Chapter 1: Introduction

The first portion of this chapter is a brief background and statement of the problem being examined in the current work, namely the characterization of the mechanical behavior of mineralized tissues at fundamental, ultrastructural, length-scales. The motivation for examining the ultrastructure of these materials will be introduced and the gaps in existing knowledge will be presented. The second portion of this chapter is an introduction to the materials studied in the remainder of the work, beginning with engineering materials. Structures of the mineralized biological tissues in bones and teeth are presented along with a brief introduction to biomimetic materials for mineralized tissue replacement. The chapter concludes with a "road map" for the organization and content of the remainder of this work.

1.1 Statement of Problem

Mineralized tissues have important structural functions in the body. Teeth are degraded by carious disease, leading to their necessary repair or removal. The bony skeleton can become structurally unsound, globally as in osteoporosis, or locally when large pieces of bone are removed from the body due to malignancies or trauma.

Because these materials have important structural functions, there is obvious motivation for studying their mechanical functioning under normal deformations as well as conditions that induce failure. An understanding of both normal and abnormal mechanical circumstances *in vivo* can assist in both the prevention of failure and in the development of remedies post-failure. This approach to mineralized tissue is inherently physics- or engineering-based, focused on the structural and mechanical functioning of the connective tissue matrix as opposed to approaches that are inherently cell biological.

Many studies have examined the behavior of mineralized tissues under a variety of mechanical loading conditions and at length-scales ranging from the macroscopic to the microscopic. Much recent attention has been on the examination of bones and teeth at ever-smaller length-scales, below the threshold for traditional mechanical testing and optical examination in white light, and incorporating techniques such as electron microscopy and atomic force microscopy for visualizing the ultrastructure and nanoindentation testing for probing mechanical behavior.

Nanoindentation is a mechanical technique for examining materials at nanometer to micrometer length-scales. A small indenter tip is brought down onto the surface of the sample, and pressed into the sample surface while the load and deformation are being continuously measured. From the load and deformation measurements, material properties (*e.g.* the elastic modulus) can be calculated for the local region of the sample contacted by the tip. This technique has great promise for the examination of inhomogeneous materials, including biological composite materials, since properties can be mapped out from point to point in one or two dimensions, allowing for the examination of biological effects at length scales comparable to the cells associated with

these biological effects.

A large number of studies have been performed, primarily in the last decade, using nanoindentation to examine local mechanical responses of mineralized tissues in bones and teeth. Studies have examined both the baseline structure-properties relationships for healthy tissues as well as the effects of disease state on nanomechanical response. It was found that in osteoporosis, although the total volume of bone drops drastically with worsening disease state, nanoindentation modulus measurements revealed no change in the local elastic modulus of the remaining tissue [Guo and Goldstein, 2000]. In contrast, in dental caries, substantial decreases in both dentin and enamel elastic modulus values have been noted, in conjunction with decreases in mineralization [Bembey et al, 2005; Angker et al, 2004].

Some studies using nanoindentation have noted substantial and surprisingly large variability in response and obtained properties. Cuy et al [2002] examined the variation in elastic modulus in the enamel of a single tooth cross-section and saw values that varied smoothly across the tooth, ranging from less than 70 GPa to greater than 115 GPa. The modulus variations appeared to related to patterns of loading during mastication (chewing). Zysset et al [1999] examined variations in bone modulus at different sites in the body and found large variations depending on tissue type and subject. The range of observed bone modulus values was from 7 to 25 GPa. Clearly at the scale of nanoindentation testing, nanometers to micrometers, mineralized tissues have extremely variable local responses. These variations have clinical implications as well. In comparing finite element models for homogeneous bone versus locally-varying bone with the same average elastic modulus, a substantial increase in local bone failure probabilities was seen with increasing variability of local modulus [Jaasma et al, 2000]. No comprehensive analysis of the structural causes of local mechanical variability, as seen in indentation testing, has been performed to date.

