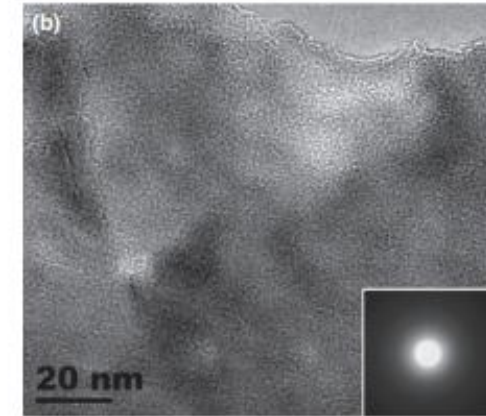
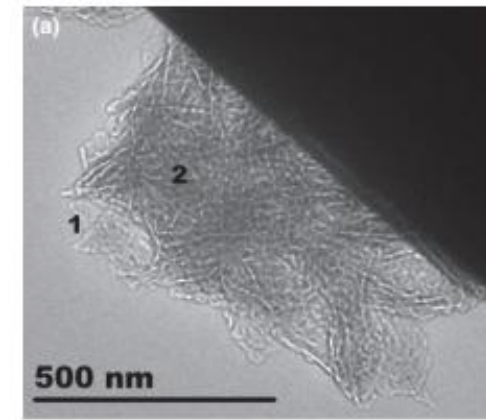
SEM/pristine aragonite

