HIERARCHY OF BONE STRUCTURE

REPORT

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Complex Polymer Morphology

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INTRODUCTION

Bones, as we all know, are hard connective tissues which constitute the major component of almost all skeletal systems in adult vertebrate animals. Bone appears to be nonliving—in fact, the word skeleton is derived from a Greek word meaning dried up. However, bone actually is a dynamic structure composed of both living tissues, such as bone cells, fat cells, and blood vessels, and nonliving materials, including water and minerals.

Bones are multipurpose structures that play diverse, vital roles in vertebrates. They provide a framework for the body, supporting it and giving it shape. They also provide a surface for the attachment of muscles and act as levers, permitting many complex movements. Many bones protect softer internal organs; for example, skull bones protect the brain, and rib bones form a cage around the lungs and heart. In addition to these structural and mechanical functions, bones also participate in the body's physiology. They store calcium, a mineral essential for the activity of nerve and muscle cells. The soft core of bone, the bone marrow, is the site of formation of red blood cells, certain white blood cells, and blood platelets.

An adult human has 206 bones, which account for 14 percent of the body's total weight. The longest and strongest bone is the thighbone, which at maturity is about 50 cm (20 in) long and 2.5 cm (1 in) wide. The smallest bone, the stirrup bone, is one of three tiny bones buried within the middle ear; it is only 0.18 cm (0.07 in) long.

Bone is a relatively hard and lightweight composite material, formed mostly of calcium phosphate in the chemical arrangement termed calcium hydroxyapatite. It has relatively high compressive strength but poor tensile strength. While bone is essentially brittle, it does have a degree of significant elasticity contributed by its organic components (chiefly collagen). Bone has an internal mesh-like structure, the density of which may vary at different points.

STUDY OF BONE STRUCTURE

Bone has a varied arrangement of material structures at many length scales which work in concert to perform diverse mechanical, biological and chemical functions; such as structural support, protection and storage of healing cells, and mineral ion homeostasis. Scale is of importance in discussing bone architecture as the structure is hierarchical and complex. Every technique of assessing bone architecture or the properties of a given structure has its own resolution, and therefore a combination of techniques is required to reveal the material structures and properties at the many different length scales. For example, electron microscopy examines bone ultrastructure at the nanometer level, Fourier transform infrared spectroscopy (FT-IR) and x-rays measure components at the Ångstrom level, light microscopy details features at the level of a few microns, and conventional mechanical testing of small specimens measures the mechanical properties of bone at the hundreds of microns or more.

In order to understand the mechanical properties of bone material, it is important to understand the mechanical properties of its component phases, and the structural relationship between them at the various levels of hierarchical structural organization. [**1-3**]

The seven levels of hierarchy in bone are

- Level 1: The Major Components
- Level 2: The Mineralized Collagen Fibril
- Level 3: Fibril Array
- Level 4: Fibril Array Patterns
- Level 5: Cylindrical Motifs: Osteons
- Level 6: Spongy vs. Compact Bone Structure
- Level 7: Whole Bone

This list of seven levels can also be shortened to 5 levels of hierarchy

A. The subnanostructure (below a few hundred nanometers): molecular structure of constituent elements, such as mineral, collagen, and non-collagenous organic proteins.
B. The nanostructure (from a few hundred nanometers to 1 μm): fibrillar collagen and embedded mineral.
C. The sub-microstructure (1–10 μm): lamellae.
D. The microstructure (from 10 to 500 μm): Haversian systems, osteons, single trabeculae.
E. The macrostructure: cancellous and cortical bone.

This hierarchically organized structure has an irregular, yet optimized, arrangement and orientation of the components, making the material of bone heterogeneous and anisotropic (Fig 1)

