Lecture 11: Molecular Design and Synthesis of Biomaterials III: Inorganic Biomaterials

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Last time:	hydrogel applications: molecular imprinting and responsive drug delivery
Today:	biomineralization and biomimectic inorganic/organic composites Inorganic biomaterials
Reading:	L.A. Estroff and A.D. Hamilton, 'At the interface of organic and inorganic chemistry: bioinspired synthesis of composite materials,' *Chem. Mater*. **13**, 3227-3235 (2001) Stephen Mann, 'Biomineralization: Principles and Concepts in Bioinorganic Materials Chemistry,' Ch. 5 pp. 68-88, Oxford Univ. Press (2001)

- 1 million orthopaedic surgeries involving bone grafting materials each year in US (R. Langer et al. Tissue Eng., 1, 151 (1995))
    - autografting is best but limited in the size of defects that can be corrected (J. South Orthop ssoc. 9, 91 (2000); Aust N Z J Surg. 69, 726 (1999))
    - allografting presents possibility of disease transmission (HIV, hep B) (S. Mendenhall; Commetnary: the bone graft market in the United States, in Bone engineering J.e. Davis, ed., em squared incorporated 2000, Torotonto, Canada p. 585-590)
    - synthetic solid hydroxyapatite resorbs very slowly (Clin Orthop. 157, 259 (1981))

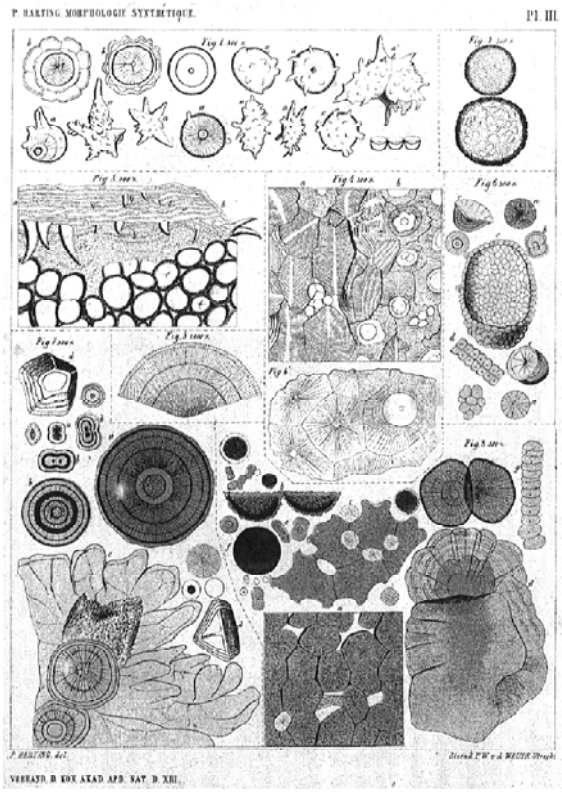
Bone Tissue Engineering:
JBRM 29, 359 (1995) Ca phosphate
JBMR 36, 17 (1997) PLGA
Biomaterials 19, 1405 (1998) PLGA
J Biomat Sci-polym ed, 7, 661 (1996) PLGA-Ca phos composite
Test

Biomineralization and biomimetic inorganic crystals[1]

Structure and synthesis mechanisms of biomimetic inorganic crystals[2-4]

- motivation for studying biomineralization
    - natural bone composite organization, with organic molecules (peptides and proteins) guiding inorganic crystal growth, allows shapes that defy the classical 230 space groups of crystalline materials
    - biomineralization processes vs. laboratory methods
        - **Biological methods -** benign synthesis methods
            1. Precisely control upon morphologies and structures over several length scales
            2. Occur at near neutral PH, ambient temperature and pressure
            3. Customize and optimize properties of materials according to the environment
        - **Laboratory methods:**
            1. Rely on extreme pH conditions form specific morphologies or patterned structures
            2. high temperature and/or high pressure synthesis
            3. Simple structure
    -
    - up to 3000X greater strength than pure inorganic crystal

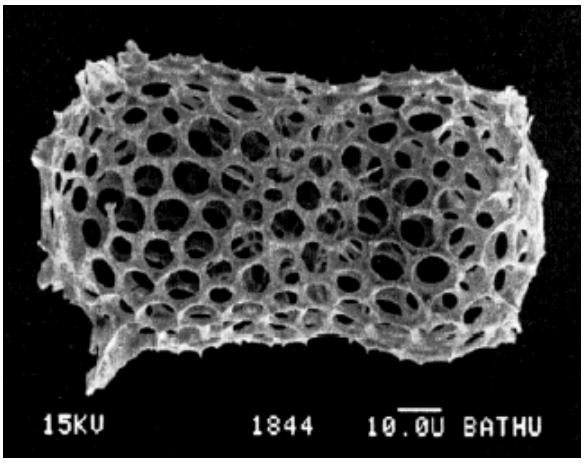
- **Applications**:
  - biomaterials for bioengineering
    - replication of trabelcular bone structure and mechanical properties is still elusive
    - low-cost, reproducible high-volume regenerative materials needed
    - biomimetic structures are readily resorbed, promote vascularization and cellular differentiation
  - biomaterials for other applications



