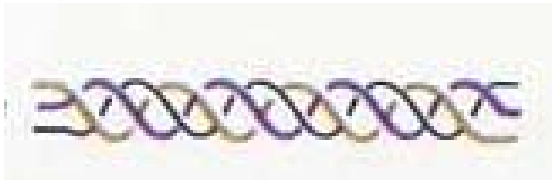
Modulators of Apatite in Bone Formation- Are They Passive or Active?

Laurie Gower, Associate Professor

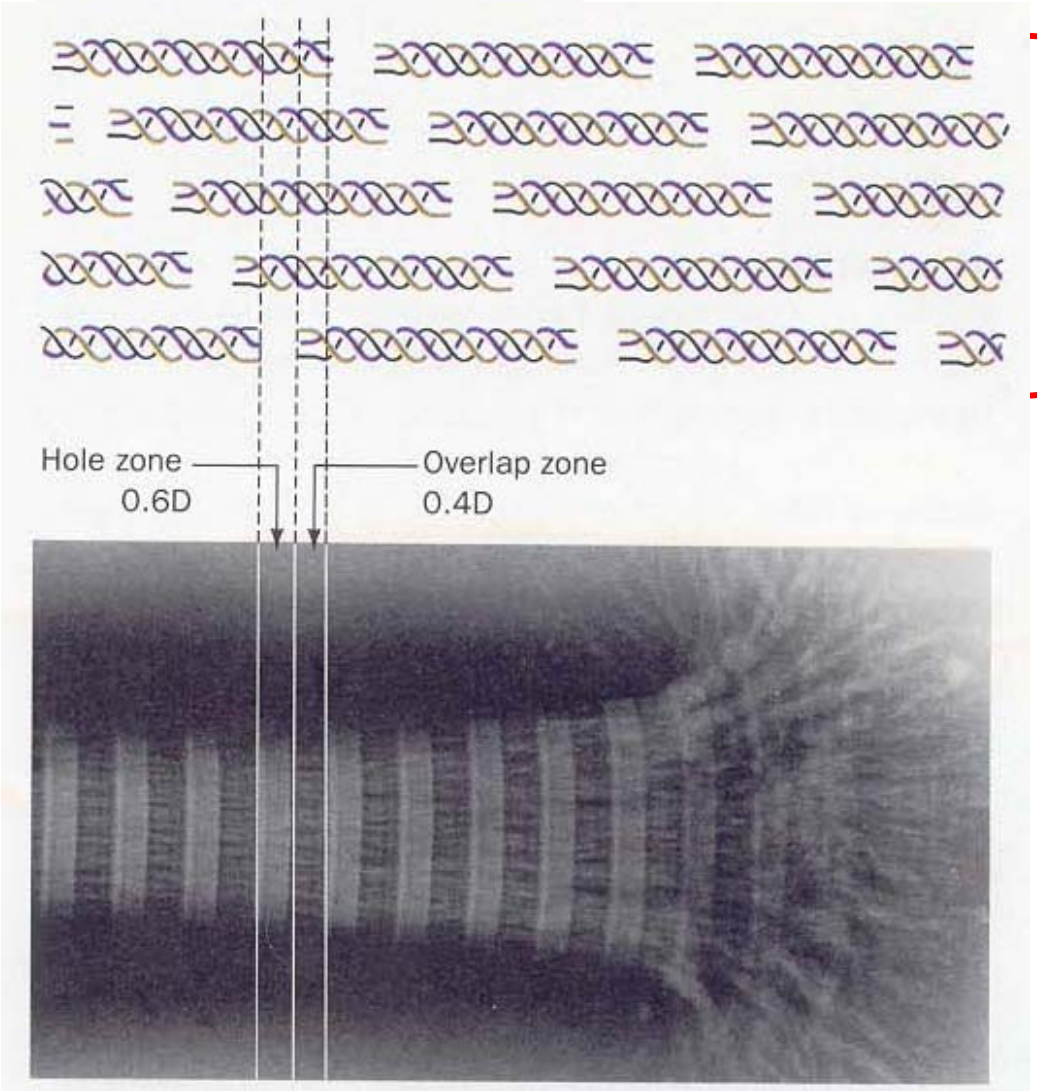
Department of Material Science and Engineering, University of Florida, Gainesville, FL, USA.

Pardee 1. Apatites I Have Known: From Man to Mars
Oct. 19, 2014 (Vancouver, CA)

Assembly of Type-I Collagen into Fibrils



Tropocollagen ‘molecule’ (triple helix)



Quarter-stagger assembly leads to periodic banding pattern from hole and overlap zones

TEM of stained collagen fibril

Voet, Biochemistry

Hierarchical Structure of Bone

Bone Composition:

~65 wt % calcium phosphate

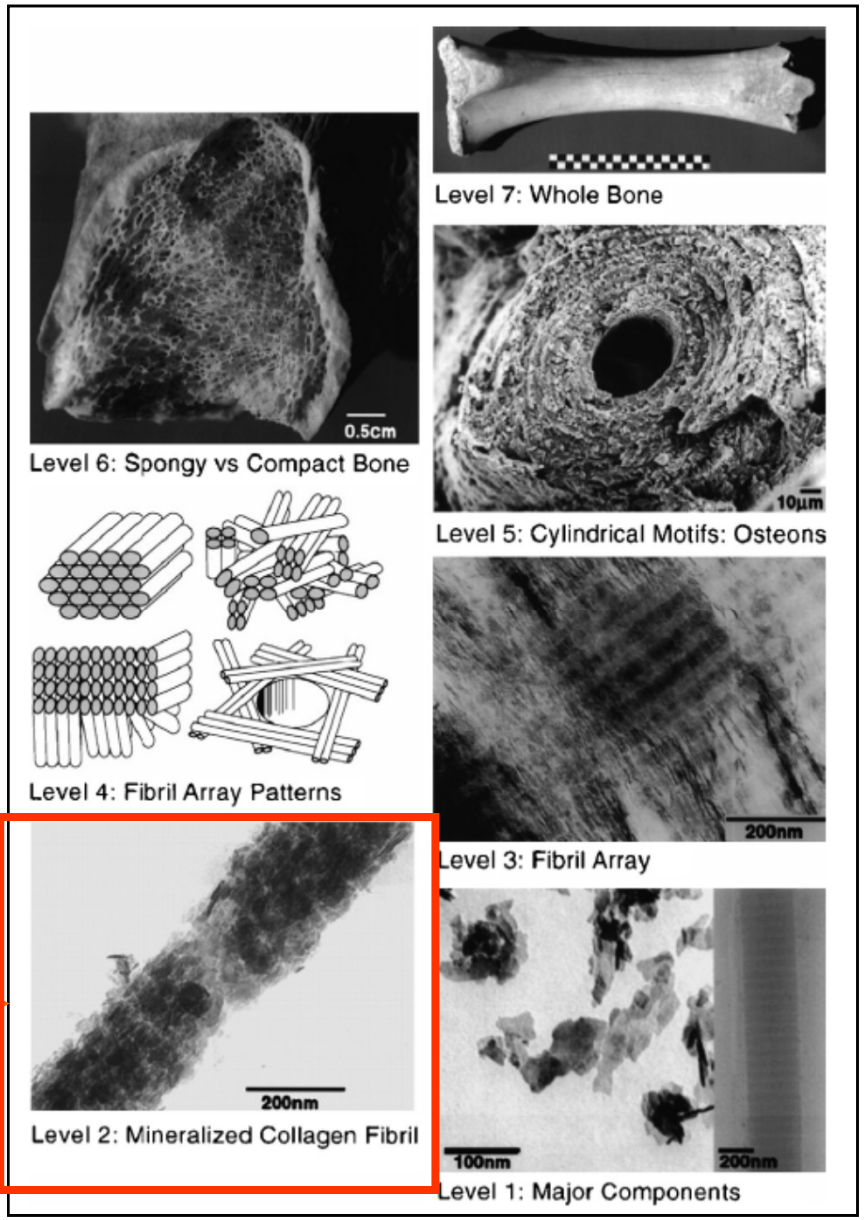
~20 wt % collagen (type I)

~10 wt % water

~5 wt % non-collagenous proteins (NCPs)

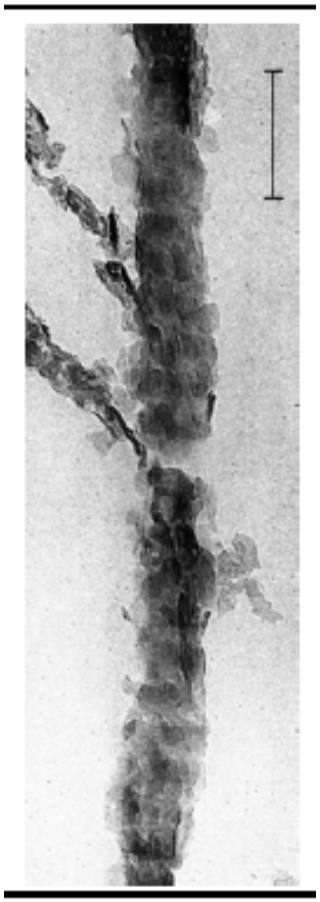
- osteopontin (aka BSP-1, SPP1)
- osteocalcin
- osteonectin
- etc.

Nanostructured architecture from intrafibrillar mineralization

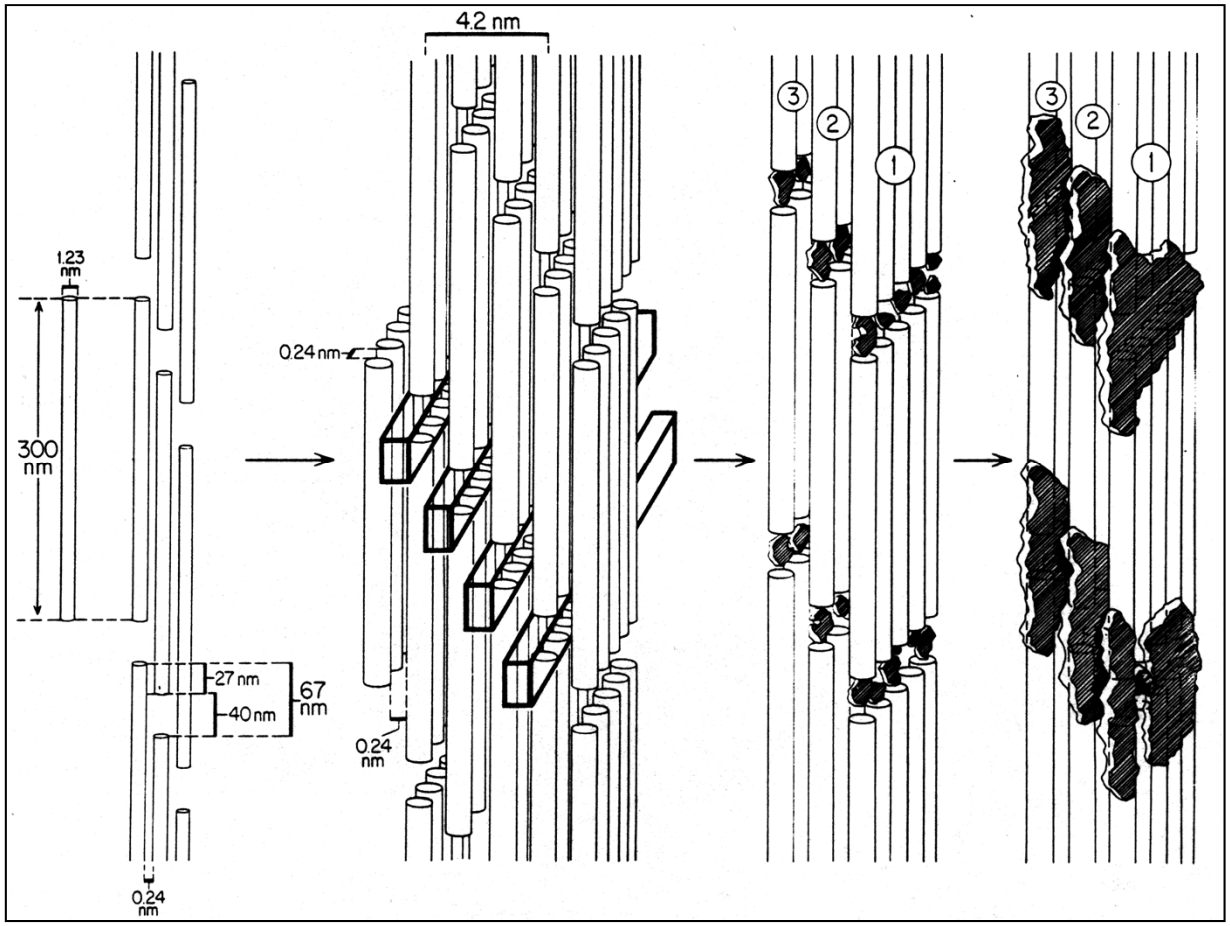


Intrafibrillar Mineralization of Collagen

Traditional view: HAp crystals nucleate on collagen domains within gap zones of collagen fibrils.