Although nanoindentation has been widely used to examine mechanical responses of mineralized tissues, there are some additional limitations in its current applicability that have not been adequately addressed by the existing literature. A basic technique to

obtain elastic modulus from raw indentation data (the Oliver-Pharr method [Oliver and Pharr, 1992]) is built into commercially-available software packages, but was designed for stiff, homogeneous engineering materials and not biological tissues. Potential problems associated with the application of this technique to more compliant materials are associated with both the fundamental physics of the deformation process as well as operational details such as calibration techniques best suited for compliant samples. Another outstanding issue is the influence of time-dependent deformation, characteristic of hydrated biological tissues, in the indentation response.

Potentially contributing to the observed variability in indentation responses, there is an intrinsic uncertainty associated with the interpretation of indentation data obtained at length-scales comparable to the nanocomposite structure of mineralized biological tissues. It has been demonstrated that in models of indentation loading of a composite engineering material, contact hardness results vary dramatically from what would be inferred from a composite homogenization scheme (in which the body is assigned uniform elastic properties calculated from the composite phase contributions) [Shen and Guo, 2001]. Since the indentation loading is localized and inhomogeneous, the individual local features in the composite structure are important. It is also well-known that at the most fundamental (sub-nm to nm) length-scales, mineralized tissues are composites composed of water, an organic protein phase, and an inorganic mineral phase. Even with the technology developed for examination of structure at these fundamental scales, there are a number of outstanding questions regarding the organization of these components and in turn how this organization leads to the observed structural and mechanical functions. Mineralized tissues have been examined at the macro-scale within the framework of engineering composite materials, and it has been shown that composition (mineral volume fraction) was insufficient to predict the elastic modulus of bone—large modulus changes are seen at nearly constant mineral volume fraction [Katz, 1971]. No study since has really improved on Katz's early work or made sense of bone (ultra)structure-properties relationships within a composite materials framework.

Given the fact that there is poor understanding of the factors contributing to the

elastic response of mineralized tissues at the macroscale, it is perhaps not surprising that little has been said on the difficulties of understanding nanoindentation responses of these materials at sub-microstructural scales. There are two practical implications of this current knowledge gap: (1) it is difficult to design biologically and clinically useful experiments when the tool (nanoindentation) being used is poorly understood; (2) it is difficult to design clinically-useful biomimetic materials, when one is attempting to mimic a structure that is poorly understood.

The current work will use a combined modeling and experimental approach to examine the indentation responses of mineralized tissues as well as more fundamental aspects of their mechanical behavior at small scales. Particular emphasis will be placed on the factors that contribute to point-to-point variability in indentation mechanical responses.

The next section of this chapter will briefly describe the composition and structure of the materials examined throughout the course of this work. Following this introductory material will be a "road map" describing the organization and layout of the remainder of this work.

1.2 Materials Background

1.2.1 Engineering Materials and Mechanical Behavior

All the world around us is constructed of materials. There are main classes of materials, and main classes of material behavior that result from different structures at the atomic scale. Engineering materials can be roughly divided into sub-categories (1) metals; (2) ceramics and glasses; (3) polymers; (4) composites composed of two or more phases of metals, ceramics, and polymers [Callister, 2000]. Under this classification, naturally-occurring biological materials are best described as composites, where the individual phases are polymers, ceramics, and water.

The response of materials to applied physical loading is the result of the structure and composition of the material. The study of the response of materials to applied external forces and deformation is the field of engineering mechanics. A number of tools are used to explore this behavior, experimental, analytical, and computational. The simplest of these is the common stretching of a dogbone-shaped piece of material: the tensile test. Fundamental quantities used in the study of these behaviors are the stress, σ and the strain, ε:

$$\sigma = P / A_0 \qquad [1\text{-}1]$$

$$\varepsilon = \Delta l / l_0 \qquad [1\text{-}2]$$

where P is the load (or force, F) applied to a piece of material with original cross-sectional area A_0 and length l_0 (Figure 1-1). The change in the specimen length is $\Delta l = l - l_0$. When mechanical behavior is described in terms of the stress and strain it is "intensive" and associated with geometry-independent material properties, while mechanical behavior described in terms of force and displacement is "extensive" and depends on the specimen geometry.