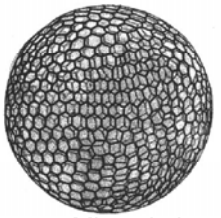
Pieter Harting's original hand drawings of calcareous microstructures (1872)

- The complex morphology and microstructure of biological inorganic materials has long been appreciated
  - E.g. Harting's hand drawings from 1872
  - natural organic/inorganic composites are used by nature as endo- and exo-sekeltons for their strong mechanical properties

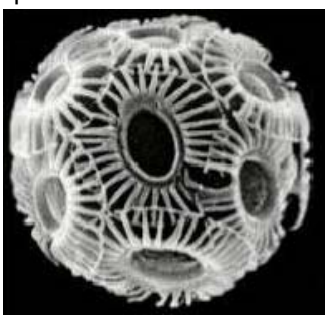
- unicellular organisms such as radiolarians and diatoms



Radiolarian: Microskeleton of amorphous silica



A. hexagona: Microskeleton of amorphous silica



Coccolith: $CaCO_3$ microskeleton

- central tenet of biomineralization:
    - organic molecules regulate nucleation, growth, morphology, and assembly of inorganic crystals (Mann, 1993)
    - molecular recognition at organic-inorganic interfaces
- two mechanisms of templating complex natural crystals:
    1. interfacial crystal growth
        - crystal nucleation at organized boundaries (Acc. Chem. Res. 30, 17 (!997); J. Mater. Chem. 7, 689 (1997))
        - utilized by unicellular organisms such as radiolarians and diatoms, where lipid vesicles compartmentalize and control solution chemistry
        - kinetically controlled crystal growth
    2. epitaxial crystal growth
        - from template proteins
        - equilibrium crystal growth dictated by template

interfacial crystal growth[5]

- Utilization of two-phase systems for directing location of mineral crystallization
- three types used by nature and one (possibly) novel approach investigated by biomimetic chemists:
    1. Vesicular biomineralization
    2. Microemulsion biomineralization
    3. micellar biomineralization
    4. Dendrimer biomineralization (novel?)

Vesicular biomineralization[6]

Biological vesicular mineralization

- Use of phospholipids structures to compartmentalize inorganic deposition
- Micrometer-sized droplets of supersaturated inorganic ions stabilized in oil by surfactant
- Nucleation and growth of inorganic phase occurs at surfactant headgroups, grows into microdroplet
  - Nonspecific (non-epitaxial) growth- headgroups don't match perfectly to crystal structure
- Lipid mesophases provide mulitiple organized micro- and nano-structures for crystal deposition

- ***Characteristics of biological vesicular mineralization:***
  1. Construction of enclosed, organized reaction environment
     a. Often using lipid bilayer vesicles
     b. Mineralization can occur inside or outside a boundary layer
  2. Control of physicochemical conditions inside reaction environment via transmembrane ion channels, transporters, and selective permeability
  3. Control of nucleation kinetics
  4. Production of complex crystal shapes by varying lipid matrix during growth

- ***Most common minerals produced by this method in biology:***
  - Silica (SiO_2) (amorphous) from $Si(OH)_4$ silicic acid – algae and bacteria
  - Calcium carbonate ($CaCO_3$) from $CaHCO_3$ – algae and bacteria
  - Hydroxyapatite (calcium phosphate, $Ca_5(OH)(PO_4)_3$) from Ca^{++} and PO4- - human bone

- ***Vesicle reactors used in biology: phospholipids bilayers***
  - Lipids have 2 hydrocarbon tails, so they can't pack into spherical micelles (molecules must have a shape complementary to this organization- wedge-shaped with 'big heads' and 'small tails'
  - Form ubiquitous bilayer structure instead