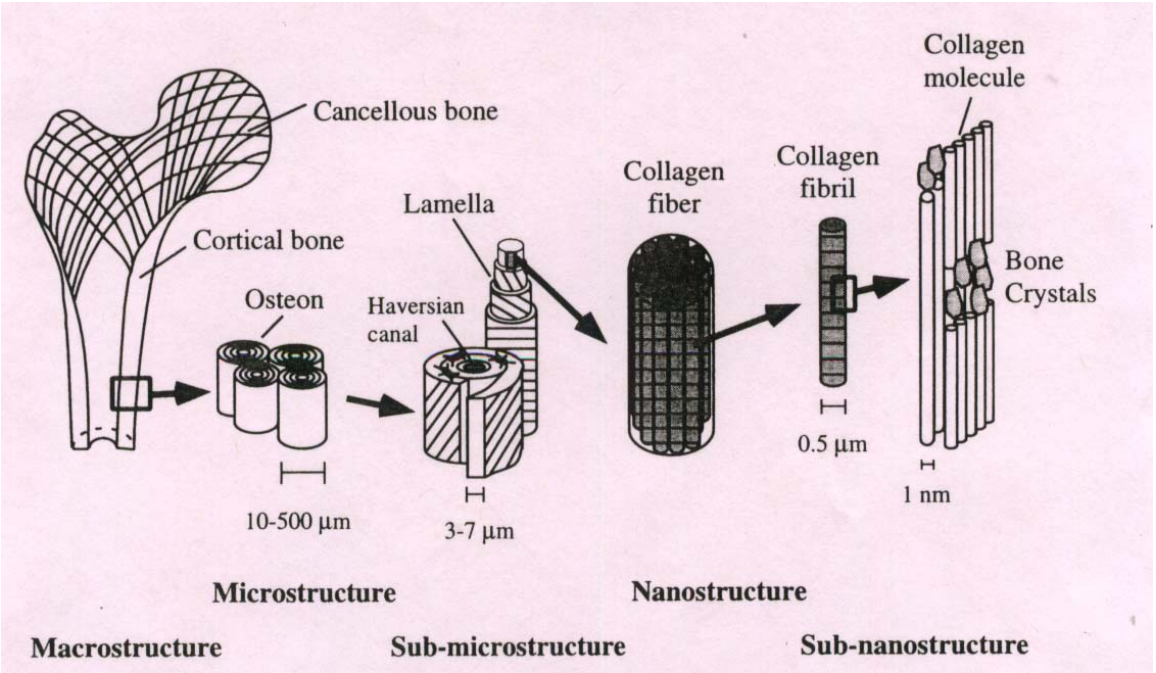


Fig 1 (Fig 1 in Ref 3)

This report discusses the above mentioned model of the levels of hierarchy in bones and gives an insight into the practical application of this model. I have tried to include the seven level hierarchy into the 5 level hierarchy, basically showing that they are more or less the same

A. MACROSTRUCTURE (LEVEL 7 & LEVEL 6)

At the macrostructure level, bone is separated into the cortical (or compact) and cancellous (or trabecular) types. In cross-section, the end of a long bone (*see Fig 2*) such as the femur has a dense cortical shell with a porous, cancellous interior. Flat bones such as the calvaria have a sandwich structure: dense cortical layers on the outer surfaces and a thin, reinforcing cancellous structure within. Although both types of bone (cortical and cancellous) are easily distinguished by their degree of porosity or density, true differentiation comes from histological evaluation of the tissue's microstructure. However, in compact coarse-cancellous bone [**4, 5**] the structure is fuzzy and it is difficult to distinguish between the two types of bone with any clarity. This bone is produced by cortical bone wrapping around the struts of cancellous bone, without replacement or remodeling of the old cancellous bone. The microstructure produced by the compaction of cancellous bone is composed of irregular, sinuous convolutions of lamellae. In contrast, the microstructure of cortical bone is composed of regular, cylindrically shaped lamellae. Therefore, reliable differentiation can only be achieved by microscopy methods. As it is clearly seen, the macrostructure of bone encompasses Level 7 and Level 6 of the seven level hierarchy.

Spongy/Cancellous Bone/Trabecular Bone	***Compact/Cortical Bone***
Highly porous	Much less porous
High concentration of blood vessels and cell-to-bone ratio	Few blood vessels and low cell-to-bone ratio
Low density	Fairly high density
Lower mechanical properties	Higher mechanical properties