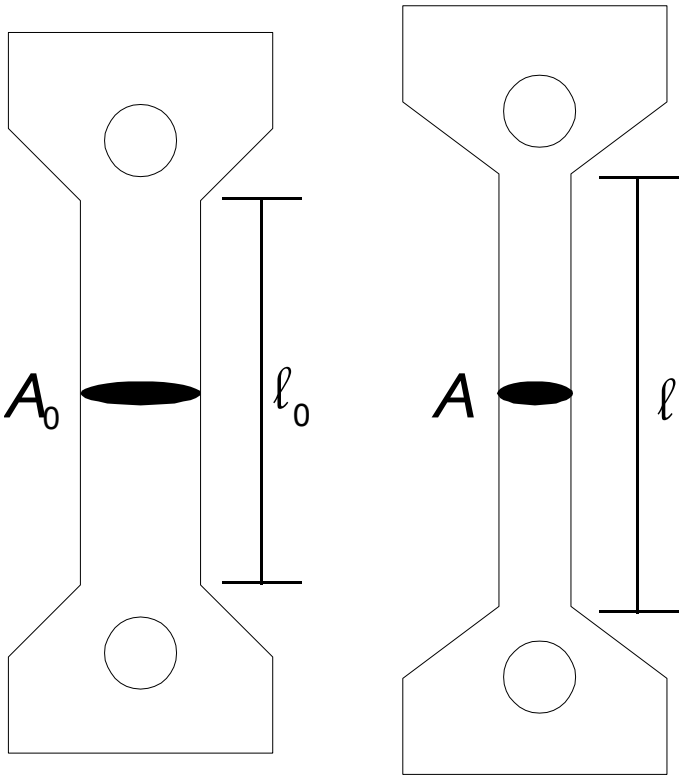

Figure 1-1: Uniaxial tension testing geometry (left) original sample; (right) stretched sample. The cross-sectional area is *A* and the gage length is *l*. The subscript "0" is for the original specimen geometry.

The most fundamental mechanical response is the elastic response of materials, defined at the most fundamental level in terms of the strength of interatomic bonds in materials with simple structures at the atomic scale (such as metals) and describing the reversible response of a material to small applied loads or deformations, such as those encountered in regular performance of the material. Linear elastic behavior also requires the stress to be a unique function of the strain [Mase and Mase, 1992], and is illustrated in Figure 1-2. For comparison purposes, nonlinear elastic and nonlinear inelastic responses are also shown in Figure 1-2. The slope of the linear-elastic stress-strain (σ–ε) response is the modulus of elasticity, E, an intensive material property. The extensive stiffness (k) is the slope of the load-displacement (P-Δl) curve, and is related to the elastic modulus for the tensile specimen shown in Figure 1-1 as $k = EA/l_0$.