Optical microsc/pristine aragonite

24



SEM/ aragonite crystals soaked in Lac-SBF formed Ap-CaP on their surfaces



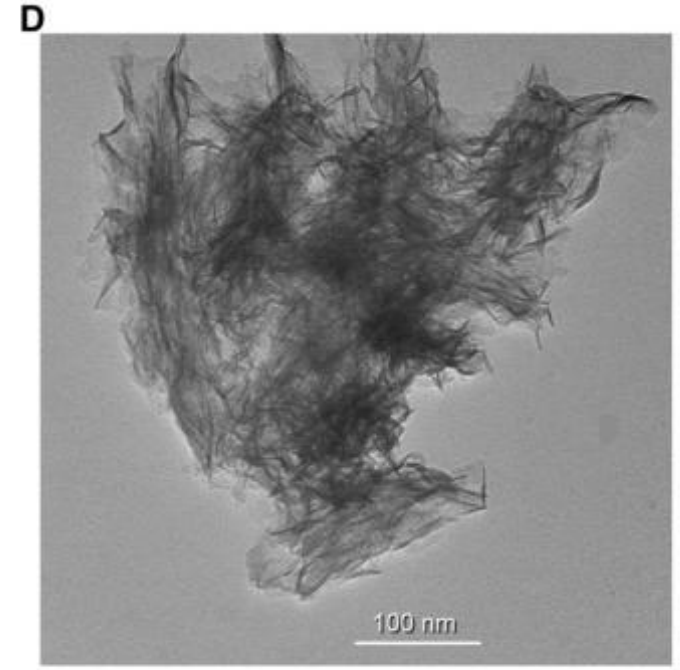
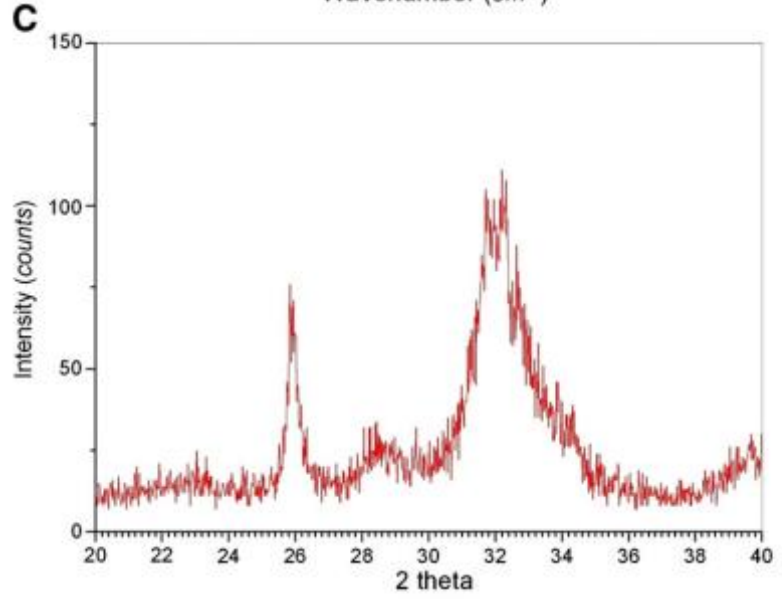
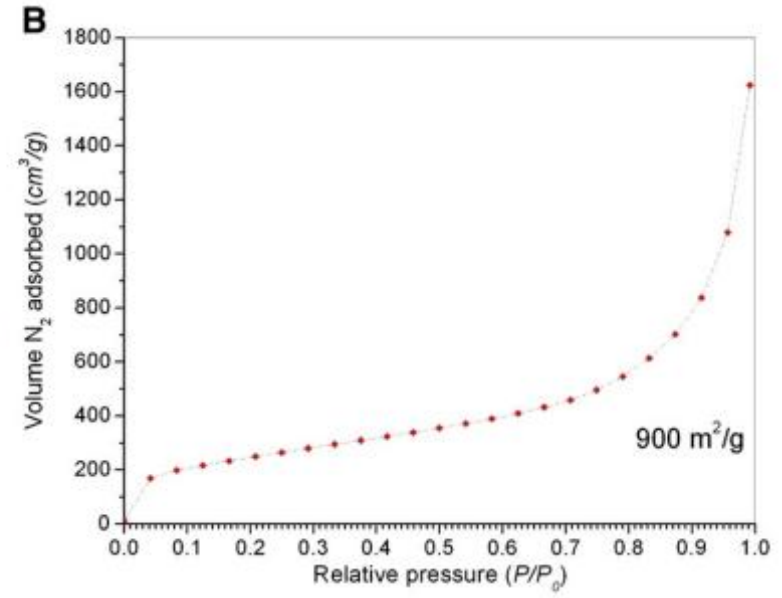
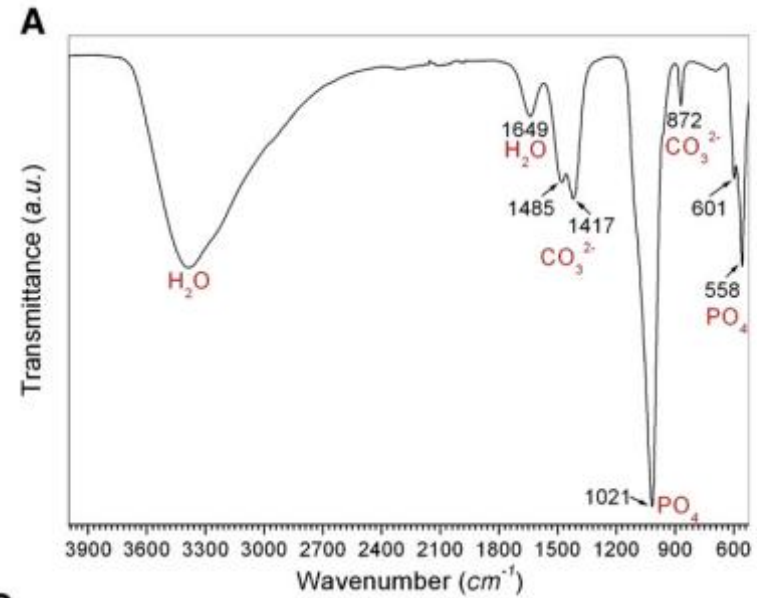
TEM/ analysis of Ap-CaP formed on aragonite surfaces upon staying in Lac-SBF at 37°C for 96 h

Fig. 6. (a) Low magnification TEM photomicrograph of a carnation-like apatitic CaP deposit formed on the surface of an A-2 aragonite (w/1050 ppm Mg) particle soaked in Lac-SBF for 96 h at 37°C; (b) point 1 of (a) with its selected area electron diffraction (SAED) image in the inset; (c) point 2 of (a) with its SAED.