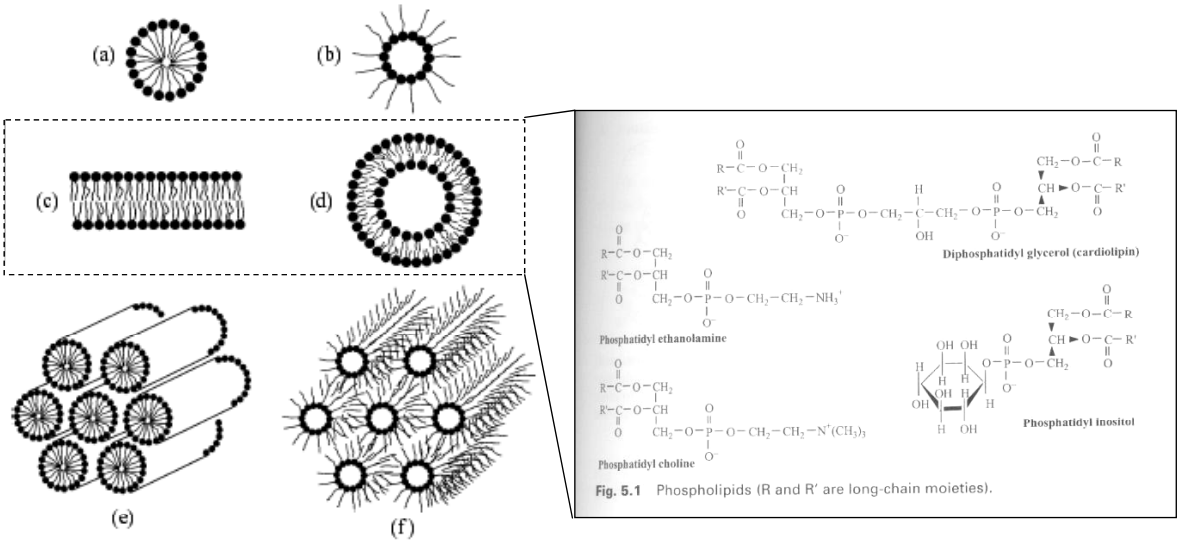


Figure 1
Schematic illustration of mesophases formed by lipid self-assembly: (a) micelle, (b) reverse micelle, (c) lamellar bilayer, (d) bilayer vesicle, (e) hexagonal, (f) inverse hexagonal.

- ***Control of crystal growth:***[7]
  - Intracellular mineral deposition is controlled using microtubules and scaffolding proteins that allow the cell to pull on the vesicle, changing its shape and orientation during inorganic growth
  - Chemical deposition can also be controlled by nm-scale variation in spatial distribution or reactants
    - E.g. sequentially-activated ion transporters

Control of vesicle morphology:

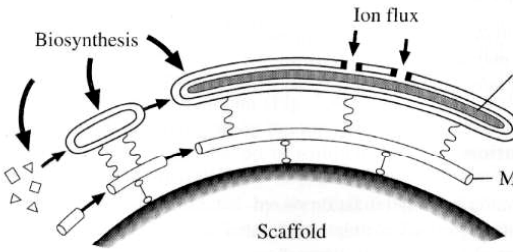
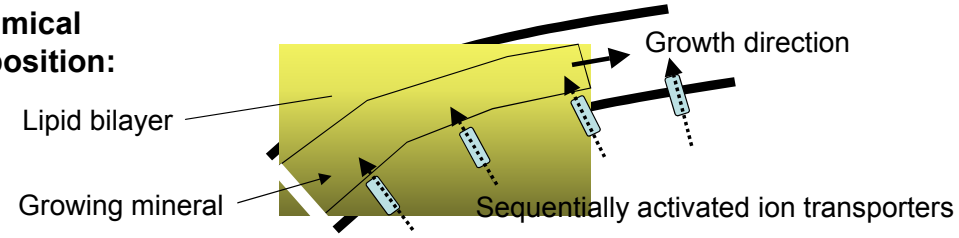


Fig. 7.6 Pattern formation in intracellular biomineralization. B, biomineral; V, vesicle; MT, microtubule.

Spatial control of chemical deposition:



(Mann, 2001)

- ***Example: coccolith skeleton***
  - Coccolithophorids: major group of calcifying algae[8]
  - Production of lipid vesicles to confine calcite formation
  - Influx of calcium and carbonate ions
  - oriented nucleation of calcite crystals within enclosed vesicles
  - Control of crystal shape by active change in vesicle shape by cell's cytoskeleton

- ***Example: radiolarian skeleton***[7]
  - Silica-skeleton algae

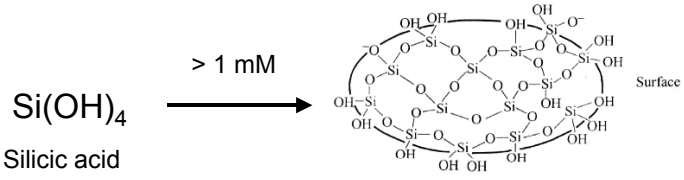


Fig. 2.18 The chemical nature of amorphous silica.

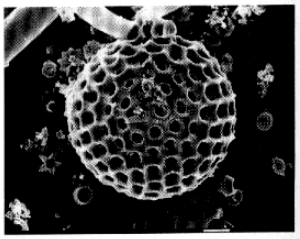
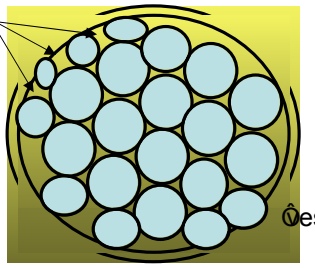


Fig. 7.14 Radiolarian silica micro-skeleton. Scale bar, 10 μm.