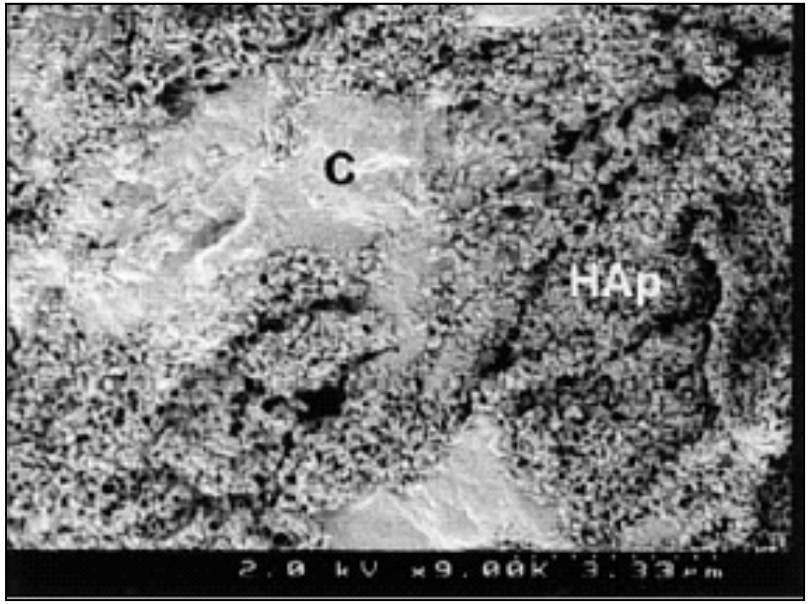
Weiner & Traub (1981)

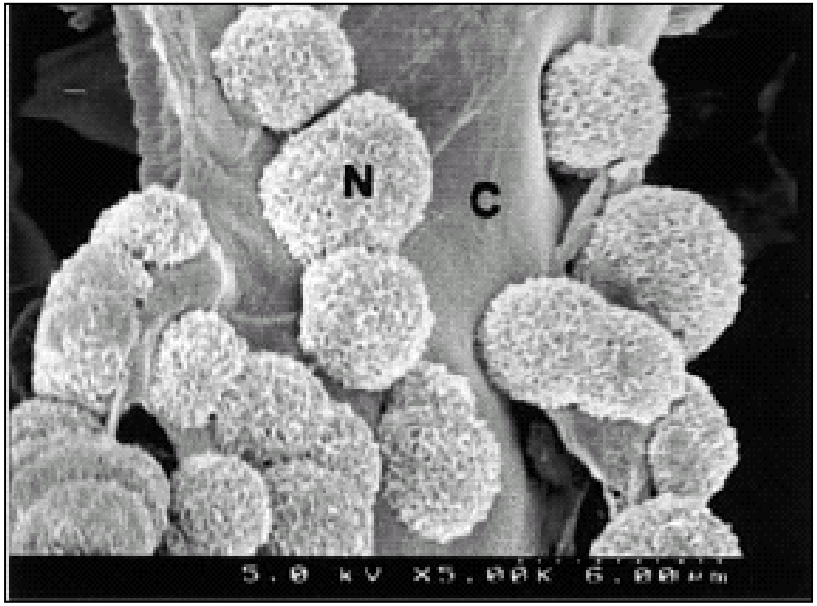


Landis, WJ et al., *J. Struc. Biol.* 110: 39-54; 1993

But why can't this be reproduced in vitro?

Mineralization of Collagen via the Conventional Solution Crystallization





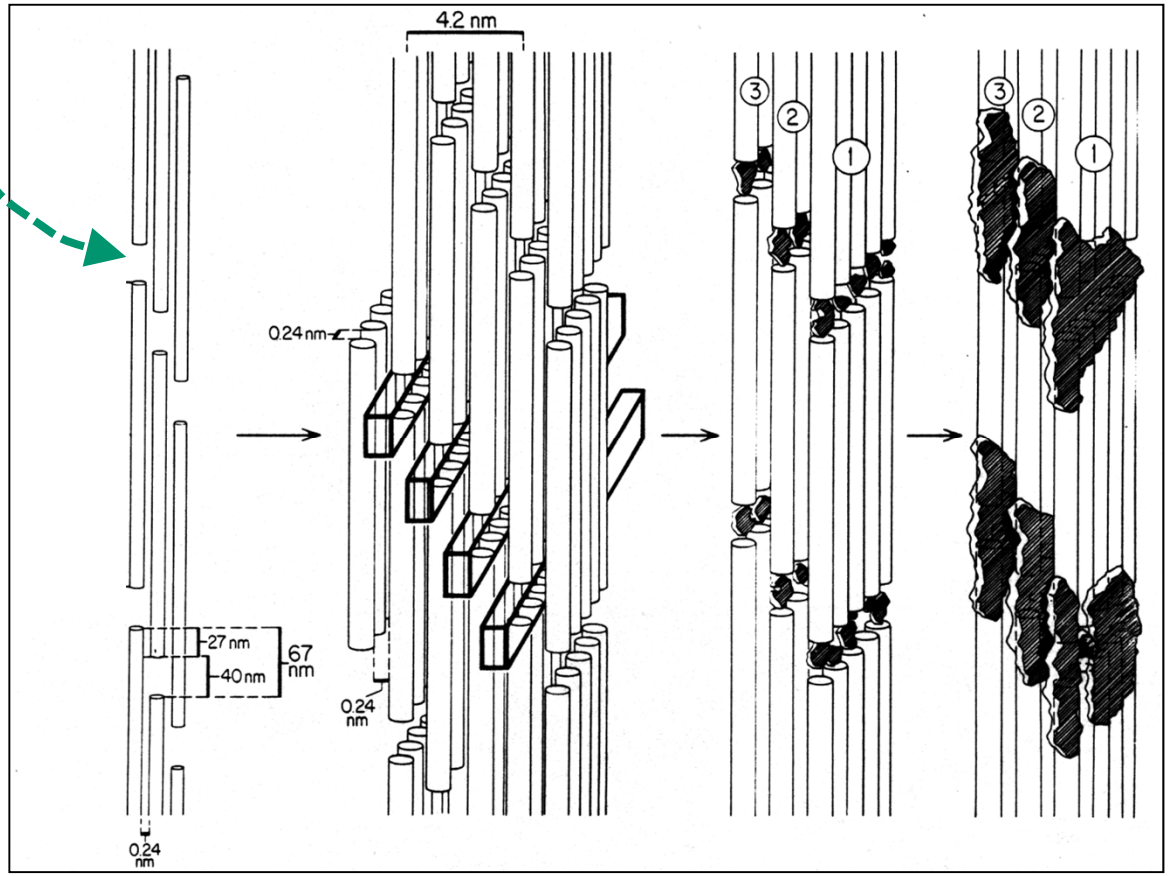
HAp spherulitic clusters nucleate heterogeneously on surface of collagen scaffold

Lickorish, David, et al. "Collagen-hydroxyapatite composite prepared by biomimetic process", *JBMR Part A*, **68A**, 19 (2004).

Is this structure (or process) biomimetic?

Intrafibrillar Mineralization of Collagen

Modified view: If collagen alone doesn't work, then perhaps NCPs migrate into the hole zones and promote epitaxial nucleation of HAp.



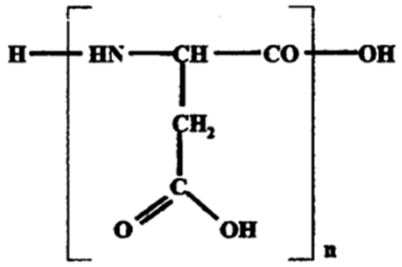
Schematic from:

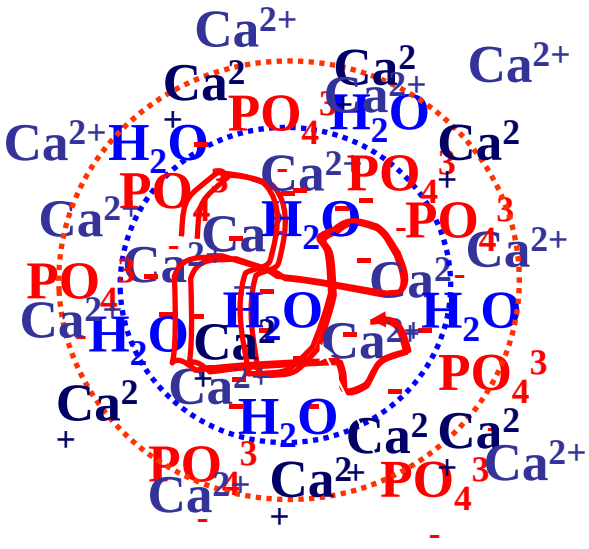
Landis, W. J., Song, M.J., Leith, A., McEwen, L., McEwen, B.F., *J. Struc. Biol.* 110: 39-54; 1993

Our Hypothesis: Intrafibrillar mineralization can be achieved with the PILP process

Polymer-Induced Liquid-Precursor (PILP) Process

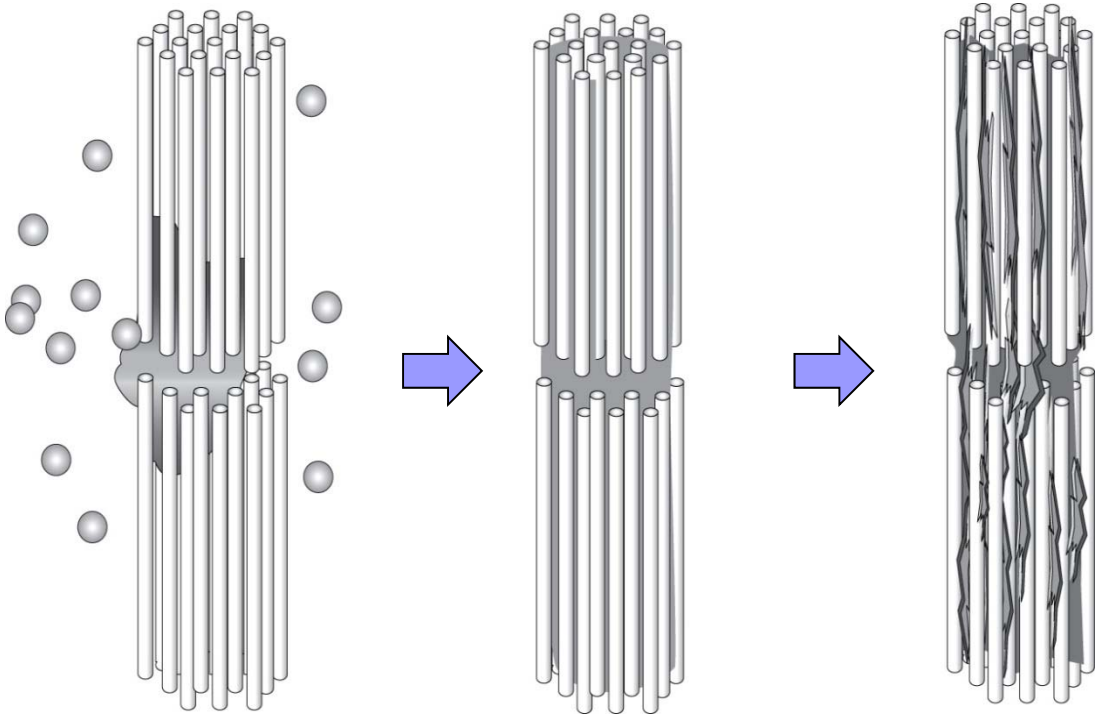
Poly(aspartic acid) → simple mimic of the acidic NCPs found in bone