[26] A. C. Tas *et al.* "Synthetic aragonite as a potential additive in calcium phosphate cements: Evaluation in Tris-free SBF at 37°C," *Journal of The American Ceramic Society*, 97, 3052-3061 (2014).

Ap-CaP synthesis from a pristine [Tas-SBF](#) solution kept undisturbed in an ordinary refrigerator (+4°C) for 4 months [27]

25



TEM image of precipitates

Fig. 8. FTIR (A), BET surface area (B), XRD (C) and TEM (D) of *in situ* forming cryptocrystalline apatitic calcium phosphate (Ap-CaP) precipitates recovered from an SBF solution kept undisturbed, in a sealed glass bottle, in a refrigerator at 4 °C for 120 days.

[27] A. C. Tas , “Grade-1 titanium soaked in a DMEM solution at 37°C,” *Materials Science and Engineering C*, 36, 84-94 (2014).

26

ACP or cryptocrystalline CaP (PCA) synthesis at RT by using calcium metal [28, 29]

Table 1
Preparation of mineralization solutions (MS) 500 mL H₂O basis.

Chemical	g	mM cation	mM anion
KCl	0.1865	5 K ⁺	5 Cl ⁻
MgCl ₂ ·6H ₂ O	0.1525	1.5 Mg ²⁺	3 Cl ⁻
NaCl	2.7760	95 Na ⁺	95 Cl ⁻
NaHCO ₃	1.1341	27 Na ⁺	27 HCO ₃ ⁻
<i>Choices:</i>			
(1) Na ₂ HPO ₄	0.7098	20 Na ⁺	10 HPO ₄ ²⁻
(2) Na ₂ HPO ₄	0.3549	10 Na ⁺	5 HPO ₄ ²⁻
(3) Na ₂ HPO ₄	0.0710	2 Na ⁺	1 HPO ₄ ²⁻

Table 2
Details of selected experiments.

Experiment	P source	Ca source	CO ₃ source	P (mM)	Ca (mM)	CO ₃ (mM)	Final pH	Phases/XRD	Medium
1	-	Ca	-	-	25	-	12.6	Ca(OH) ₂ + CaCO ₃	H ₂ O
2	-	Ca	NaHCO ₃	-	25	27	9.9	CaCO ₃	5 KCl + 1.5 MgCl ₂
3	-	Ca	NaHCO ₃	-	25	27	9.9	CaCO ₃	H ₂ O
4	-	Ca	NaHCO ₃	-	25	27	12.3	CaCO ₃	95 NaCl
5	-	Ca	NaHCO ₃	-	25	27	12.3	CaCO ₃	MS
6	Na ₂ HPO ₄	Ca	-	10	25	-	12.3	PCA + CaCO ₃	H ₂ O
7	Na ₂ HPO ₄	Ca	-	10	16.667	-	12.2	PCA	MS w/o HCO ₃
8	Na ₂ HPO ₄	Ca	-	10	25	-	12.4	PCA	MS w/o HCO ₃
9	(NH ₄) ₂ HPO ₄	Ca	-	10	16.667	-	11.3	ACP	MS w/o HCO ₃
10	(NH ₄) ₂ HPO ₄	Ca	-	10	25	-	12.0	PCA	MS w/o HCO ₃
11	Na ₂ HPO ₄	CaCl ₂ ·2H ₂ O	-	10	25	-	5.9	DCPD + PCA	H ₂ O
12	(NH ₄) ₂ HPO ₄	CaCl ₂ ·2H ₂ O	-	10	16.667	-	6.5	DCPD	MS w/o HCO ₃
13	(NH ₄) ₂ HPO ₄	CaCl ₂ ·2H ₂ O	-	10	25	-	6.5	DCPD + PCA	MS w/o HCO ₃
14	(NH ₄) ₂ HPO ₄	CaCl ₂ ·2H ₂ O	-	10	50	-	5.7	DCPD + PCA	MS w/o HCO ₃
15	(NH ₄) ₂ HPO ₄	CaCl ₂ ·2H ₂ O	-	10	16.667	-	6.1	DCPD + PCA	H ₂ O
16	Na ₂ HPO ₄	Ca	NaHCO ₃	1	2.5	27	9.2	ACP	MS
17	Na ₂ HPO ₄	Ca	NaHCO ₃	5	12.5	27	10.3	ACP	MS
18	Na ₂ HPO ₄	Ca	NaHCO ₃	10	25	27	12.0	ACP + CaCO ₃	MS
19	Na ₂ HPO ₄	Ca	NaHCO ₃	10	25	27	9.0	No ppt ^s	H ₂ O
20	(NH ₄) ₂ HPO ₄	Ca	NaHCO ₃	10	25	27	10.4	ACP + CaCO ₃	MS
21	Na ₂ HPO ₄	Ca	NH ₄ HCO ₃	10	25	27	10.1	ACP + CaCO ₃	MS
22	(NH ₄) ₂ HPO ₄	Ca	NH ₄ HCO ₃	6.667	16.667	27	9.4	ACP	MS
23	(NH ₄) ₂ HPO ₄	Ca	NH ₄ HCO ₃	10	16.667	27	9.3	ACP	MS
24	(NH ₄) ₂ HPO ₄	Ca	NH ₄ HCO ₃	10	25	27	9.5	ACP	MS