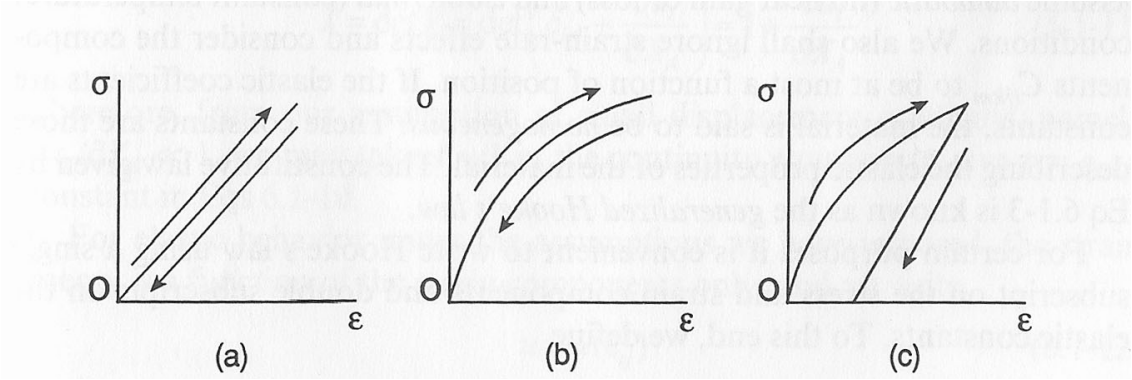

Figure 1-2: Uniaxial stress-strain (σ-ε) curves for loading and unloading of three materials types: (a) linear elastic, (b) nonlinear elastic, and (c) nonlinear, inelastic. [from Mase and Mase, 1992]

For a homogeneous material with the same mechanical behavior in all three dimensions in space ("isotropic"), the fully three-dimensional stress-strain behavior can be uniquely described by just two coefficients, the elastic modulus E and the Poisson's ratio ν. The Poisson's ratio describes the degree of transverse contraction in response to an applied axial strain ϵ_a and is defined as:

$$\nu = -\frac{\epsilon_t}{\epsilon_a} \qquad [1\text{-}3]$$

where ϵ_t is the transverse strain. The sign of the two strains are opposite, such that the Poisson's ratio is positive for most bulk solid materials. Elastic behavior is time- and rate- independent.

Engineering materials used frequently in nanoindentation investigations of mechanical behavior are shown in Table 1-1, with the elastic modulus and Poisson's ratio values typically reported for these materials [Oliver and Pharr, 1992; Chang et al, 2003b].

Table 1-1: Isotropic elastic properties for common engineering materials

Material	*Class*	*Elastic Modulus, E (GPa)*	*Poisson's Ratio, ν*
Fused silica[1]	glass	72	0.17
Aluminum[1]	metal	70	0.35
PL-1[2]	polymer	3	0.36

[1]Oliver and Pharr, 1992
[2]Chang et al, 2003b

In metals, after an initial region of elastic behavior, there is an onset of permanent deformation or yielding (Figure 1-3) typically seen in a tensile experiment as a substantial decrease in the slope of the stress-strain response. The limit of elastic behavior is very small, on the order of 1%, while plastic deformation can occur over a very large range of strains prior to final sample failure. Permanent deformation is frequently called "plastic", especially in metals, and can be time-independent or time-dependent. Plastic deformation is categorized by definable changes in local structure at the molecular scale, such as dislocation motion in metals or molecular chain unfolding in polymers.

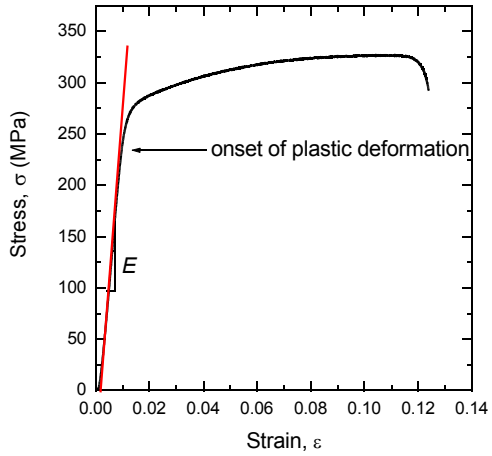

Figure 1-3: Uniaxial stress-strain (σ-ε) response of aluminum under tensile loading. The response is initially linear, characterized by the elastic modulus (*E*) but transitions to a plastic deformation-dominated response at approximately 1% strain.

Behavior of polymers is frequently inelastic from the onset of loading. Polymers are typically characterized as "viscoelastic", an intrinsically time-dependent deformation mode, by virtue of being a combination of elastic deformation and viscous flow. Viscoelastic behavior, and time-dependent mechanical behavior in general, is a focus of this work and will be described in more detail in Chapter 2. However, as a simplified introduction to time-dependent mechanical behavior, there are two characteristic deviations from elastic behavior: (a) changes in stress (force) or strain (deformation) during a hold-time under fixed applied loading conditions (Figure 1-4), and (b) differences in the *apparent* elastic modulus at different applied loading rates (Figure 1-5).