PILP droplet

PILP Mineralization of Collagen

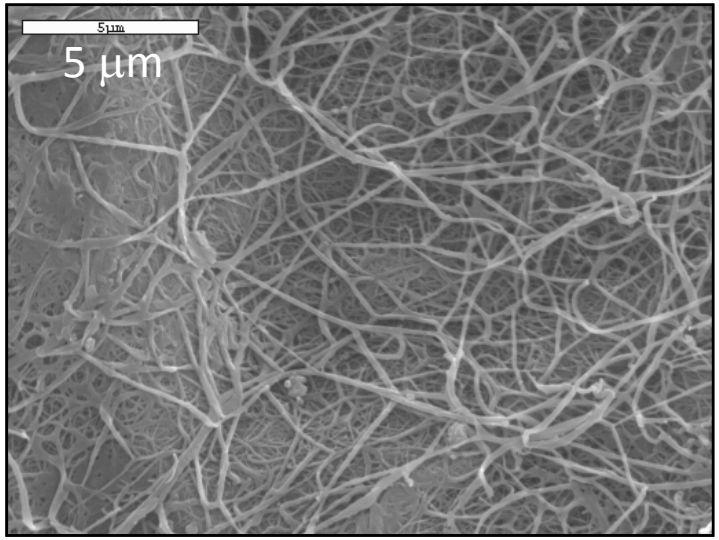


Infiltration of PILP

Solidification to ACP

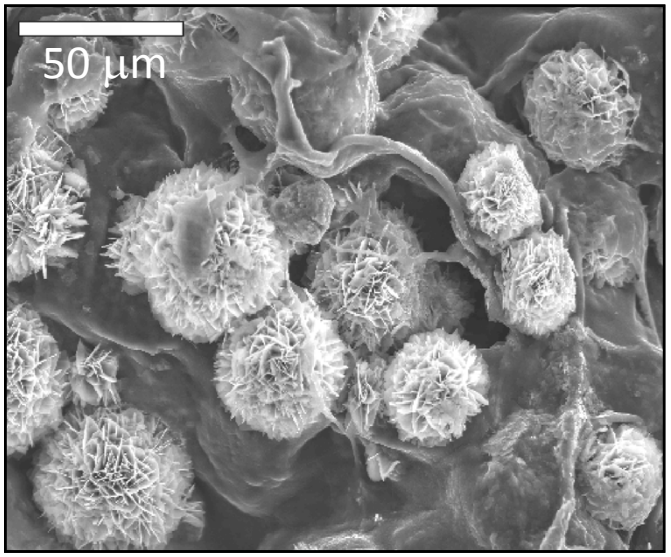
Crystallization to HA

Mineralization of Collagen with Hydroxyapatite

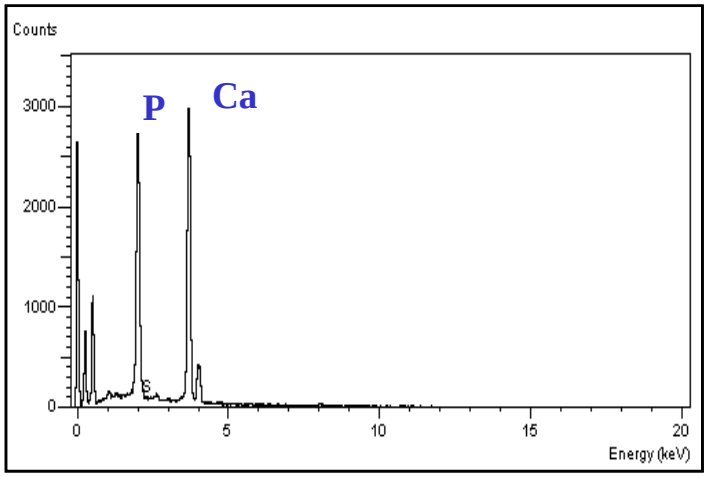


Type I Collagen Scaffold

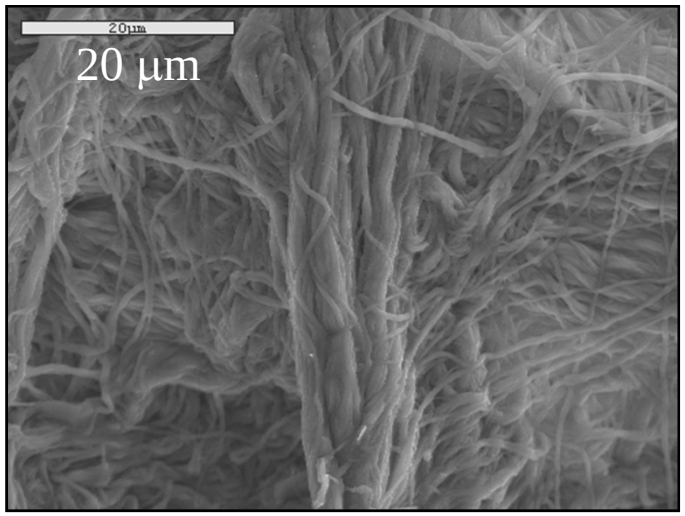
+ pASP



Conventional Crystallization

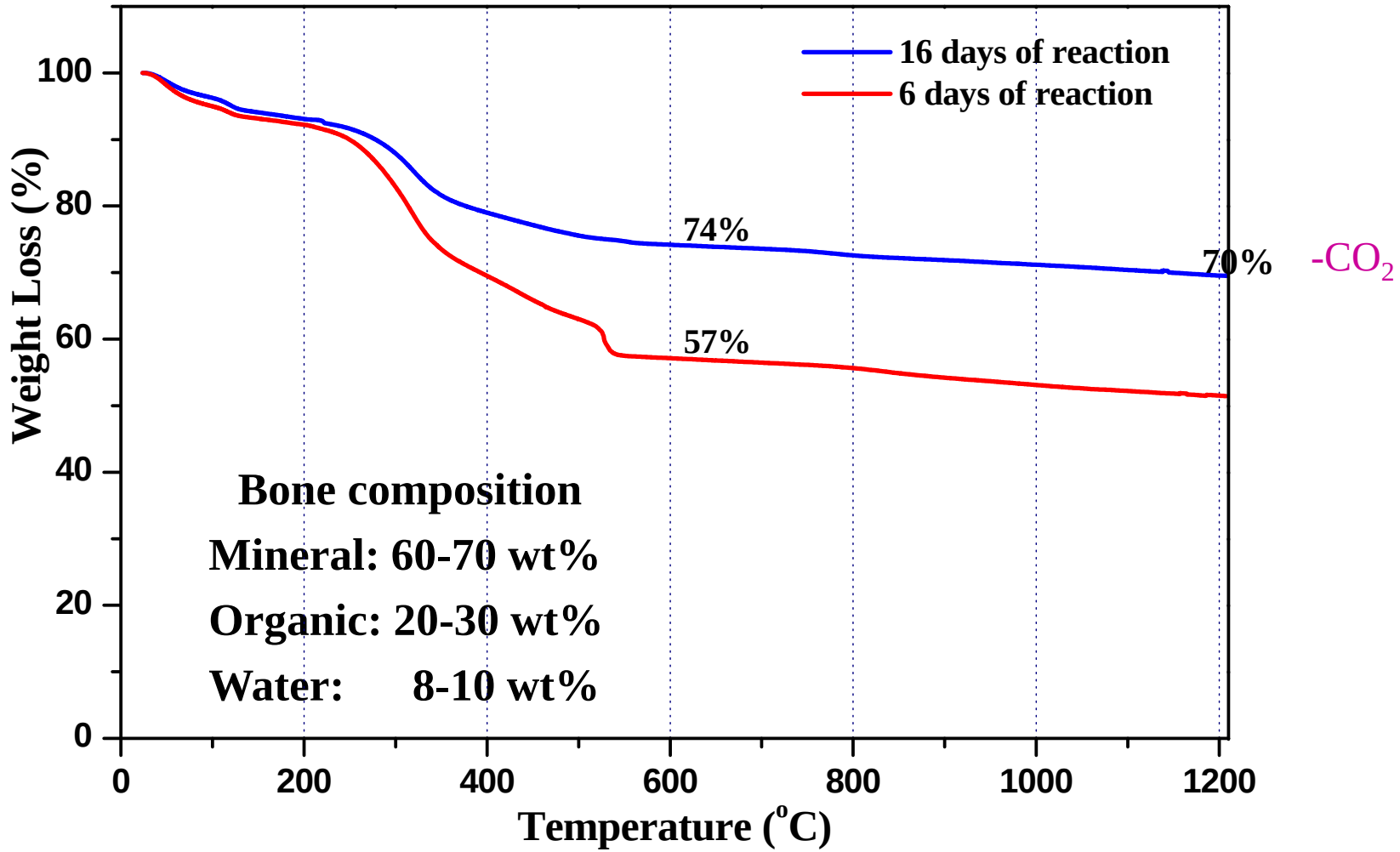


EDS Spectrum



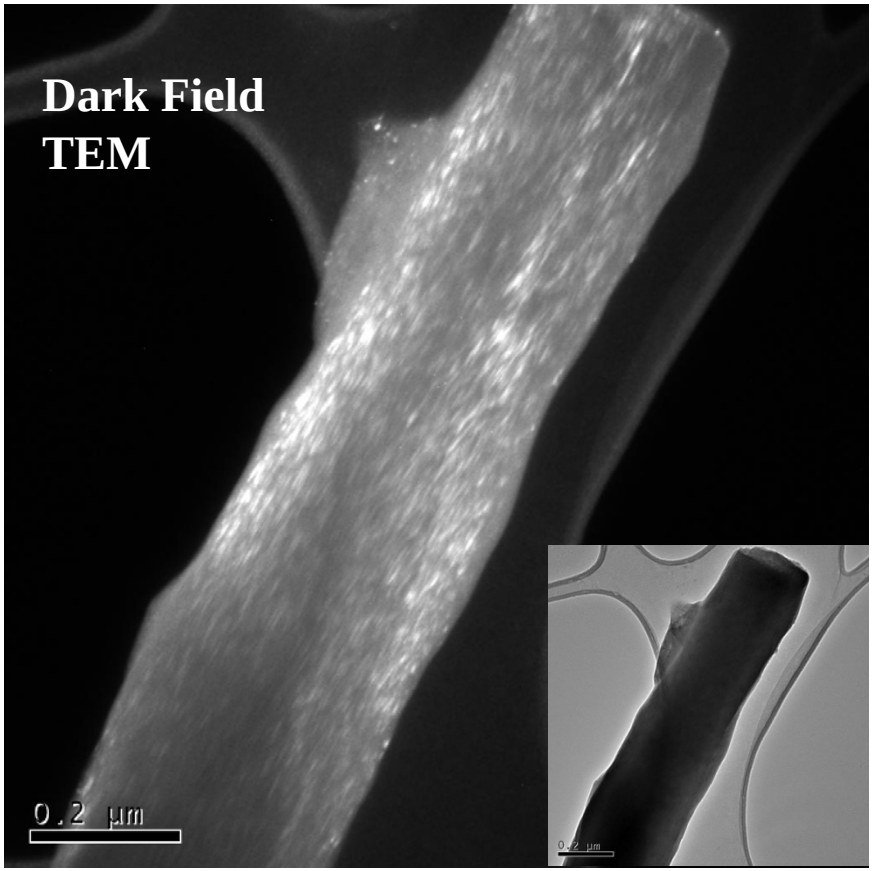
PILP Mineralization

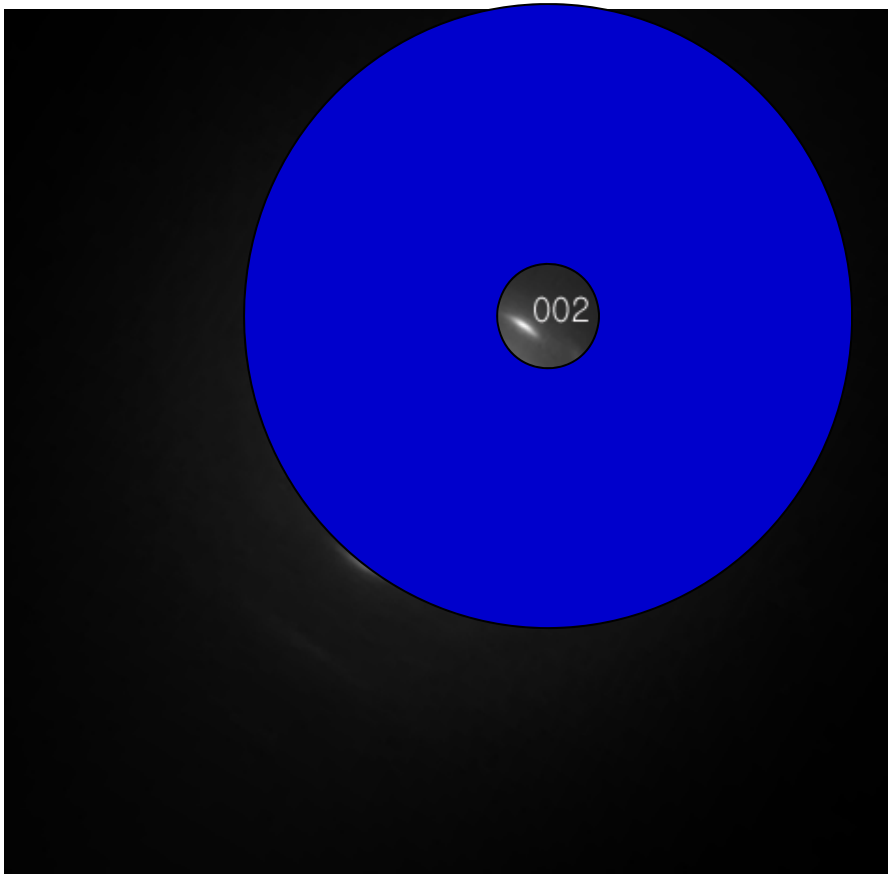
Thermogravimetric Analysis → Mineral Content