The design of this unique study combined solution chemistry and deprotonation (of electrochemical origin) together.

The very first reaction caused by Ca metal is “*in situ* deprotonation” and H₂(g) evolution, which autogenously increases the solution pH.

[28] A. C. Tas, “Calcium metal to synthesize amorphous or cryptocrystalline calcium phosphates,” *Materials Science and Engineering C*, 32, 1097-1106 (2012).

[29] U.S. Patent No. [9,108,860 B2](#), “Synthesis of amorphous calcium phosphate or poorly crystalline calcium phosphate by using Ca metal,” August 18, 2015, Inventor & Assignee: A. C. Tas

Epilogue

This document provided the highlights of 27 of my peer-reviewed Journal publications which were overwhelmingly (26 out of 27) focused on the Ap-CaP (apatitic calcium phosphate) or ACP (X-ray or TEM-amorphous calcium phosphate) synthesis and processing. The hyperlinks used throughout the text provide easy access to the experimental methods/data/results/discussion.

The reader could see that Ap-Cap synthesis is not difficult at all and it can take as the starting material a bioceramic (*e.g.*, CaCO₃ (calcite, vaterite or aragonite), crystalline brushite, monetite, struvite, HA, TCP, biphasic HA-TCP or biphasic Plaster of Paris+monetite ceramic I happened to study), biometal (titanium and its alloys) or a macroporous biopolymer (collagen foam).

The synthesis procedures highlighted here used temperatures $\leq 90^{\circ}\text{C}$ (mostly RT or 37°C), therefore, they are quite easy to scale-up. The reactors (made out of glass, HDPE or polycarbonate) were not agitated and the procedures proposed did not involve the use of pressures higher than 1 atm.

The conversion of a material system under investigation resulted in the formation of nanoneedles of Ap-CaP mostly aggregated to submicron or micron sizes, which meant increased surface roughness. Osteoblast cells do love rough surfaces and they spread their filopodia during their proliferation. The materials I presented here are different from some products on the market, *e.g.*, MagnetOS /Needle-Grip product family of Kuros Biosciences that have high crystallinity CaP needles on their surfaces despite their small dimensions. As crystallinity of an apatite-like CaP increases, its resorbability decreases. The Ap-Cap or ACP materials I published from 2004 to 2015 (they do not exhibit the 3571 cm^{-1} IR band thus they are not hydroxyapatite) all had low crystallinity.

Please note that all procedures shared in this document are either published or patented.