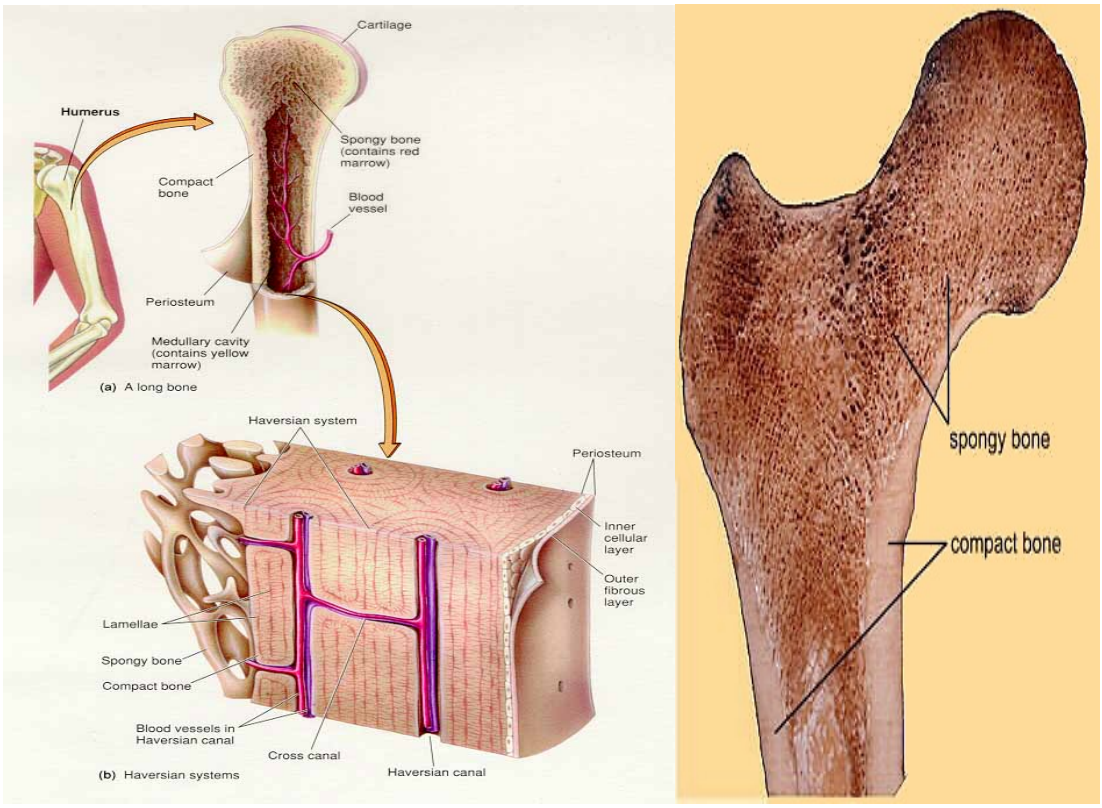
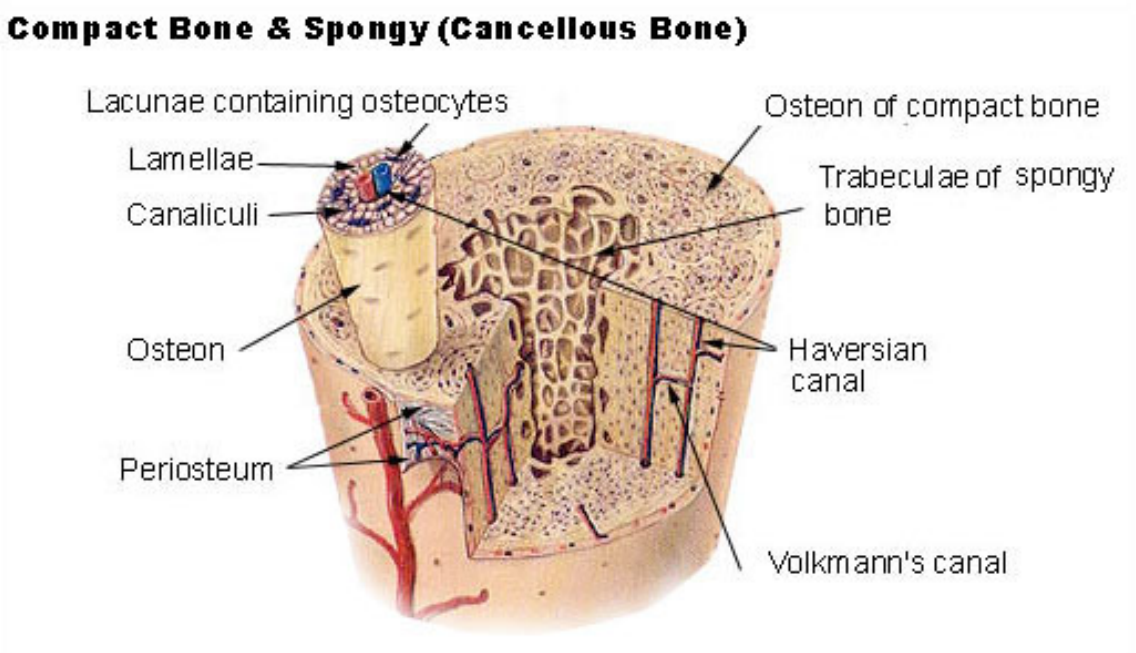