→ **High degree of mineralization (compositions match bone)**

[001] Crystallographic Orientation of HA



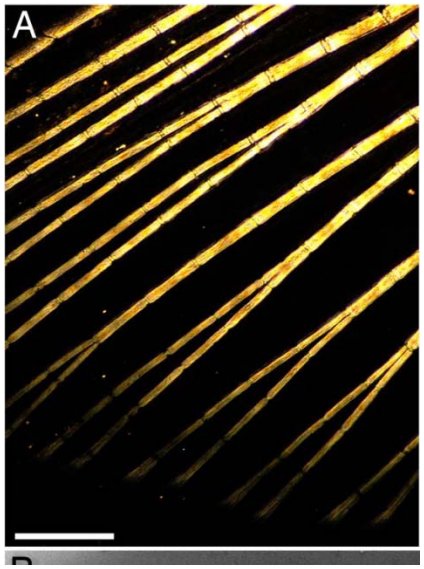


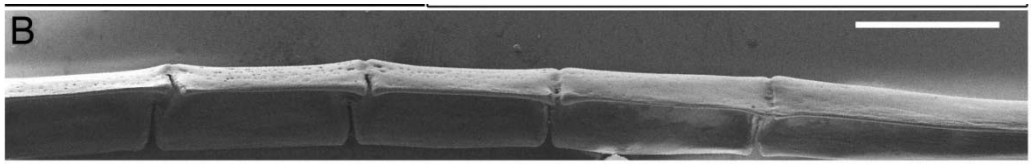
Bright-field TEM **SAED**

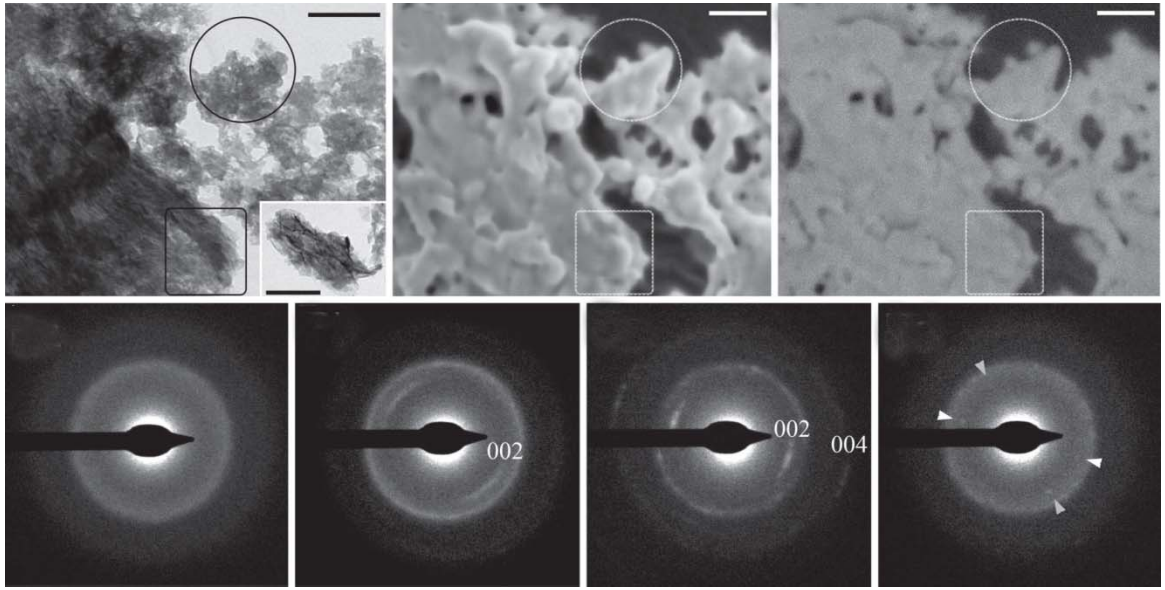
Olszta, M.J., Cheng, X.G., Jee, S.S., Kumar, R., Kim, Y.Y., Kaufman, M.J., Douglas, E.P., and Gower, L.B. (2007) **Bone structure and formation: A new perspective**. *Materials Science & Engineering R-Reports 58, 77-116*

Amorphous calcium phosphate is a major component of the forming fin bones of zebrafish: Indications for an amorphous precursor phase

Julia Mahamid*, Amnon Sharir†, Lia Addadi*‡, and Steve Weiner*

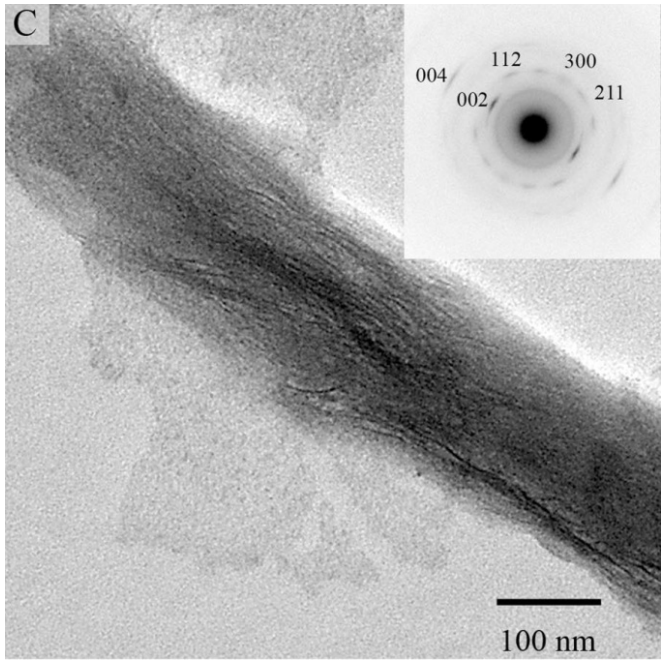






12748–12753 | PNAS | September 2, 2008 | vol. 105 | no. 35

Mineralization of isolated fibrils on TEM grids



→ They proposed that mineralizaton occurs via inhibitor (polymer) exclusion from the fibril

Deshpande AS, Beniash E., Bioinspired Synthesis of Mineralized Collagen Fibrils. CG&D 2008;8(8):3084-3090.

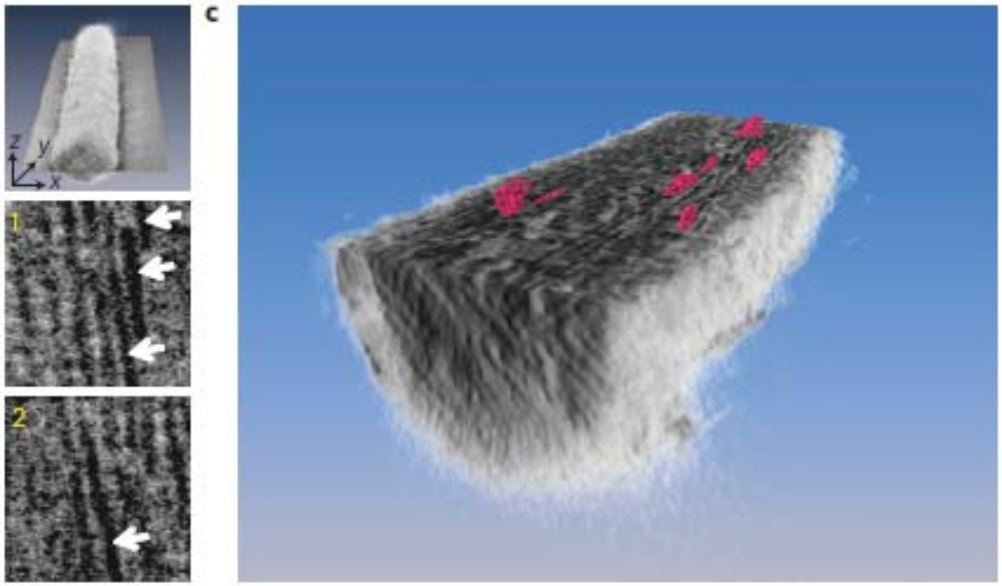
nature materials

LETTERS

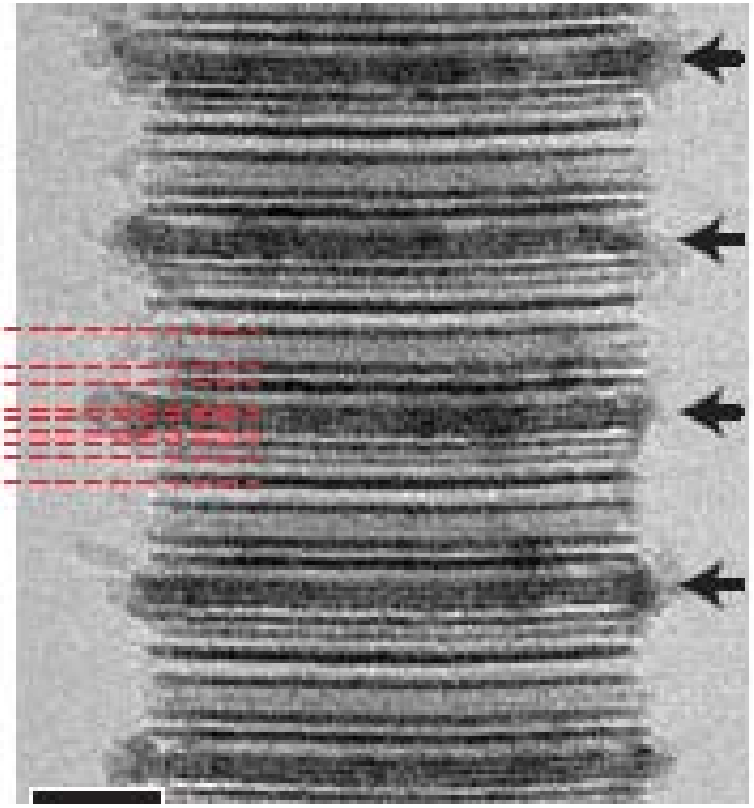
PUBLISHED ONLINE: XX MONTH XXXX | DOI: 10.1038/NMAT2875

The role of collagen in bone apatite formation in the presence of hydroxyapatite nucleation inhibitors

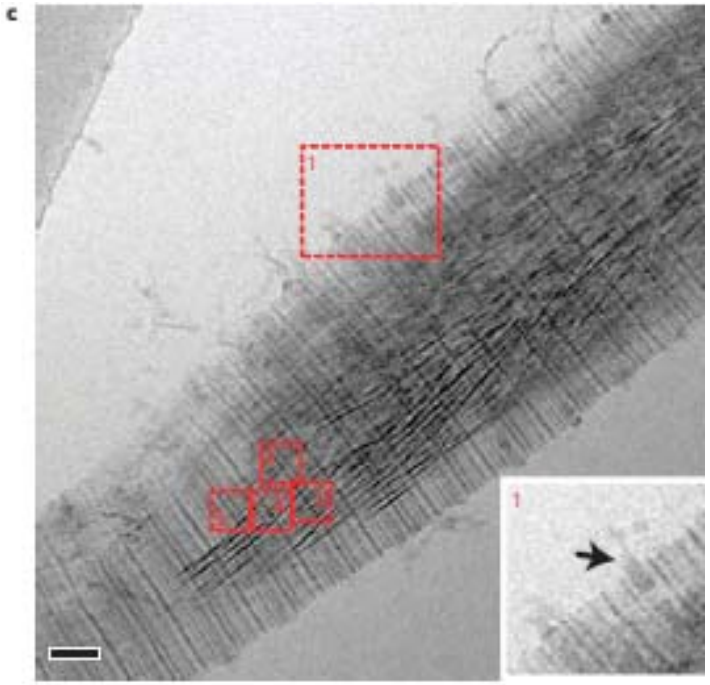
Fabio Nudelman[1], Koen Pieterse[2], Anne George[3], Paul H. H. Bomans[1], Heiner Friedrich[1], Laura J. Brylka[1], Peter A. J. Hilbers[2], Gijsbertus de With[1] and Nico A. J. M. Sommerdijk[1]*