Mineralization nucleated at *exterior* surface of vesicles



Õesicle foamÕ

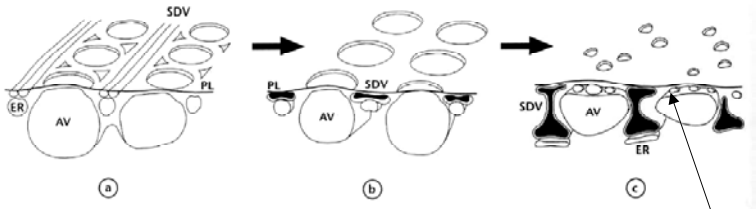


Fig. 7.15 Stages in the morphogenesis of the diatom frustule. See text for details.

nanovesicles

SDV - silica deposition vesicles
AV - aleolar vesicles
PL - plasmalemma (lipid bilayer cell wall)
ER - endoplasmic reticulum

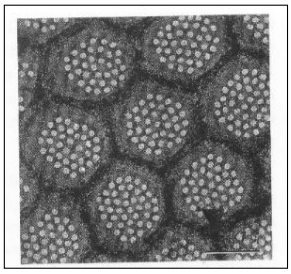
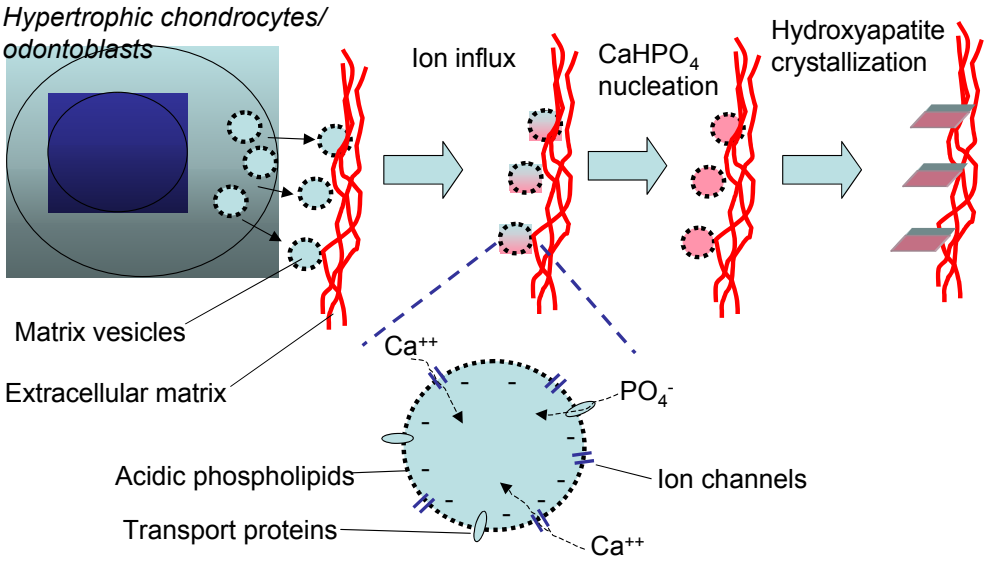


Fig. 7.16 Patterning of small holes, 40 nm across, within the hexagonal framework of a diatom frustule. Scale bar, 400 nm.

- ***Example: human bone formation***
  - Growth plate cartilage, tooth dentine (Clin. Orth. Re. R 314, 266 (1995))

1. Matrix vesicles 100-200 nm diameter secreted, attach to matrix
2. Calcium and phosphate ions influx into vesicle nanoreactors
3. Calcium phophate supersaturates
4. Controlled precipitation in amorphous structure
5. Crystallizes into hydroxyapatite
6. Growing crystals burst vesicles
7. Growth continues, controlled by extracellular conditions



synthetic (biomimetic) vesicular mineralization

Vesicular mineralization

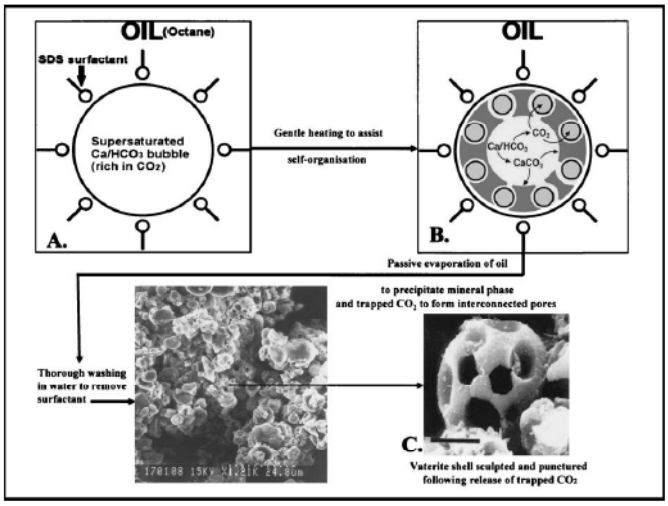
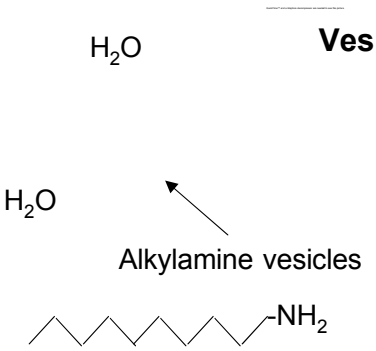