Fig 2: (Left)Anatomy of the long bone and Haversian systems. (Right) A human long bone showing the compact and spongy bone. (Below) Anatomy of Cancellous bone



B. MICROSTRUCTURE

Mineralized collagen fibers form into planar arrangements called lamellae (3–7 μm wide). In some cases these sheets (lamellae) of mineralized collagen fibers wrap in concentric layers (3–8 lamellae) around a central canal to form what is known as an *osteon* or a *Haversian* system (Fig 2, 3). The osteon looks like a cylinder about 200–250 μm in diameter running roughly parallel [**6**] to the long axis of the bone. Other forms of cortical bone where the mineralized collagen fibers are less well registered and no pattern can be distinguished are called *woven* bone. In some forms of bone, the lamellae are overall tangential to the outer surface of the bone (without forming osteons), and together with woven bone tissue, form a larger plywood-type stacking of thicker layers (150–300 μm) around the complete circumference of the bone in what is called *lamellar* bone. Cancellous bone (Fig 2) is made of an interconnecting framework of trabeculae in a number of combinations, all comprising the following basic cellular structures: rod–rod, rod–plate, or plate–plate. A trabecular rod is about 50–300 μm in diameter. This corresponds to Level 5 in the seven level hierarchy which can be summarized as

- Also called Haversian systems.
- Created by the excavation of large tunnels by osteoclasts.
- These tunnels are then refilled by osteoblasts, beginning with a thin layer of cement and followed by layers of lamellar bone until the channel is almost filled.
- Remaining channel functions as a blood vessel to the surrounding bone and has smaller channels (canaliculi) branching off it, which are filled with osteocytes.
- Overall appearance is an onion-like structure with a central hole surrounded by multiple lamellar layers.
- Balances pre-existing asymmetry.
- Modifies/improves mechanical properties.

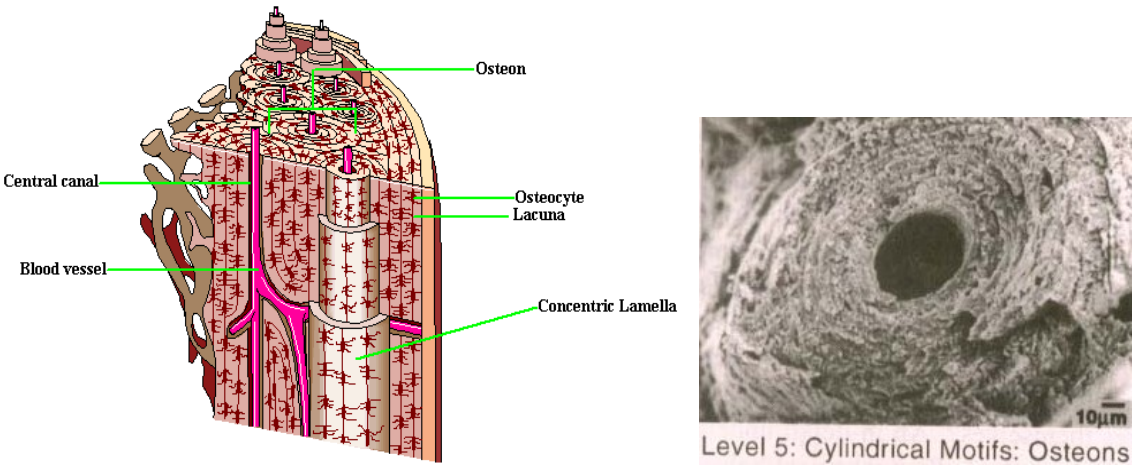


Fig 3: Osteons or Haversian systems. (Left) Anatomy of a Haversian system. (Right) SEM image of Osteons.