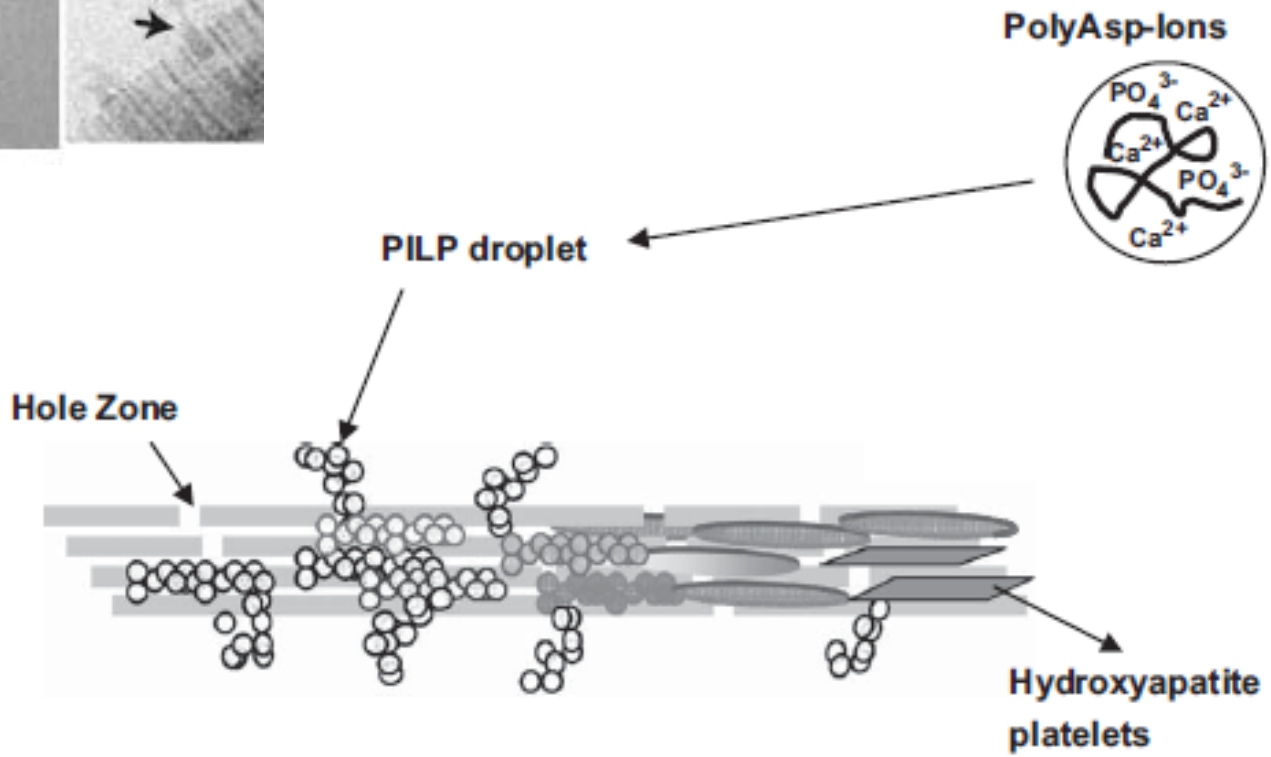
Cryo-TEM tomography

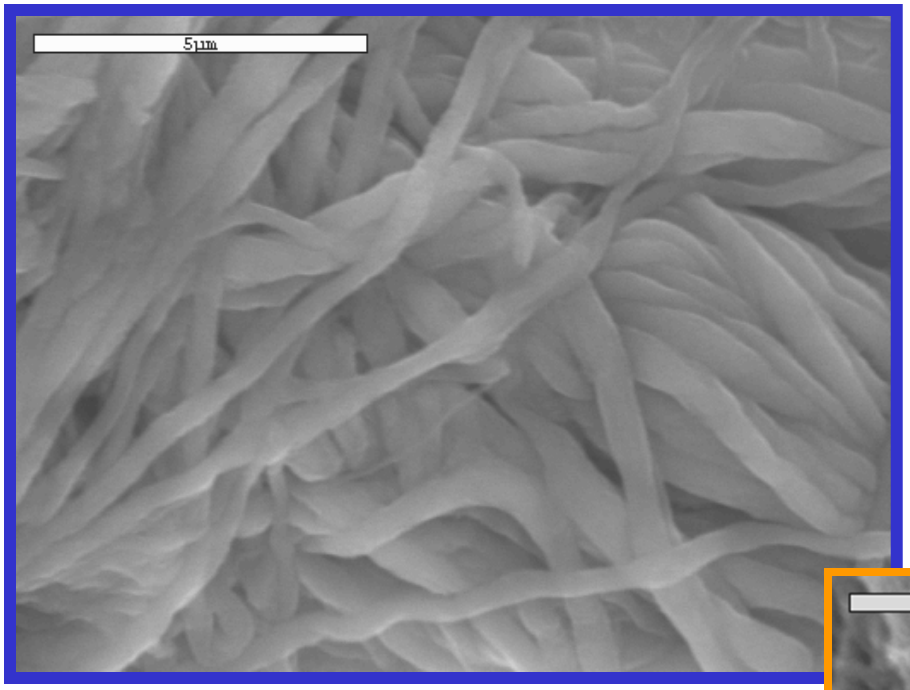


Amorphous calcium phosphate enters fibril at the *a*-bands

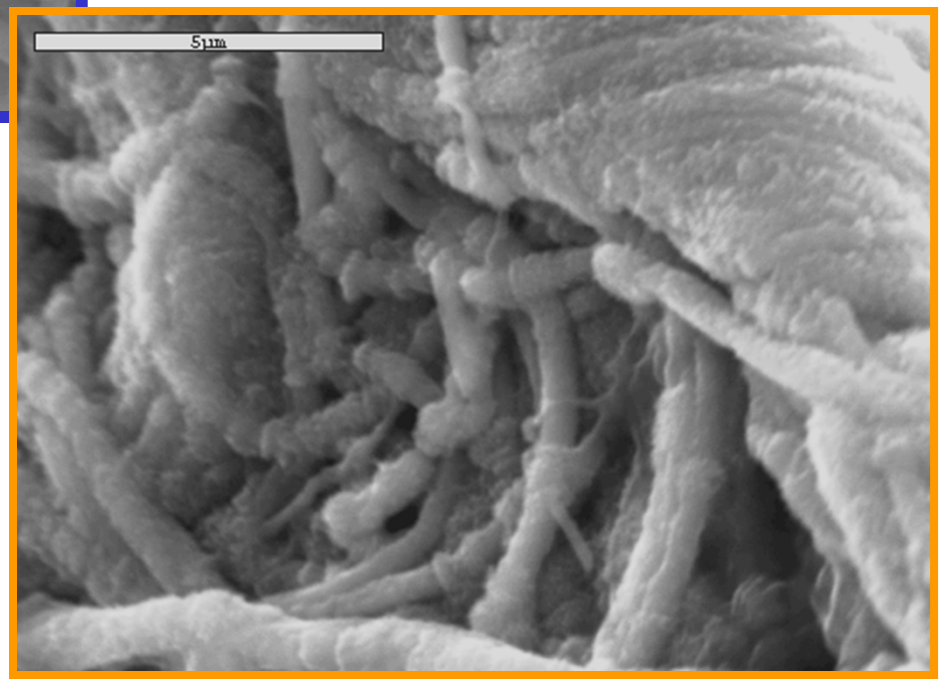


Jee SS, Culver L, Li Y, Douglas EP and Gower LB: Biomimetic mineralization of collagen via an enzyme-aided PILP process. Journal of Crystal Growth. *312: 1249–1256, 2010.*



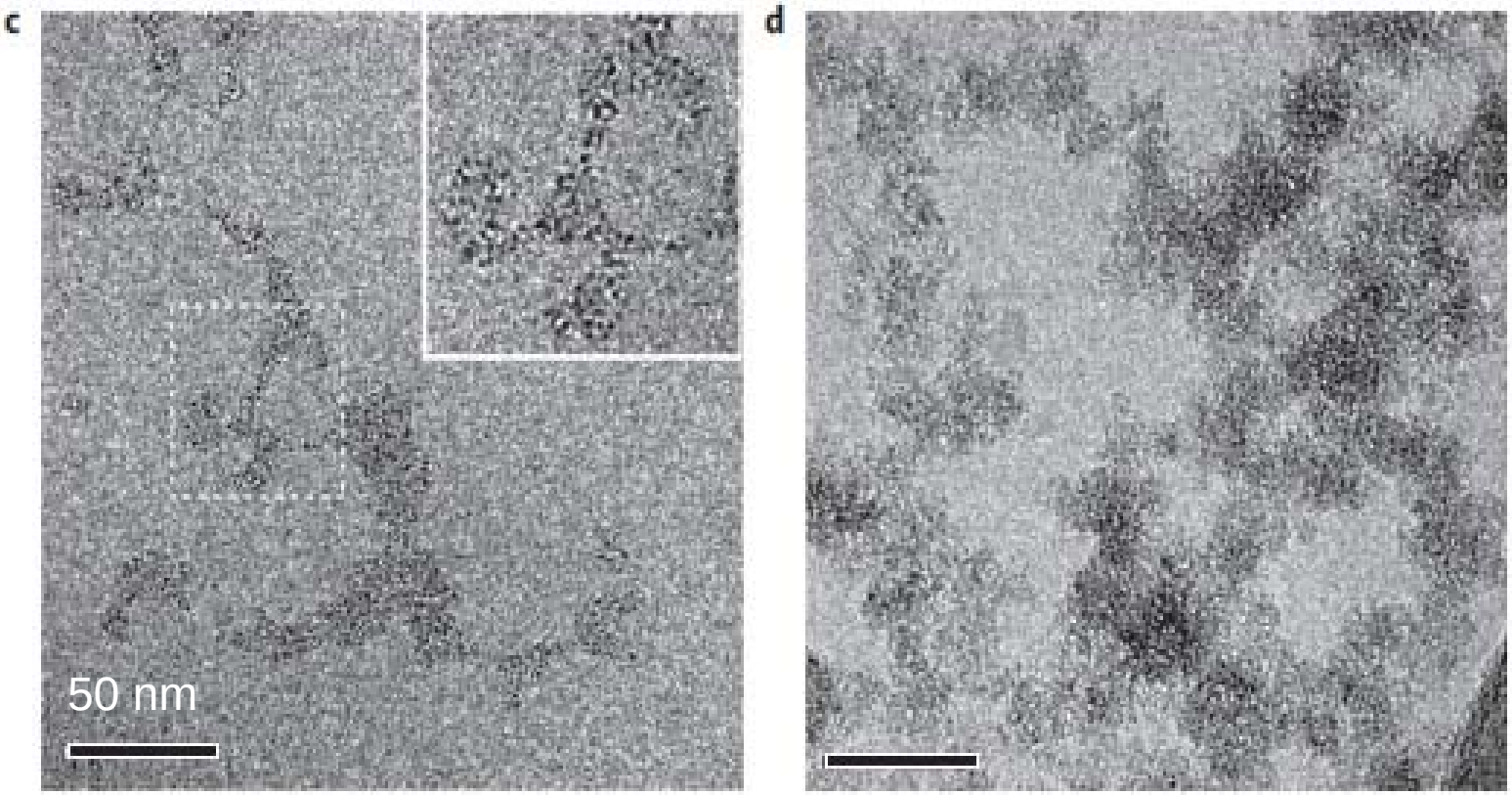


Pure intrafibrillar mineral



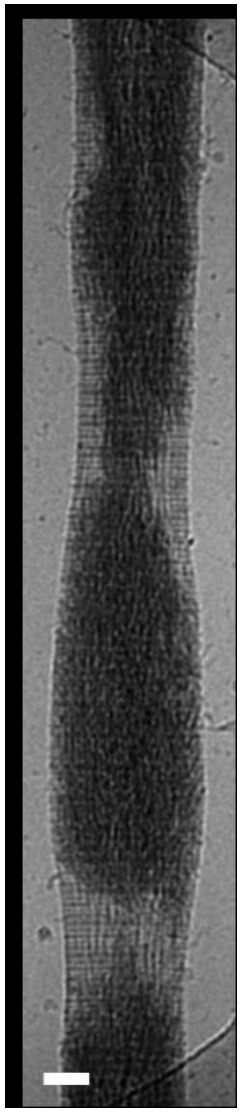
Extrafibrillar coating

CryoTEM image of calcium phosphate aggregates of pre-nucleation clusters formed with PolyAspartate