Viscoelastic behavior differs fundamentally from plastic behavior. Plastic deformation is irreversible, being associated with permanent changes in the material, while viscoelastic flow is recoverable.

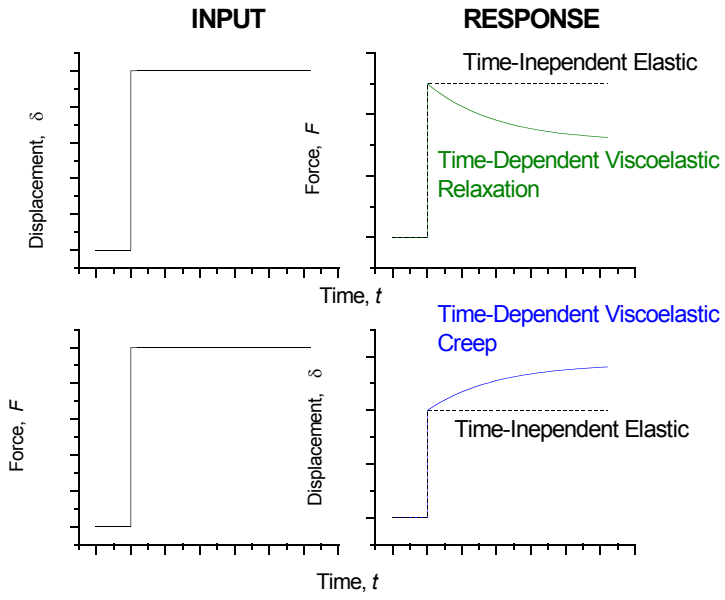

Figure 1-4: Time-dependent mechanical responses under fixed loading inputs: (top) force-relaxation and (bottom) length extension (creep) in a viscoelastic material.

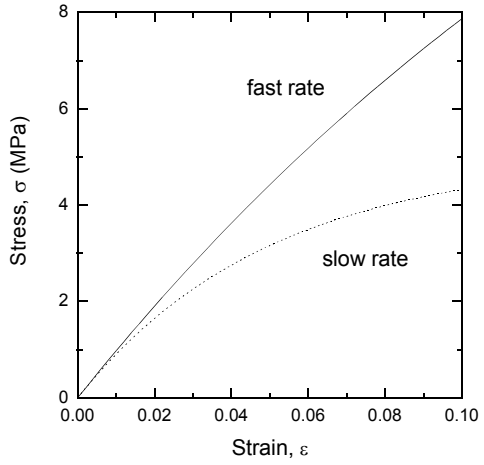

Figure 1-5: Stress-strain (σ-ε) responses for a viscoelastic (Maxwell) material loaded at constant strain rate at two rates (differing by a factor of four). Apparent variations occur with rate in the numerical value of the elastic modulus. In addition, the responses are not linear.

1.2.2 Bone

Bone is an extremely complicated material, with a hierarchical structure on many different levels of organization. Details of the composition and structure of bone will be discussed next, both in the context of *de novo* bone formation and in bone healing.

1.2.2.1 Bone Composition

Bone is a living composite material comprised of cells and a multi-component structural extracellular matrix (ECM). The ECM of bone is composed of three phases: inorganic mineral phase, organic phase, and water and will be discussed in more detail in following sections. The ECM is created and maintained by active bone cells. There are three cell types in bone, osteoblasts, osteoclasts, and osteocytes. Osteoblasts and osteocytes are involved in bone formation and maintenance while osteoclasts are the major resorptive cells of bone [Kaplan et al, 1994]. Bone is, in general, dynamic and

constantly remodeling via the action of these cells. The cells in bone respond to a variety of signals including chemical, mechanical, and electrical signals. The mineral phase is a carbonated apatite.