C. SUB- MICROSTRUCTURE

Bone lamellae are 3–7 μm thick [**7**], but the arrangement and orientation of the substance of a lamella is not well known. There may be differences in the lamellae encountered in cortical and cancellous bone. The most common perception of the arrangement of the collagen fibers in a lamella of an osteon is that they lie in parallel in each lamella, with a change in the orientation of fibrils from one lamella to the next described figuratively as a twisted plywood or helicoidal structure. The osteonal lamellae are wrapped around a central canal, and sequential concentric lamellae have fiber orientations alternating with each other, spiraling around the central canal. Lamellae with alternate orientations are seen as alternately bright, dark, or intermediate in cross-section under a polarized light microscope (PLM) with the intensity of the transmitted light depending on the collagen content, its degree of alignment, the presence of a mineral fraction, and on the orientation of the section [**8**]. The orientations envisaged in this kind of modeling are transverse, longitudinal, or oblique.

Overall, the collagen has a basically parallel orientation but the fibers form a continuum both within single lamellae and between lamellae. The description fits with Level 4 in the seven level hierarchy.

The four known types of fibril array pattern in bones are listed below.

a. Arrays of Parallel Fibrils
b. Woven Fiber Structure
c. Plywood-like Structures
d. Radial Fibril Arrays

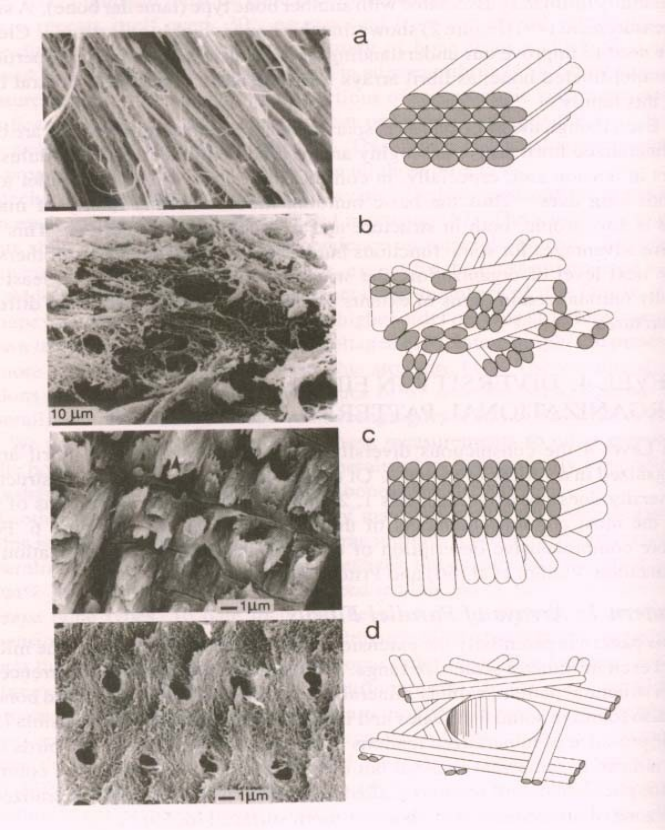


Fig 4: Four types of fibril array patterns.

D. NANOSTRUCTURE: COLLAGEN FIBERS FROM 100NM TO 1 MM

The mineralized collagen fibril is the basic building block of bone. The most prominent structures seen at this scale are the collagen fibers, surrounded and infiltrated by mineral. The attachment sites of macromolecules onto the collagen framework are not distinctly

known, although several immunohistological studies have shown preferential labeling of some macromolecules in a periodic fashion along the collagen molecules and fibers [**9**]. This constitutes Levels 2 and 3 in the seven lever hierarchy.

- ***Fibrous Composition:***
    - Fibrils are composed of individual collagen fibers, oriented parallel to each other.
    - Each fiber is separated by 68 nm from the fibers before and after it, and by 1.5 nm from the fibers to either side.
    - Fibers are assembled with two orthogonal layers, one identical, and one identical, but offset by 28 nm.