10 minutes

6 hours



Tomographic TEM image of mineralized collagen fibril

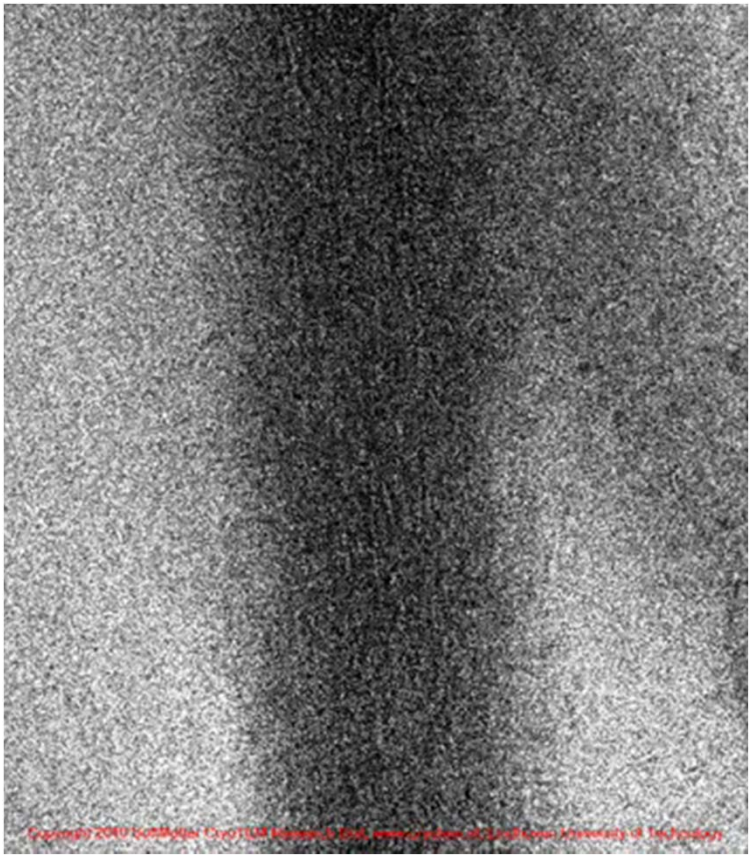
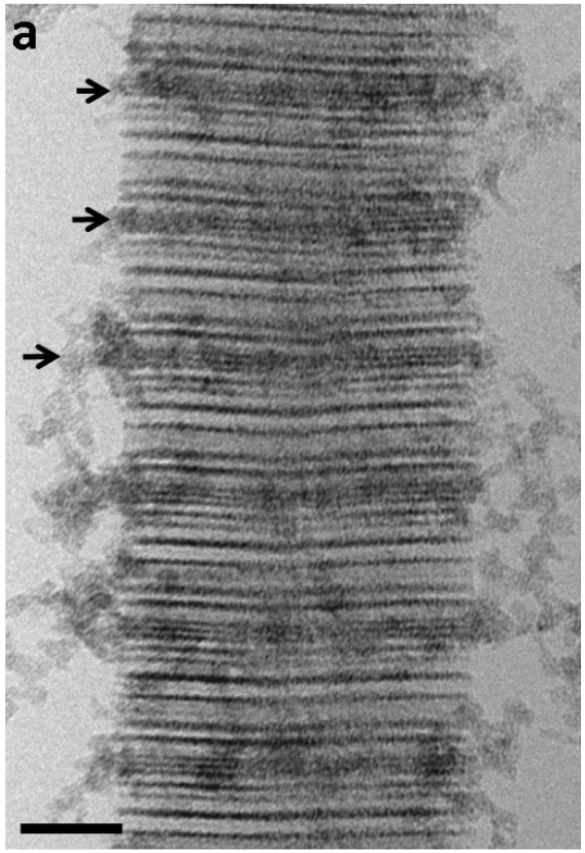
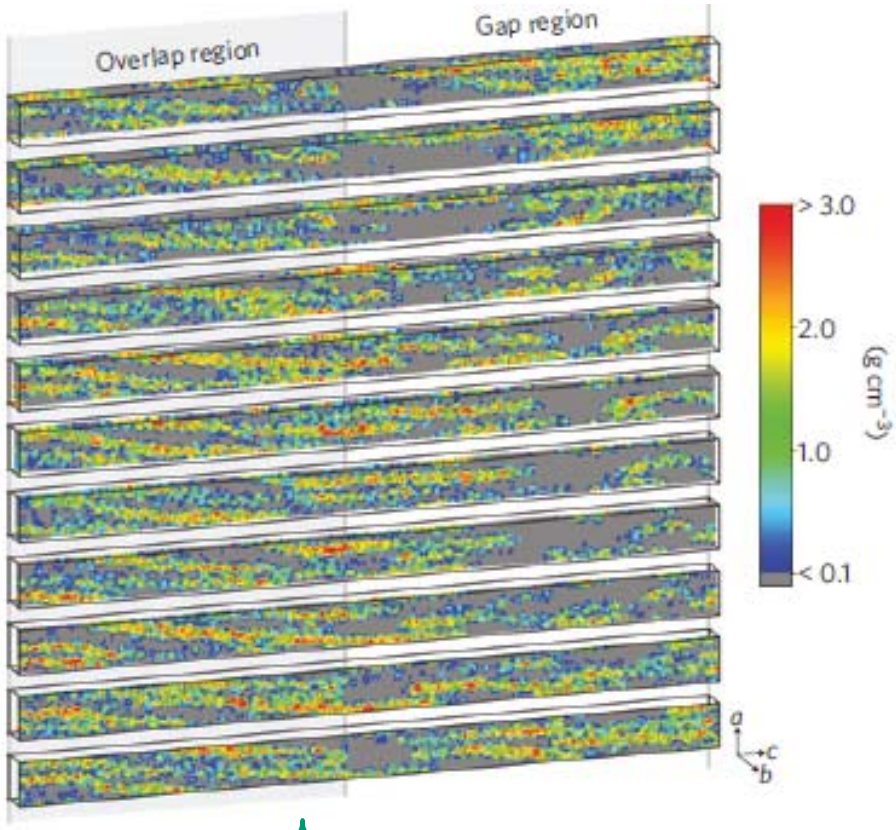


Fig. S6. Fibril mineralized for 48 h, where the deformation caused by the presence of mineral can be observed.

c-DMP-1 plus Polyaspartate



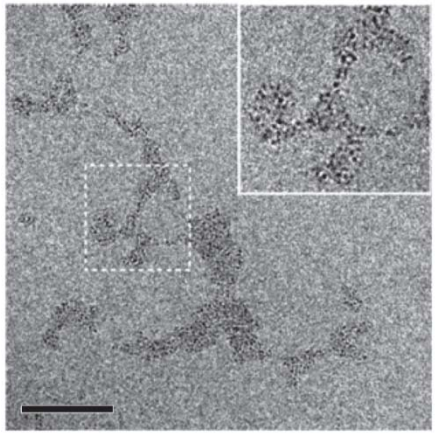
Mass Density Map of Fibril



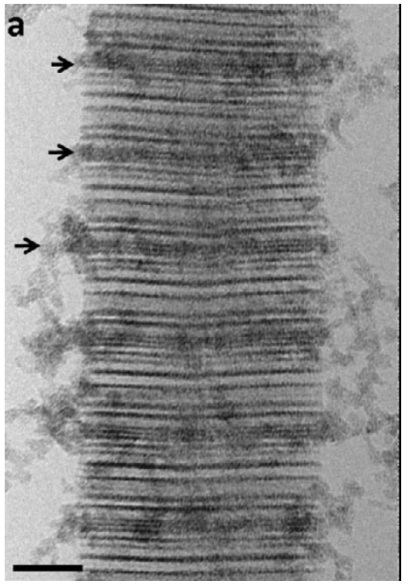
Mass density map of a microfibril, depicting slices through the *b axis of the crystal unit cell. Grey areas demonstrate the space within the microfibril through which the mineral* phase could potentially diffuse.

Particles vs. Droplets? Diffusion vs. Capillarity?

1. Precursor phase appears to be **coalesced** phase or particles.

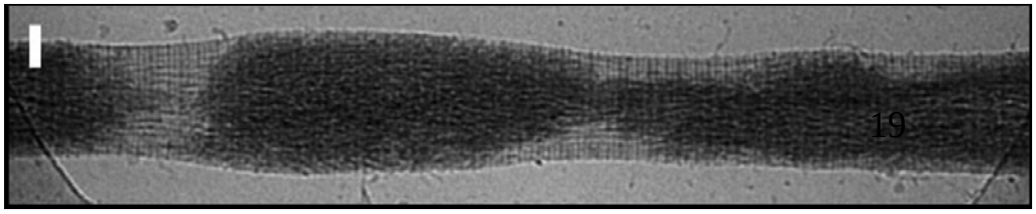


2. Precursor phase **densifies** with time to become ACP.

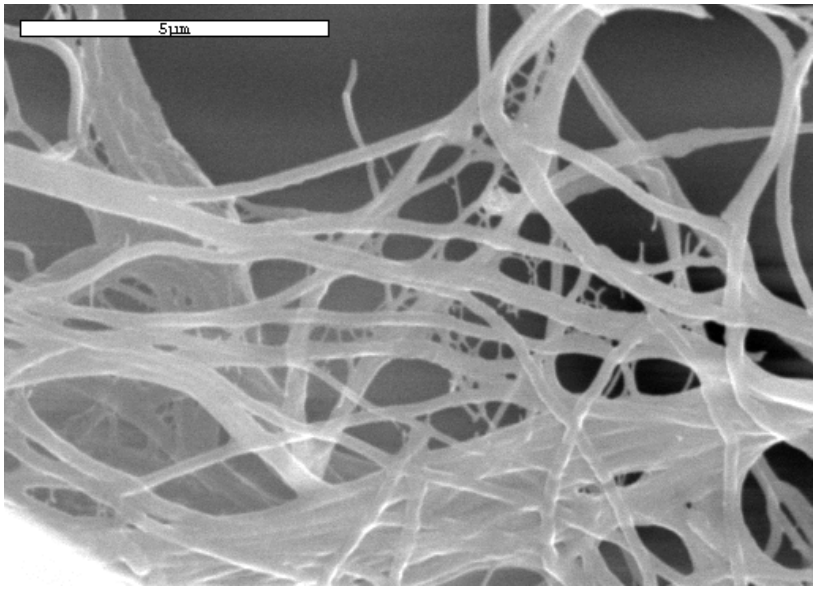


3. Chains of “particles’ seem too **large** to enter gap zones and diffuse throughout.

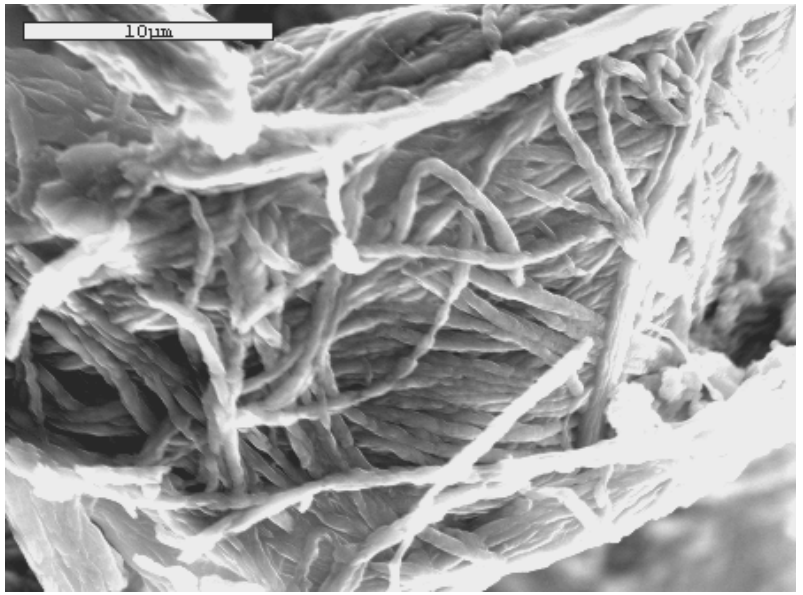
4. Crystals seem to form at the **interior** of the collagen fibril first.



