BE.342/442 Tuesday, October 25, 2005
Topic: Biomineralization: Bones and Teeth

Bones

Bones and teeth can be mineralized and fossilized. The insoluble calcium and bones and teeth is held together with proteins, the crystal structure of which is not entirely known.

Read "The Material Bone: Stucture-Mechanical Function Relations" by S. Weiner and H. D. Wagner, on the course website. This article discusses the structures found within bone proteins. The mineralized collagen fibrils of bone have highly complex structures described in terms of up to 7 hierarchical levels of organization. The materials have evolved to fulfill a variety of mechanical functions. The article discusses structure-mechanical relations at each of the hierarchical levels, and discusses gaps in present knowledge and potential for novel discoveries.

The Young's modulus of bone increases with calcium content – so calcium content is important for strength, but the structure is mediated by proteins in the extracellular matrix of bone.

The 7 hierarchical levels span from the components of collagen fibrils to the whole bone.

- Level 1: Components, including proline and hydroxyproline.
- Level 2: Mineralized collagen.
- Level 3: Arrays of fibers.
- Level 4: Fiber array patterns, e.g., spirals that may remind you of alpha-helical structures!
- Level 5: Osteons: cylindrical motifs in bone structure
- Level 6: Spongy vs. compact bone
- Level 7: Whole bone

Comparison of human, baboon, cow, and rat bones have been studied by means of SEM micrographs of lamellar fracture surfaces. These do not highlight the differences very clearly: clearly the hierarchical varies are quite complex and span a wide range of length scales.

A closer view of the scaffold in a bone reveals porous fibers. On the scale of a few hundred nanometers, bones can be observed and analyzed using x-ray diffraction. The diffraction rings at this resolution can reveal the distance between collagen bundles, but little other information. Collagen bundles can organize into parallel groups, interwoven fiber structures, plywood-like alignment, and radial fibril arrays. The various organizations optimize the mechanical properties of the fibers for a particular application.

With new advances in x-ray of crystallography, estimates can be made of the alignment of fibers in bone samples.

The article proposes a model of collagen packing in which fibers arrange holes in their structure to form channels. Collagen bundles with oval-shaped cross-section may or may not orient in a crystalline structure (aligning the directions of the ovals). The fibril axis, too, may or may not align: just as wood has fibers with different alignments to provide strength in different directions,

collagen fibers can align to determine their strength. Measurement of Young's moduli of bone versus the angle of the measurement shows significant angle-dependence.

Teeth

Source: Steve Weiner, et al. "Peritubular Dentin Formation: Crystal Organization and the Macromolecular Constituents in Human Teeth." *Journal of Structural Biology* **126**, 27-41 (1999).

Dentinogenesis involves a stacked structure containing: a cell-rich zone, a cell-free zone, and an osteoblast layer. The odontoblasts manufacture predentin, which mineralizes into teritubules of dentin. The *odontoblast process* fills the tubules of predentin produced by odontoblasts, and has relatively little structure inside. The fibril then sprouts predentin, which branches like a tree and becomes mineralized to form dentin.

The fiber orientation in dentin can be observed through SEM images of fracture surfaces. The odontoblast processes that migrate into the dentin region leave behind non-mineralized columns, surrounded by treelike structures of branching fibers. Staining of mature tooth proteins revels protein content and fiber alignment. Stains can distinguish manel dentin, globular dentin, and circumpulpal dentin. A TEM taken through a tubule cavity shoes plate-shaped crystals lining the cavity. X-ray diffraction patterns of different bone samples roughly suggest the organization of the fibrils, although these patterns have thus far been very low-resolution.

The amino acid composition of peritubular dentin shows high quantities of glysine and serine (about each of150-200 glysine and serine residues per 1000 amino acid residues), as well as elevated quantities of threonine, phenylalanine, and gutamic acid. The article provides the precise amino acid content. It also provides the protein structure for dentin matix acidic phosphoprotein, a positively-charged protein that carries phosphates and other charged ions.

Nature's biomineralization scaffolds and their locations:

Taxon	**Material**	**Anatomical location**	**Multi-purpose**	**Tailor-made**
Mollusca	Nacre	Inner shell layer		X
	Prismatic (single xtal prisms)	Outer shell layer		X
	Foliated			X
	Crossed lamellar		X	
Vertebrata	Parallel-fibered bone	Usually a component of fast-growing long bones		X
	Woven bone	Fetal bones, wound repair		X
	Mineralized tendons	Mainly legs of large bones		X
	Dentin	Inner layer of teeth		X
	Lamellar bone	Widely distributed w/i phylum	X	
Echinodermata	Stereom	Tests, spines, ossicles	X	
Plantae, Protoctistae, Animalia	Silica	Cell walls, cell infillings, extracellular skeletons	X	

This class is intended to stimulate your thinking about design of organic and inorganic scaffolds. For more information about solving structural problems in biomineralization, read: Steve Weiner, Lia Addai, and H. Daniel Wagner, "Materials design in biology." *Materials Science and Engineering C* **11** (2000) 1-8.

We learn from nature than negatively charged scaffolds can attract positively-charged ions to facilitate biomineralization. Prof. Zhang's research attempted the placement of positively-charged residues at regular locations along peptide sequences. The peptides contained positively-charged Y, L, P, or F residues with spacer sequences of D. Varying the number of D spacers between Y, L, P, or F residues charges the rates of mineralization. Incorporation of negatively-charged groups or alternating sequences of positively- or negatively-charged groups also may facilitate biomineralization. This research project was never completed (due to lack of funding) – perhaps one of you will find the answer!

Prof. Sam Stupp at Northwestern University found a potential solution to the problem of disease transmission from scaffolds made of animal protein (e.g., collagen).

> Read: Jeffrey D. Hartgerink, Ella Beniash, and Samuel I. Stupp, "Self-Assembly and Mineralization of Peptide-Amphiphile Nanofibers," *Science* **294** (23 November 2001) 1684.

Prof. Stupp's group designed short peptides with a C-terminus containing the ubiquitous cell adhesion motif RGD. At the N-terminus, the peptides terminate in hydrophobic hydrocarbon tails that cause them to self-assemble into fibers. These synthetic fibers deliver a molecule such as RGD to the scaffold surface, without the potential problem of disease transmission from natural

scaffolds. The mechanical properties of the self-assembled fiber hydrogel can be made sensitive to pH, oxidation, and point mutations in the sequence. In the solid gel form, the structure is fibrous, like that of collagen. Mineralization on these scaffolds leads to formation of hydroxyapatite plates similar to the crystals found in bone.
However, the price of this material was recently recorded at: $10,000 for 5 mg!

Another interesting example in proteins for biomineralization:
Nature **425** (30 Oct. 2003) 977-980.
This article describes pOC, a protein that orients and binds hydroxyapatite.

Look out for research on this topic here at MIT, and around the world! (E.g., Prof. Zhang's colleagues in the UK working ot designer peptide scaffolds for bone cells.)