- ***Mineralized Composition:***
    - Dahllite crystal are initially formed in the holes between fiber rows.
    - Dahllite crystals grow over time, eventually expanding out of the holes and forming continuous sheets throughout the fiber.
    - Expansion of dahllite decreases fiber spacing from 1.5 nm to 1.1 nm.
    - Dahllite crystals grow parallel to each other within a specific collagen fibril, following the orientation of the fibril channels.
    - Water initially fills the space between fibers and is removed as the dahllite concentration increases.

- ***The Fibril Array***
    - Individual collagen fibers are arrayed parallel to their neighboring fibers.
    - Two possible fibril arrangements: parallel crystal layers and non-parallel crystal layers.
    - Fibrils are not distinct; they often merge with neighboring fibrils.
    - Mineralization of fibrils seems to occur linearly, beginning near one end.

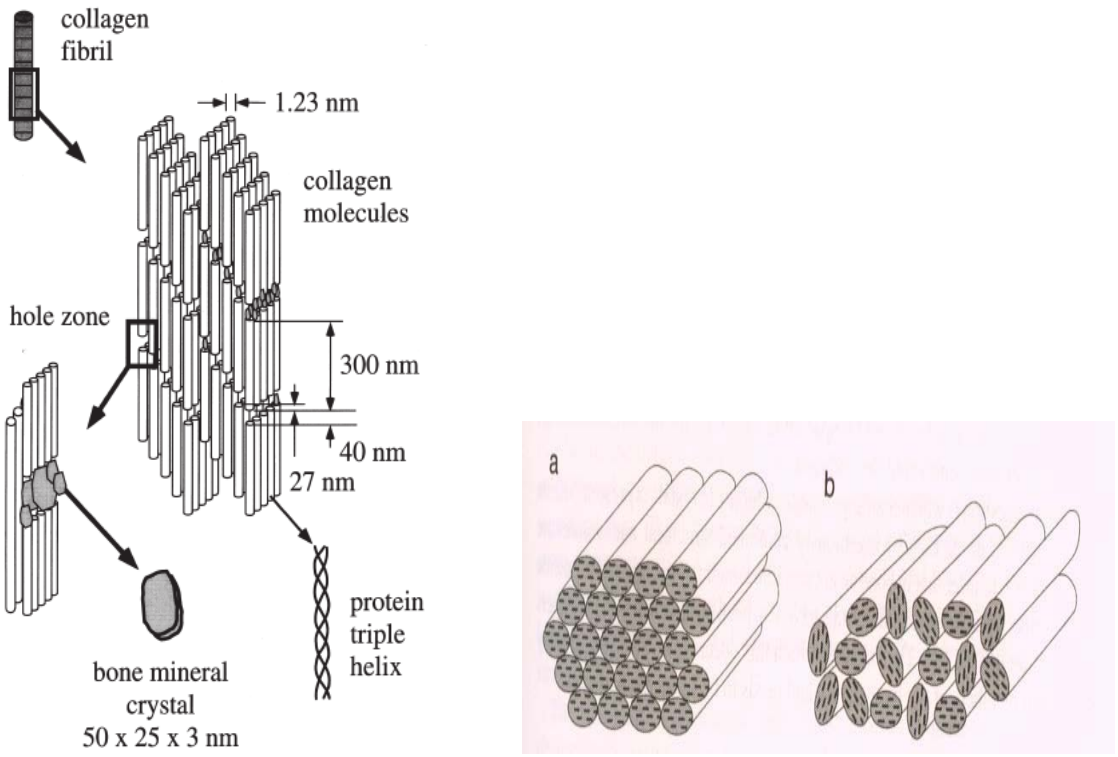


Fig 5: (Left) A schematic diagram illustrating the assembly of collagen fibrils and fibers and bone mineral crystals. The well known 67 nm periodic pattern results from the presence of adjacent hole (40 nm) and overlap (27 nm) regions of the assembled molecules. **(Right)** Parallel and non-parallel crystal layers. (Fig 2 in Ref 3)