Mineralization of Collagen with Osteopontin



Reconstituted Collagen Sponge



Mineralized with 100 ug/ml OPN

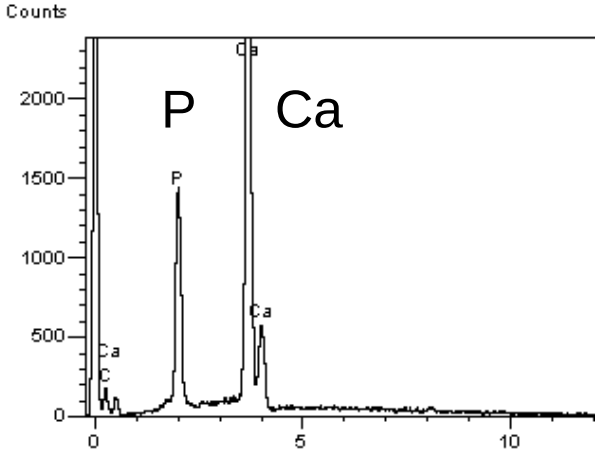
- 4 day mineralization

100 ug/ml OPN

4.5 mM $CaCl_2 \cdot 2H_2O$

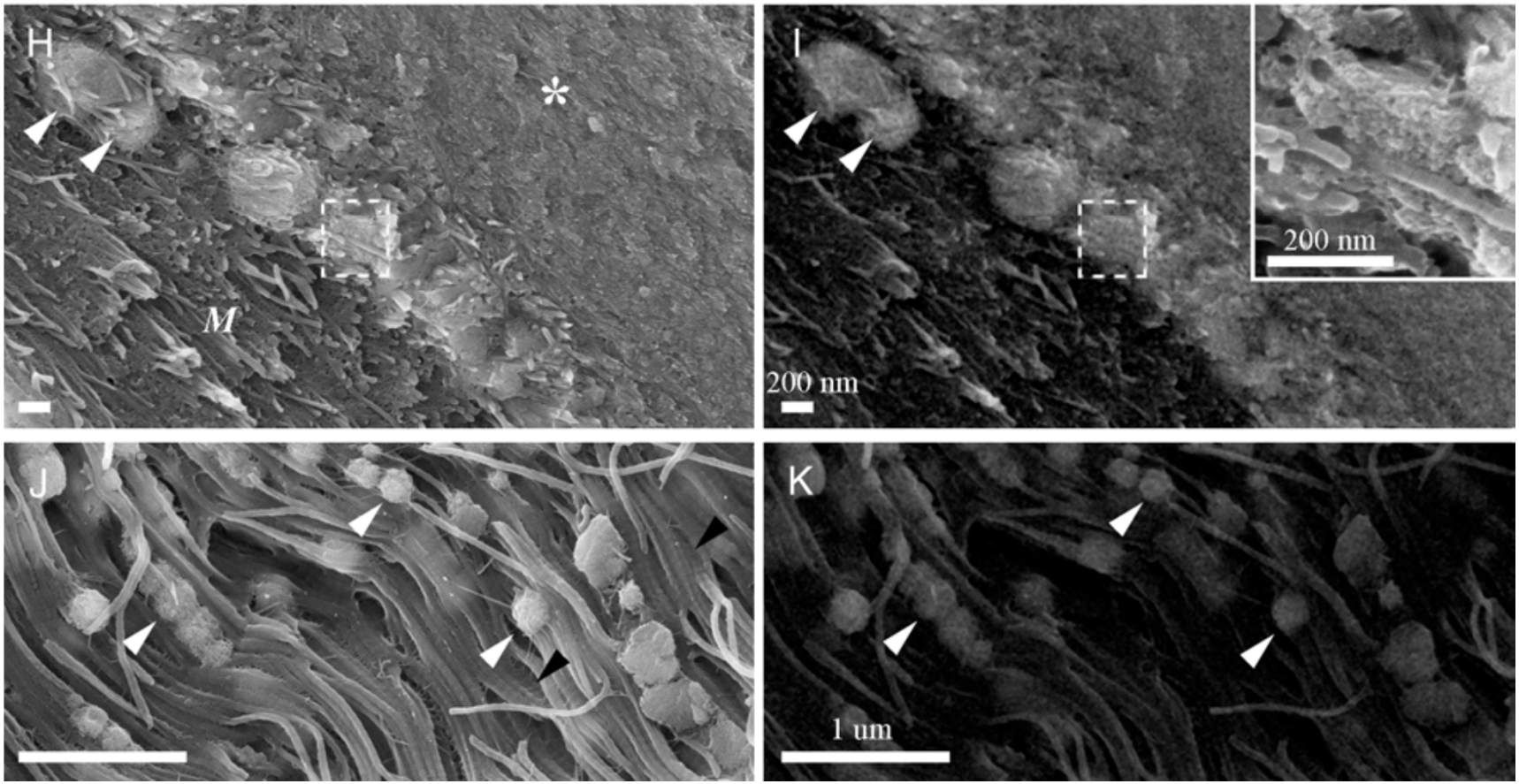
2.1 mM KH_2PO_4

EDS



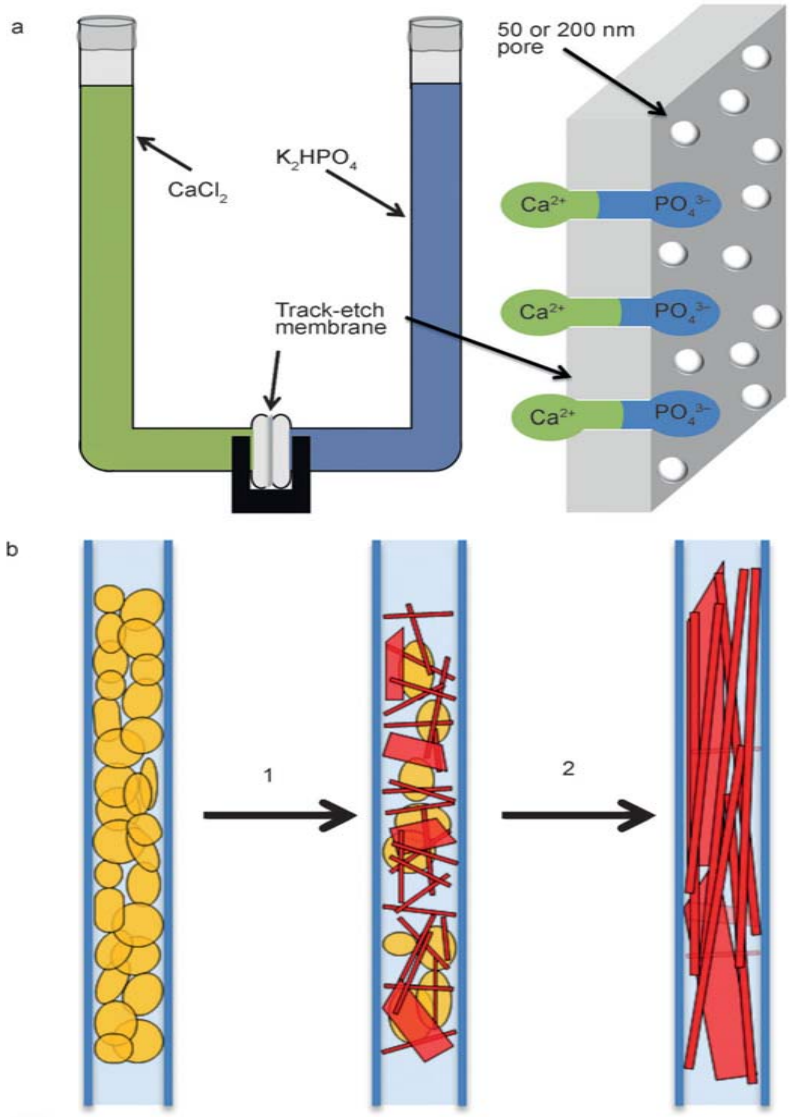
Rodriguez et al. *Acta Biomaterialia.* 10, 494 (2014)

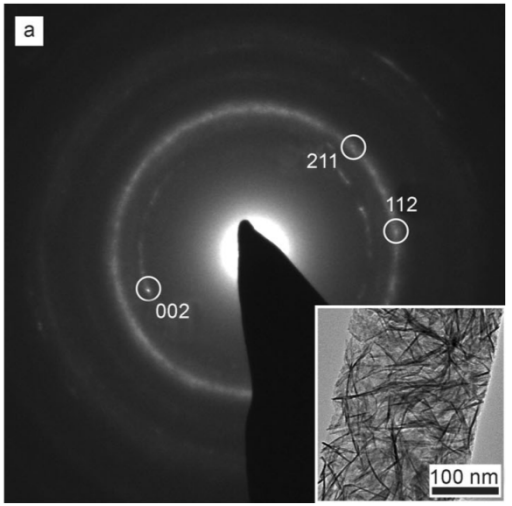
Weizmann group: Mahamid et al, *PNAS* 105, 12748 (2008)



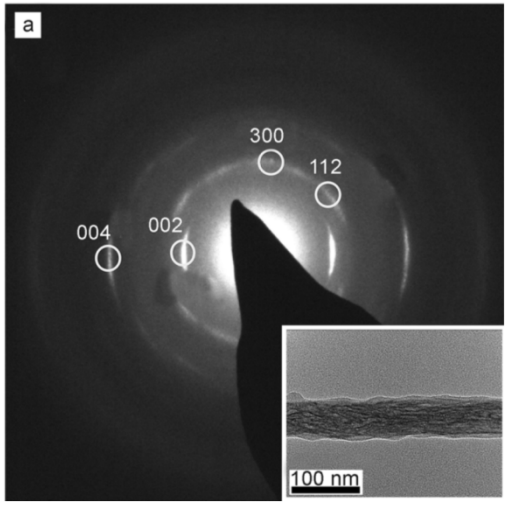
(H & J) Cryo-SEM images on bone growth zone in caudal fin of zebrafish,
(I & K) are backscattered electrons images of the same areas
→ The newly deposited, nonmineralized bone matrix (M) contains large, "mineral-bearing globular entities". The globules "fuse" into the mineralizing bone matrix.

Nanoscale Confinement Controls the Crystallization of Calcium Phosphate: Relevance to Bone Formation (Cantaert & Meldrum)





300 nm pores → random orientation



50 nm pores → [001] orientation

Cantaert, Bram; Beniash, Elia; Meldrum, Fiona C. *Chemistry – A European Journal*, 19 (2013): 14918

Summary of PILP System

- Intrafibrillar mineralization of collagen can be achieved

  → interpenetrating HAp-collagen nanostructured composite

- A high degree of mineralization can be achieved

  → 60-70 wt% mineral loading (matching bone)

- Broad XRD peaks match bone (nanocrystals)
- The [001] crystallographic orientation matches bone

  → no ‘epitaxial’ nucleating proteins were used

- Many polyeletrolytes and type I collagen scaffolds work
- Recent literature shows bone is formed from globular amorphous precursor that fuses into the matrix

➢ Does this evidence suggest that bone formation may be more passive than originally perceived?

Acknowledgements

Funding:

NSF-BES

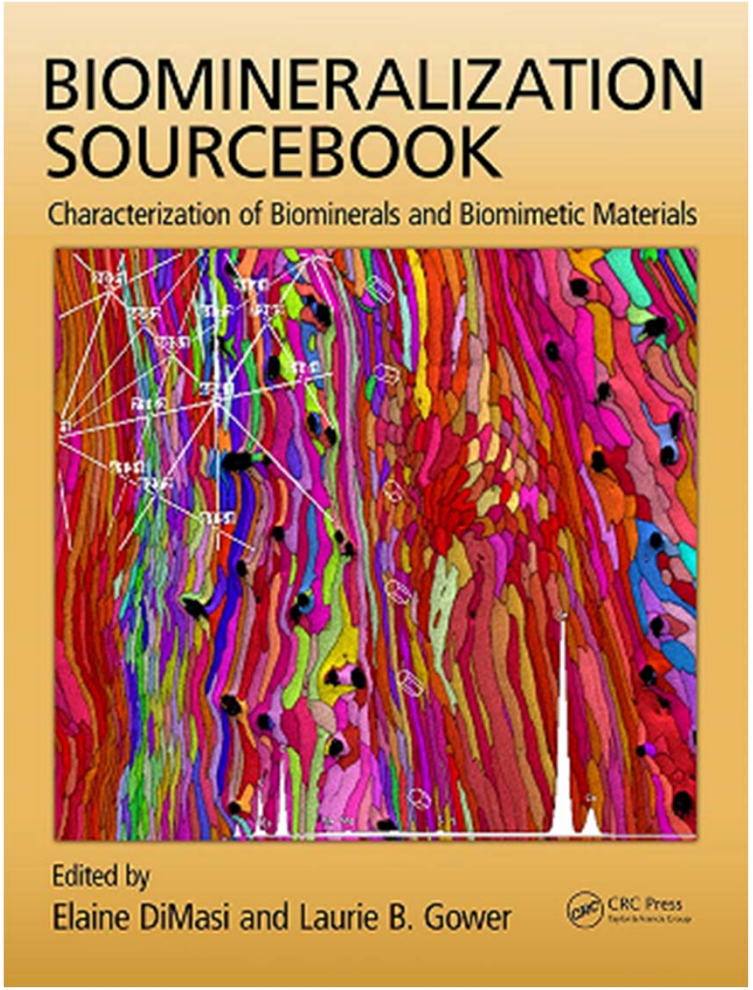
DMR-BMAT (NIRT)

Graduate students:

- Matt Olszta
- Sang Soo Jee

Post-Docs:

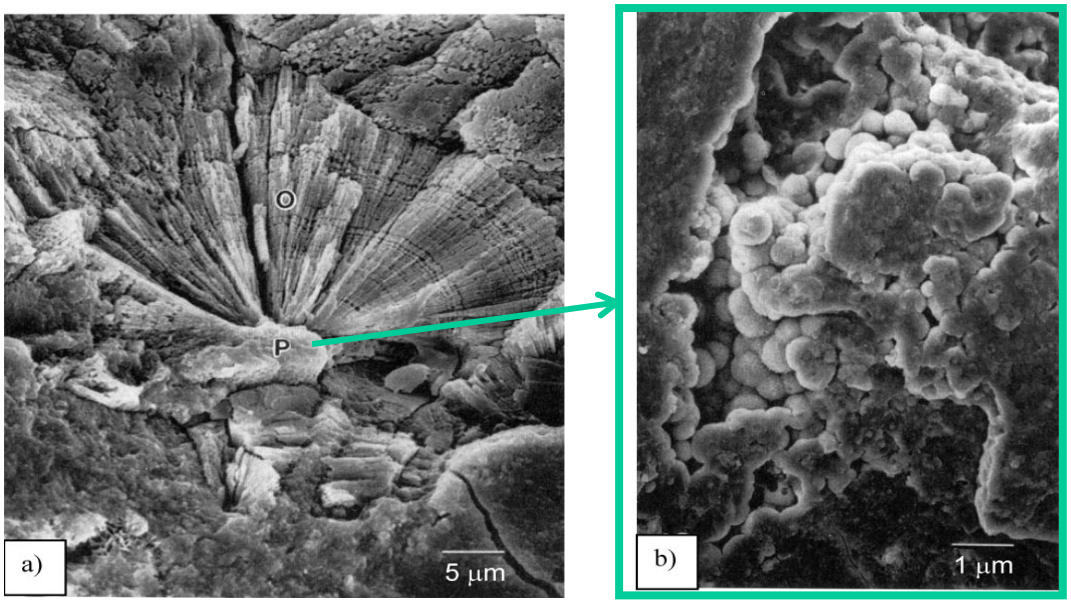
- Yi-Yeoun Kim
- Taili Thula
- Douglas Rodriguez