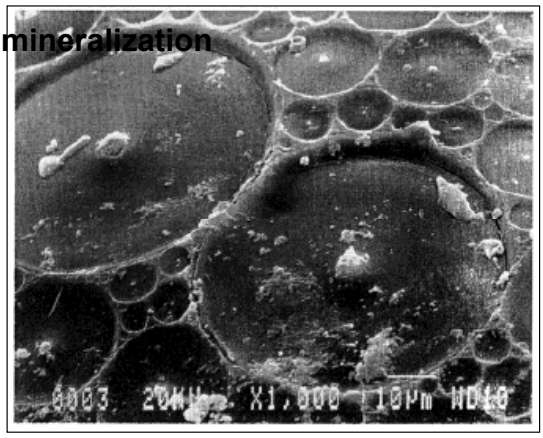
Figure 2. Porous vaterite microspheres generated by self-organizing media. (A)–(C) Schematic mechanism for the formation of vaterite microspheres. Droplets of surfactant contain a solution of calcium bicarbonate ($CaHCO_3$) enriched in carbon dioxide (A). The droplets become supersaturated when the carbon dioxide slowly escapes from the solution. Bubbles of carbon dioxide become trapped at the oil-water interface (B), which leaves an imprint as the emulsion is air dried (C).

(Green et al. 2002)

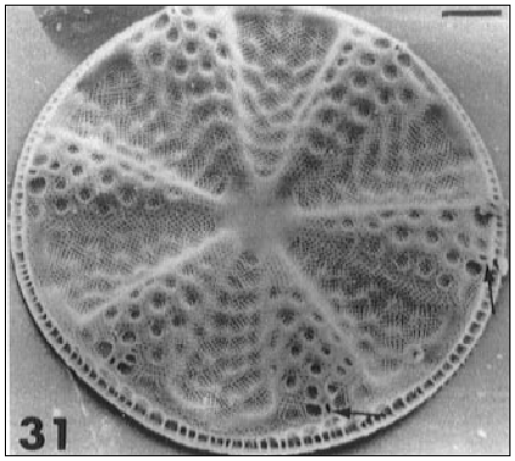
- chemistry of $CaCO_3$ deposition in vesicles:
  - $Ca^{++}(aq) + 2HCO_3^-(aq) \rightarrow CaCO_3(s) + CO_2(aq) + H_2O$
  - $$K_{eq} = \frac{[H_2O][CO_{2(aq)}][CaCO_{3(s)}]}{[Ca^{++}_{(aq)}][HCO_{3(aq)}]^2} = constant$$
  - e.g. algae: remove dissolved CO_2 to induce more $CaCO_3$ precipitation
  - supersaturation highest near bubbles of CO_2, nucleation preferentially at surface of vesicle and grows inward



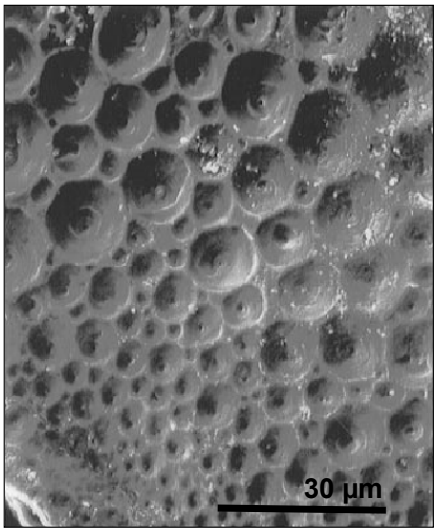
Vesicular mineralization



- Synthetic vesicular growth does not yet exhibit the control seen in nature:
  - Lack control over ion introduction into local spaces, ability to manipulate vesicle shape on the scale performed by microtubules and other cytoskeletal elements



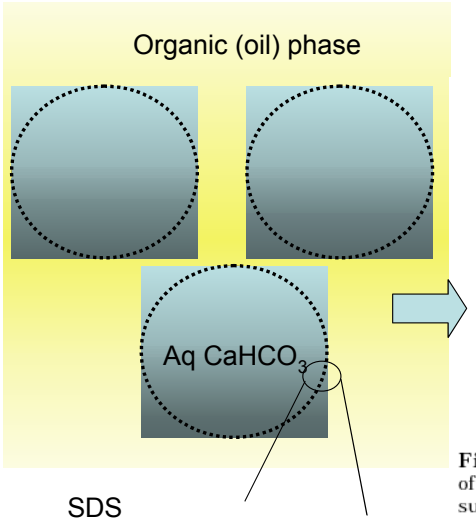
Disk-shaped diatom



Vesicular growth diatom facsimile

Microemulsion biomineralization

- Aqueous solutions of biomineral emulsified by an organic surfactant in an organic phase
- Mineralization occurs from surface of droplet stabilizer and grows inward into aqueous droplet