Osteoid

The organic phase of bone, called osteoid, is approximately 90% Type I collagen and 10% noncollagenous proteins and other macromolecules [Kaplan et al, 1994].

Type I collagen is a triple helix comprised of two $\alpha 1(\mathrm{I})$ chains and one $\alpha 2(\mathrm{I})$ chain. As in other fibrillar collagens, individual triple helices (tropocollagen molecules) about 300 nm long are grouped in a quarter-staggered array with periodic banding every 67 nm due to regions of overlap (Figure 1-6). This regular arrangement of tropocollagen molecules forms larger collagen fibrils, which are themselves in turn grouped to form large collagen fibers. Collagen is highly cross-linked with both inter-molecular and intramolecular crosslinks [Alberts et al, 1996] (Figure 1-7). This cross-linking in part makes bone collagen highly insoluble [Hayes, 1991]. Recent estimates for the strength of this crosslinking effect are apparent in the mechanical behavior of isolated collagen: the individual tropocollagen molecule is estimated to have an elastic modulus of around 1 MPa while collagen in the form of crosslinked fibers has an elastic modulus of several hundred MPa [Freeman and Silver, 2004].

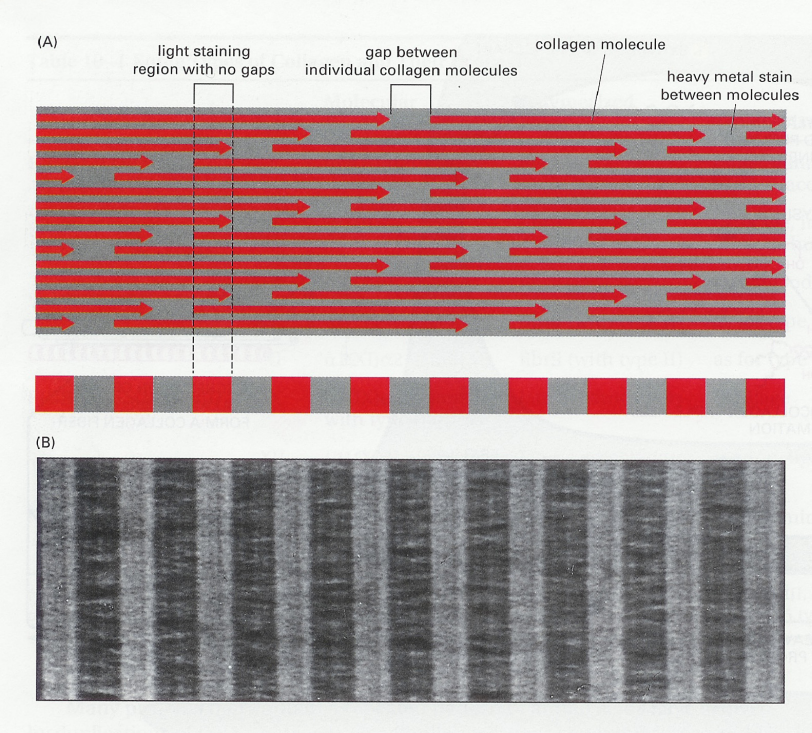

Figure 1-6: Collagen periodic banding [from Alberts et al, 1994]

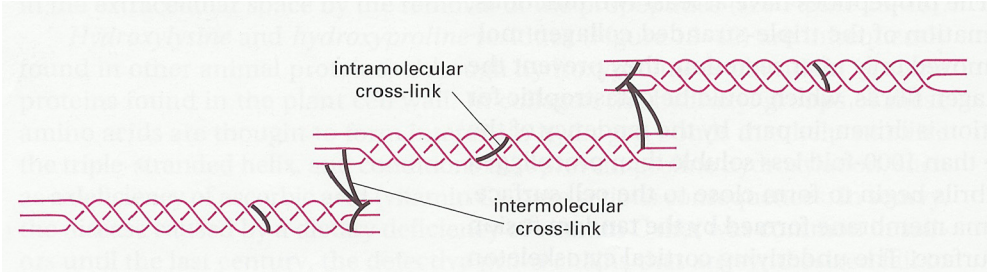

Figure 1-7: Collagen cross-linking [from Alberts et al, 1994]