Hardback: 424 pages
ISBN 9781466518353

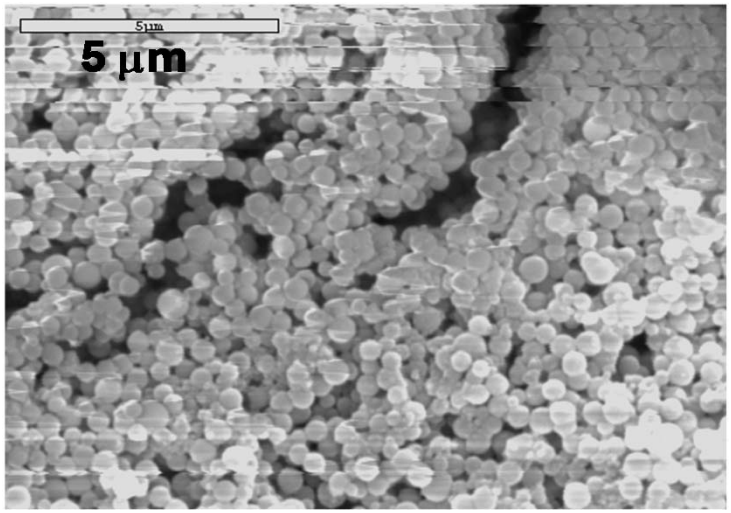
http://www.crcpress.com/product/isbn/9781466518353

Composite Kidney Stones: CaOx with CaP Nidus

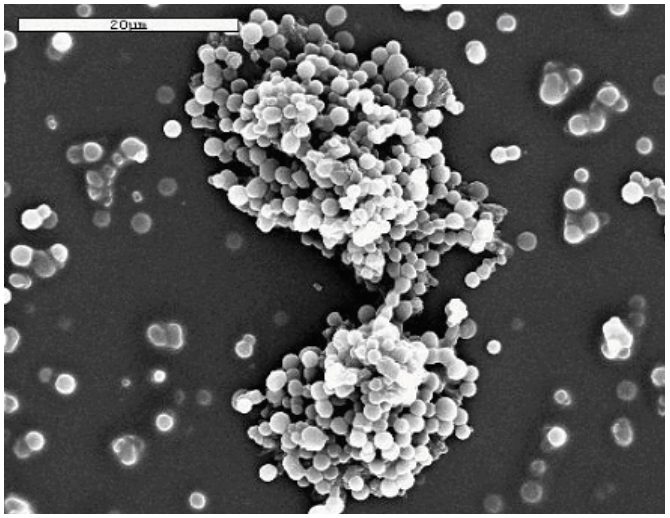


Zoom in on CaP core

Khan et al. (1997) J. Urology, 157, 376.

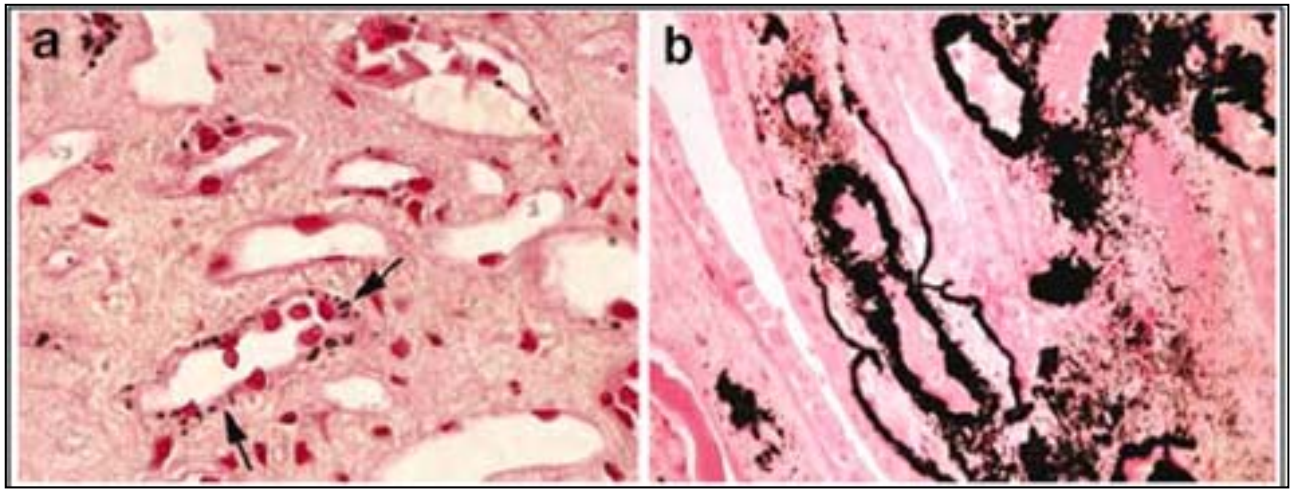


ACP globules from pAsp

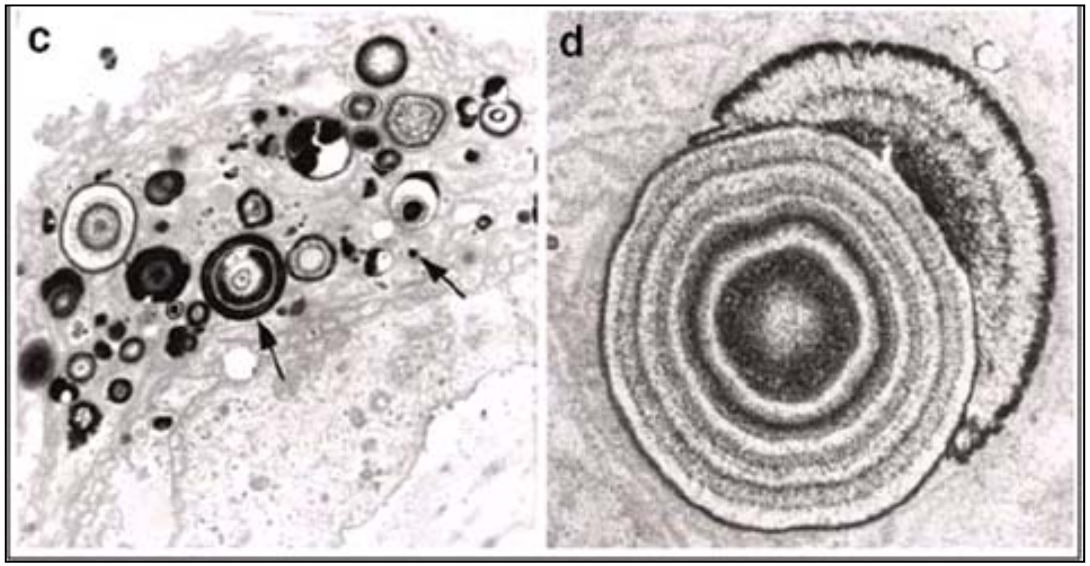


CaOx globules from pAsp

Randall's Plaque: 1st Stage of Idiopathic Stone Formation



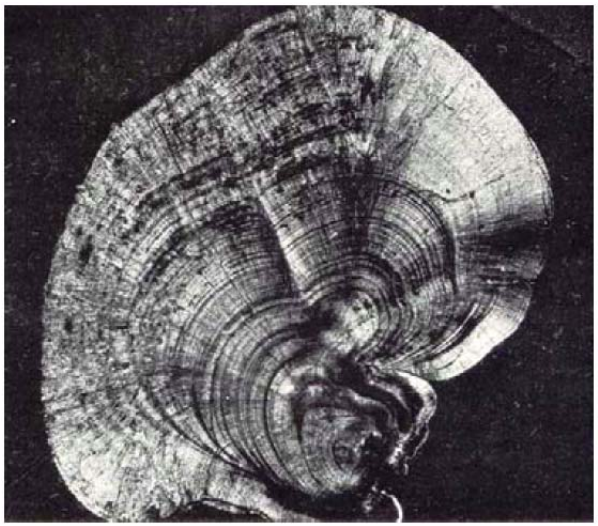
CaP deposits in papillary tissue seem to initiate in the basement membrane of the thin loops of Henle. Mineral then infiltrates neighboring interstitial tissue, extending to the urothelial lining. (Mag. x 900)



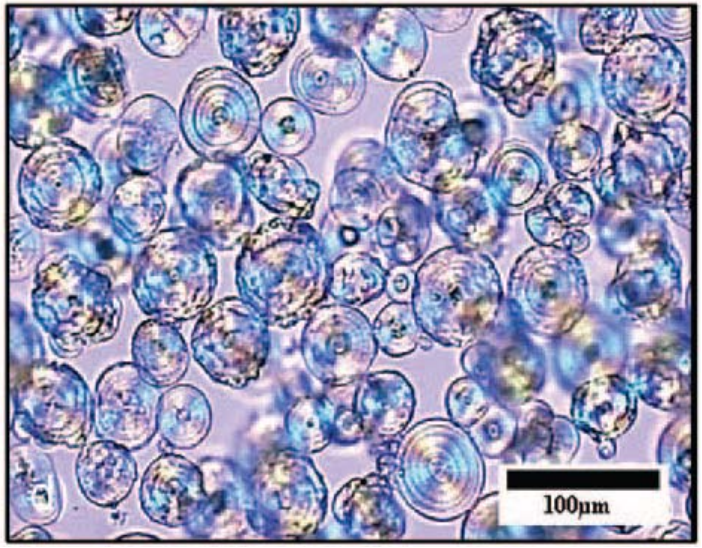
TEM shows spherules of CaP with concentric laminations of electron dense material. Organic material is enriched with OPN. (Mag x 25K and 70K)

Evan et al., Kidney Intl. 69: 1313, 2006.

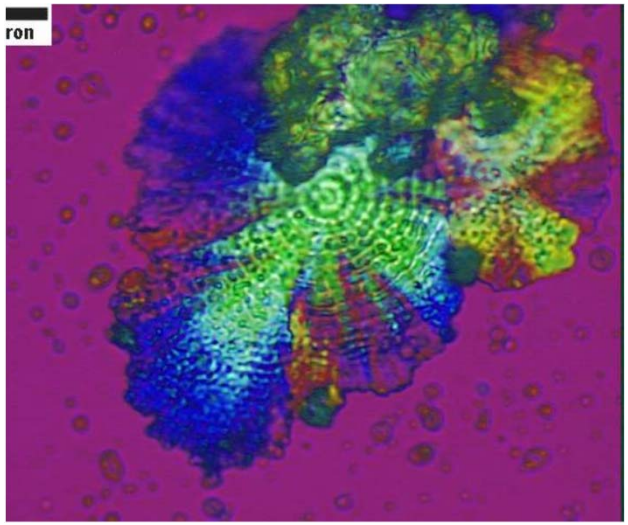
Mineral Spherulites and Concentric Laminations

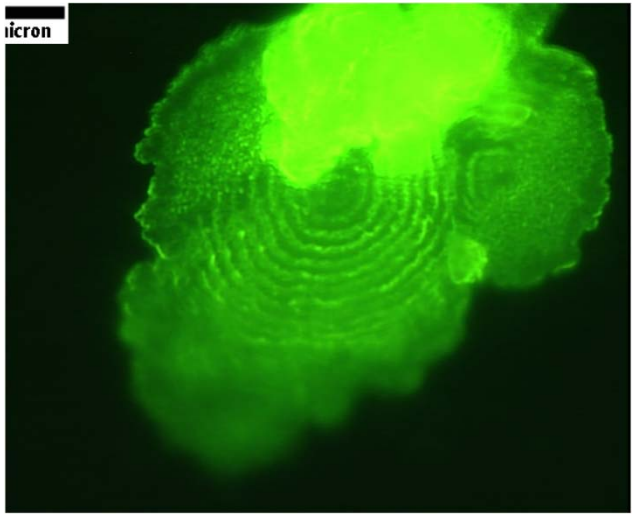


Pyrah, L. N. in *Renal Calculus*, Springer-Verlag, New York (1979)



CaP globules w concentric laminations





Concentric laminations in spherulitic $CaCO_3$ films via PILP