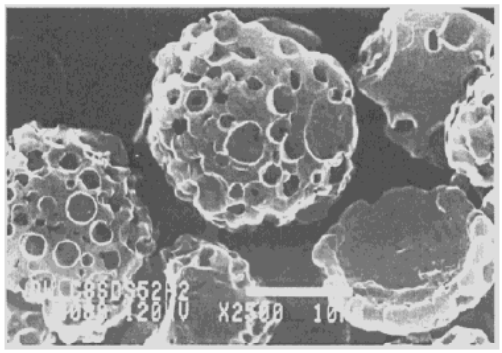


Figure 3. Vaterite spheroids formed from a microemulsion of octane:SDS:$CaHCO_3$ (71:4:25 wt %). Note the complex surface patterning and uniform size of the spheres. Scale bar = 10 μm. (Reproduced by permission from ref 14.)

Microemulsion mineralization

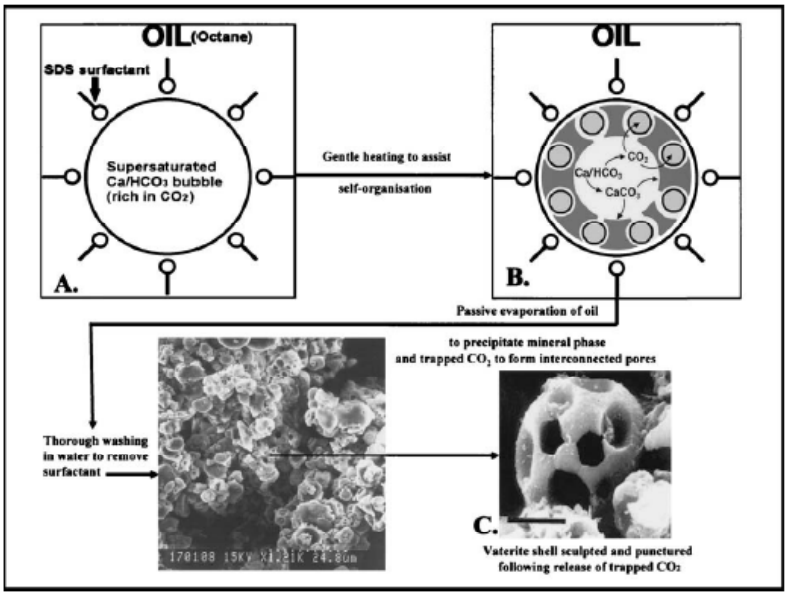


Figure 2. Porous vaterite microspheres generated by self-organizing media. (A)–(C) Schematic mechanism for the formation of vaterite microspheres. Droplets of surfactant contain a solution of calcium bicarbonate ($CaHCO_3$) enriched in carbon dioxide (A). The droplets become supersaturated when the carbon dioxide slowly escapes from the solution. Bubbles of carbon dioxide become trapped at the oil-water interface (B), which leaves an imprint as the emulsion is air dried (C).

(Green et al. 2002)

- Model of process:[6]

1. Dissolved CO_2 in the supersaturated solution separates into bubbles that are trapped at the oil-water interface
2. Loss of CO_2 in solution increases the concentration of carbonate ions and thus the supersaturation
3. local supersaturation is highest next to CO_2 bubbles, so first calcium carbonate precipitation occurs here
    - Bubbles trapped at oil/water interface dictate 'foamed' surface morphology of inorganic structure
        - Vaterite formed in this process dictated by Ostwald's rule of stages:
            - Polymorph with highest solubility preferentially forms under kinetically-controlled conditions

Bicontinuous microemulsions: interconnected microemulsion templates for mineralization:

- Formed and then oil phase frozen to limit dynamic changes in liquid crystal nanochannel network during inorganic calcium phosphate crystallization
- Nanochannels formed with supersaturated solution; allowed to crystallize in surfactant-encapsulated water channels.
- Final structure composed of fibers with dimensions much larger than original nanochannels:
  - Growing calcium phosphate expands surfactant structure with time

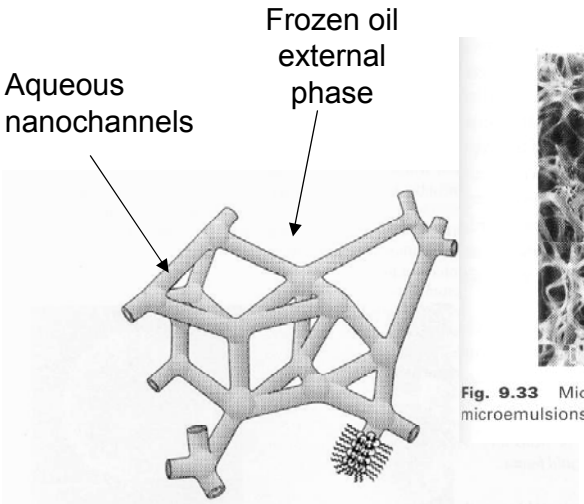


Fig. 9.32 Idealized structure of a bicontinuous microemulsion. For clarity, only the nanoscopic water channels are shown along with surfactant molecules. The oil channels are of similar dimension and interpenetrate the water conduits.