Mineral

The mineral in bone is a member of the apatite family. Apatites have the composition

$Ca_5(PO_4)_3[F, Cl, OH]$

and a hexagonal crystal structure (Figure 1-8). Mineralogical apatite is fluroapatite

while biological apatite is a member of the hydroxyapatite family. Bone mineral is sometimes called dahllite or carbonated apatite due to appreciable concentrations of $C0_2$ in the structure [Deer et al, 1966]. Apatites have densities of 3.1-3.35 gm/cc [Deer et al, 1966].

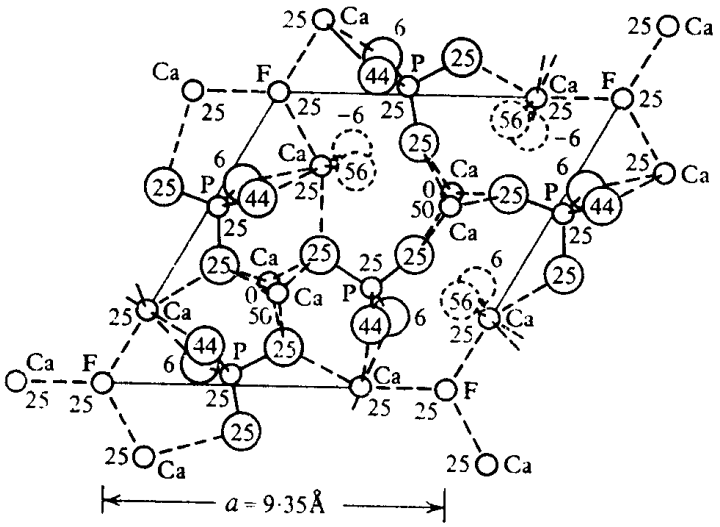

Figure 1-8: Fluoro-apatite crystal structure [from Deer et al, 1966]

Hydroxyapatite in mineralized tissues has a slightly variable composition due to diet and environment. Hydroxyapatite crystals in bone appear as plates 20-80 nm long and 4-5 nm thick although some needle-like structures have also been noted [Bonucci, 2000]. Mineral platelets fuse both laterally and longitudinally, forming long and broad sword-blade structures that seem to remain relatively thin, ~ 5 nm [Currey, 2002]. The plates are located in the gaps, both end-to-end and longitudinal, between individual collagen molecules (Figure 1-9).

Bone structure and mineralization at the microscale to macroscale is typically examined using x-ray methods, such as plane radiography and computed tomography (CT), due to the high density of the mineral phase. Much recent attention has been on local measurements of mineral content using techniques with substantially greater spatial resolution than CT, such as quantitative back-scattered electron imaging (qBSE) using a scanning electron microscope [Ferguson et al, 2003].