E. SUB- NANOSTRUCTURE: *Crystals and collagen fibrils down to tens of nanometers*

The sub-nanostructures of the three main materials are crystals, collagens, and non-collagenous organic proteins. The mature crystals are not needle-shaped, but plate-shaped. Plate-like apatite crystals of bone occur within the discrete spaces within the collagen fibrils, thereby limiting the possible primary growth of the mineral crystals, and forcing the crystals to be discrete and discontinuous. The mineral crystals grow with a specific crystalline orientation—the *c* axes of the crystals are roughly parallel to the long axes of the collagen fibrils [**10**]. The average lengths and widths of the plates are 50 × 25 nm. Crystal thickness is 2–3 nm [**11**]. The nanocrystalline bone apatite has small but

significant amounts of impurities such as HPO4, Na, Mg, citrate, carbonate, K, and others whose positions and configurations are not completely known [**10**]. The primary organic component of the matrix is Type I collagen. Collagen molecules secreted by osteoblasts self-assemble into fibrils with a specific tertiary structure having a 67 nm periodicity and 40 nm gaps or holes between the ends of the molecules (Fig. 5). Non-collagenous organic proteins, including phosphoproteins, such as osteopontin, sialoprotein, osteonectin, and osteocalcin, may function to regulate the size, orientation, and crystal habit of the mineral deposits. Through chelation of calcium or enzymatic release of phosphorus from these proteins, they may serve as a reservoir for calcium or phosphate ions for mineral formation. However, additional studies are needed to conclusively define their actions and mechanisms. This evidently represents the first level in the seven level hierarchy.

Summing the first level,

The Major Components

- ***Mineral: Dahllite, a carbonated apatite ceramic***
  - $Ca_5(PO_4CO_3)_3$
  - Nanomaterial, with dimensions ~50nm x 25nm x 2.5nm.
  - Plate-shaped despite dahllite's normal hexagonal crystal structure.
  - Estimated Young's modulus of 110 GPa.
  - XRD shows structure identical to hydroxyapatite, however, chemical analysis indicates absence of hydroxyl groups as well as presence of impurities such as HPO_4, Na, Mg, and K.
- ***Protein: Predominantly Type 1 Collagen***
  - Composed of three identical collagen fibers, each 1000 amino acids in length.
  - These fibers are woven in a triple-helix to form a cylinder, 80-300nm x 1.5nm.
- ***Water***
  - Plays an important role in the mechanical properties of bone.
  - Located between the triple-helical collagen fibers, and in the gaps found at the second level (in the seven level) of hierarchy

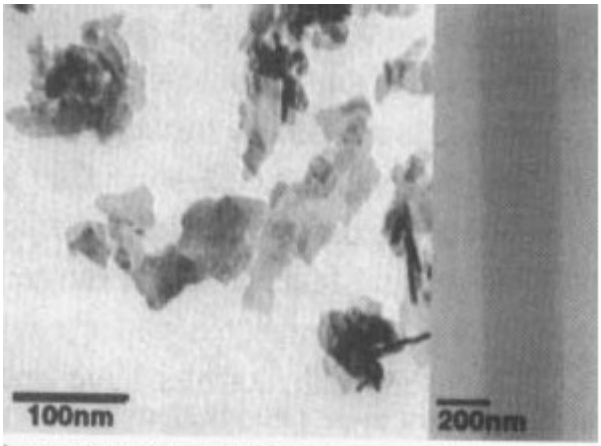


Fig 6

FUTURE PROSPECTS AND APPLICATIONS

- In the field of prosthetics, Nanoceramics have shown outstanding osteoblast proliferation.
- If a hierarchical approach that mimics natural bone can be created for nanomaterials these cellular interactions may be improved even further.
- Additionally, the corresponding increase in mechanical properties may allow previously unsuitable materials to become viable options for future implants.
- Hierarchical materials have shown vastly increased mechanical properties. By applying these hierarchical architectures to modern composite materials, significant increases in mechanical properties should result. Additionally, the understanding of these architectures will allow the properties to be varied in a way that optimizes the material for the task at hand.

The composition of bone tissue is more complex than most engineering composites. A more fundamental understanding may be achieved by models employing a collagenous matrix and mineral crystals. These organic and inorganic constituents act together to give bone its unique properties. The viscoelastic properties and resistance to fracture cannot yet be explained by explicit molecular mechanisms or commonly measured physical characteristics, but models of the elastic properties and their anisotropy using composite rules of mixtures for the elements have been suggested.

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