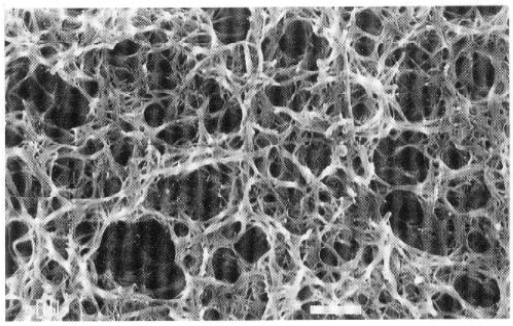
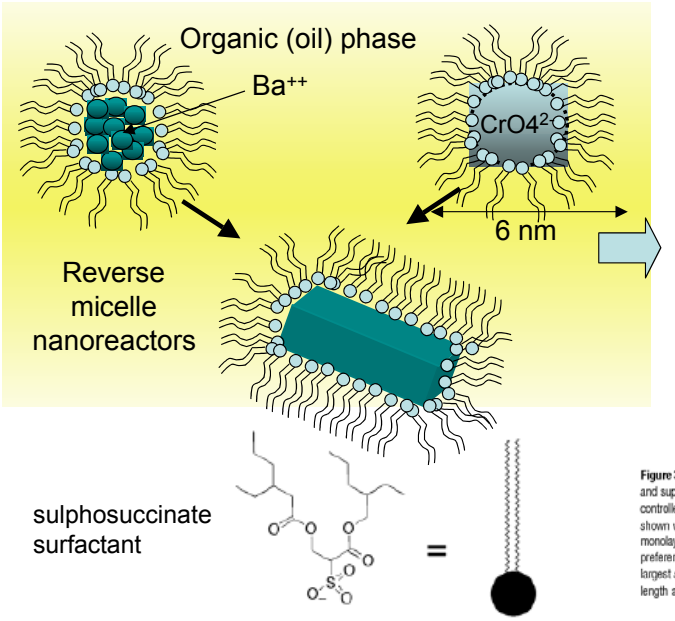


Fig. 9.33 Micro-skeletal form of calcium phosphate prepared in bicontinuous microemulsions. Scale bar, 1 μm.

Micellar biomineralization

- Growth of inorganic crystals from surface of micelles[9]
- Model for microstructure development in one case: $Ba^{++}CrO_4$—growth inside reverse micelles (water nanodroplets in oil phase)



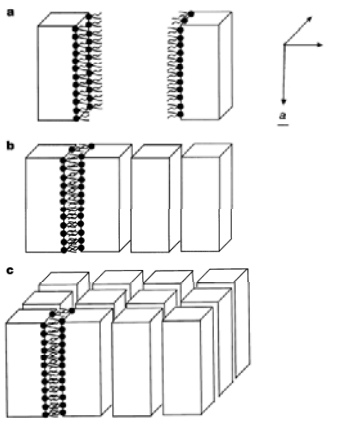


Figure 3 Proposed model for the surfactant-induced self-assembly of nanoparticle chains and superlattices. **a**, Surfactant-coated prismatic $BaCrO_4$ nanoparticles synthesized by controlled crystallization in microemulsion water droplets. For clarity, only one face is shown with associated surfactant molecules. **b**, Interdigitation of the surfactant monolayers induced as the crystal faces develop in shape and size, resulting in preferential aggregation normal to both the prism long axis (crystallographic *a* axis) and largest side face. **c**, Aggregation in two dimensions proceeds as the chains develop in length and number.

Figure 1 TEM image showing ordered chains of prismatic $BaCrO_4$ nanoparticles. The nanoparticles were prepared in AOT microemulsions at $[Ba^{2+}] : [CrO_4^{2-}]$ molar ratio ≈ 1 and $w = 10$. Scale bar = 50 nm.

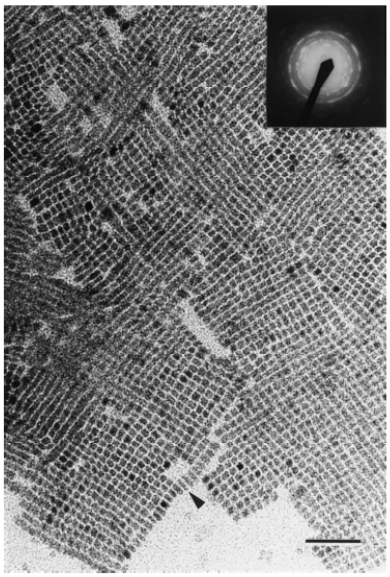


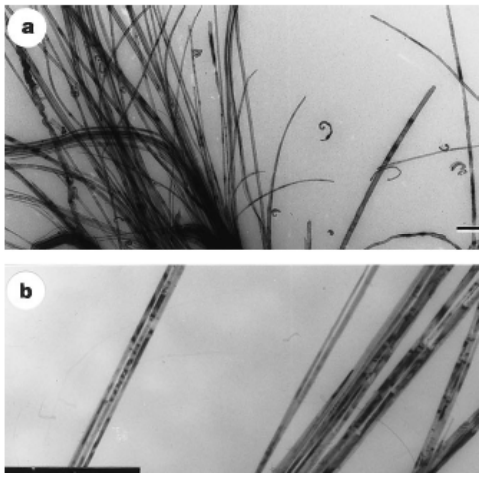
Figure 2 Rectangular superlattice of $BaCrO_4$ nanoparticles. These were formed by two-dimensional aggregation of nanoparticle chains prepared in AOT microemulsions at $[Ba^{2+}]:[CrO_4^{2-}]$ molar ratio ≈ 1 and $w = 10$. Arrow shows dislodged particles revealing the prismatic morphology of individual crystallites. Scale bar = 50 nm. Inset, the electron diffraction pattern gives the superimposition of reflections from zone axes approximately parallel to the [100] direction.

(M. Li et al. 1999)

- Changing the ratio of cations to anions in the crystal alters its interaction with the organic surfactant and thus the assembly/growth properties in the microemulsion
  - With excess barium, cationic faces form on the crystal
    - Strong ionic binding between headgroups and crystal prevents controlled growth and precipitation of growing crystals leads to eventual nanofilament formation
  - With excess chromate, anionic faces form that don't bind surfactant
    - No longer assembled by surfactant-surfactant interactions, leading to spherical nanoparticles

5:1 Ba^{++}: CrO_4^{2-}
cationic faces

1:5 Ba^{++}: CrO_4^{2-}
anionic faces



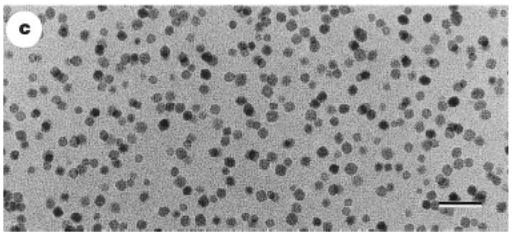
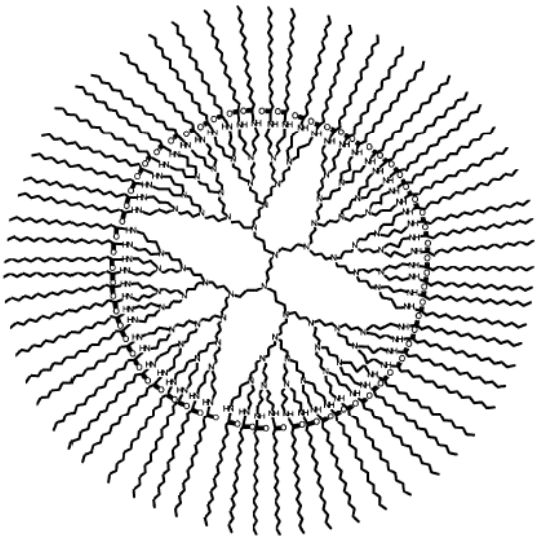
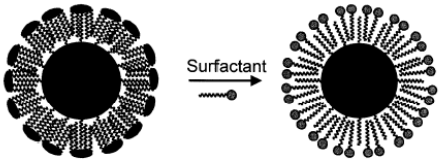


Figure 4 Characterization of $BaCrO_4$ nanostructures formed at different reactant ratios. **a**, TEM image of bundles of $BaCrO_4$ nanofilaments prepared in AOT microemulsions at $[Ba^{2+}] : [CrO_4^{2-}] \approx 5 : 1$ and $w = 10$; scale bar = 500 nm. **b**, Higher magnification image of single filaments elongated along the crystallographic *a* axis; scale bar = 200 nm. Inset, [0$\bar{2}$1] zone electron diffraction pattern recorded from an individual fibre. **c**, Spherical $BaCrO_4$ nanoparticles prepared at $[Ba^{2+}] : [CrO_4^{2-}] \approx 1 : 5$ and $w = 10$; scale bar = 50 nm.

- Dendrimer biomineralization[10]
  - Approach:
    - Hydrophobic surfactant tails self-assembled on the surface of dendrimer particles

- Surface-modified dendrimers mixed with aqueous biomineral solutions to drive crystallization





Scheme 1 Schematic representation of the modification of the outer layer of dendrimer-based aggregates using single chain surfactants.

  - After 4 days, calcite rhombohedrons grown around amorphous calcium carbonate spheres were observed
    - Coexistence of these 2 phases previously only observed in biological systems
    - Significant role of organic component in both nucleating and stabilizing amorphous phase?

References

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