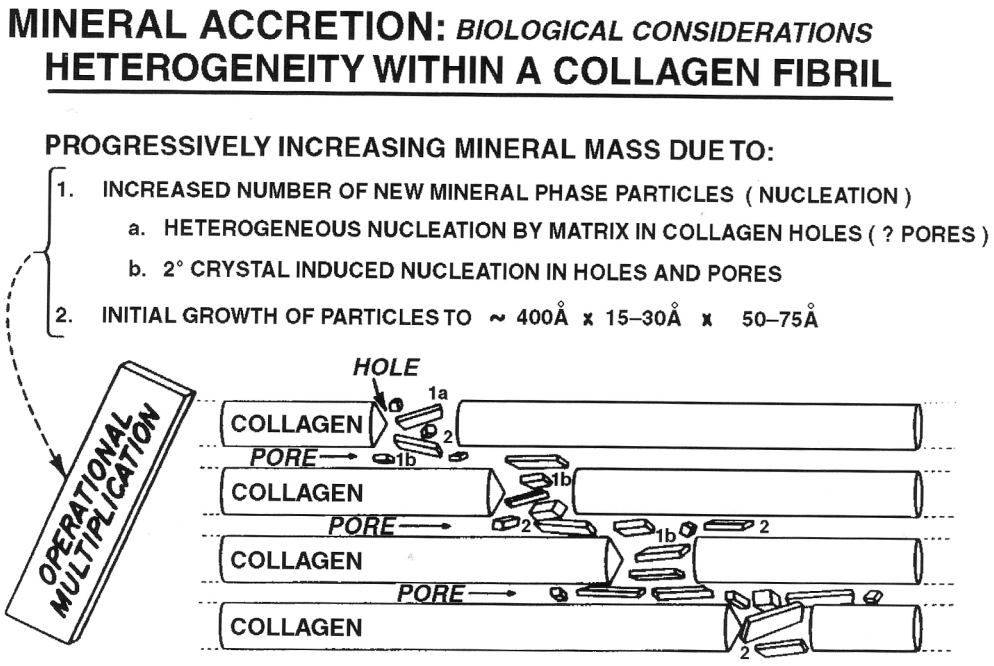

Figure 1-9: Bone mineralization by apatite crystal nucleation and growth in spaces between collagen fibrils [from Kaplan et al, 1994].

1.2.2.2 Bone Hierarchy and Microstructure

At very small length-scales, 10s to 100s of nm, the ultrastructural scale, the structure of bone is dominated by individual collagen fibrils and hydroxyapatite crystals. The precise arrangement of the components at this ultrastructural length-scale is poorly understood [Currey, 2002]. There are many levels of structural organization in bone, at much longer length-scales, and which have been well-studied. Although different investigators use different definitions of the primary length-scales and organizational structures in bone, one useful illustration comes from Weiner and Wagner [1998], who use a categorization system with seven quantifiable levels of structural hierarchy, approximately evenly distributed (Figure 1-10).

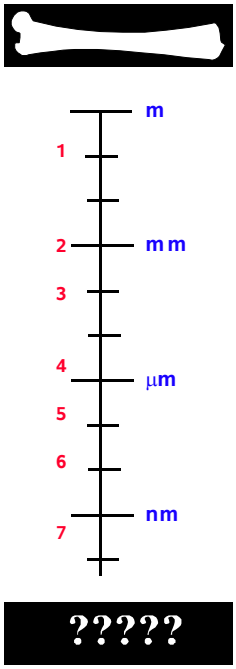

Figure 1-10: Quantification of the scales of bone hierarchy, after Weiner and Wagner [1998]. The seven levels of hierarchy are defined as:

1. **Whole bones (cm – m)**
2. **Cortical and cancellous bone (mm)**
3. **Haversian, osteon systems (100s of μm)**
4. **Patterns of fibril arrays (plywood structures) (μm)**
5. **Arrays or bundles of fibrils (100s of nm)**
6. **Mineralized collagen fibrils (80-100 nm)**
7. **Collagen fibrils, mineral crystals, water (nm)**

The collagen matrix can be dominantly organized and parallel-fibered or dominantly irregular (particularly in young bone called "woven") [Bonucci, 2000]. Parallel-fiber bone is more mature and typically results from the remodeling of woven bone [Kaplan et al, 1994]. There are layers (lamellae) of parallel-fibered bone and layers rolled into cylinders to form structures called osteons (Figure 1-11).

At a microscopic structural level, bone is roughly divided into cortical (compact, dense) and trabecular (spongy, cancellous). The density of cortical bone is substantially higher than that of trabecular bone, and the surface area of trabecular bone is

substantially higher due to the porous structure. Cortical and cancellous bone have approximately the same material density, but due to macroporosity trabecular bone is frequently represented as a porous material with relative density (structure density normalized by density of the fully dense material) less than 0.7 [Gibson and Ashby, 1997].

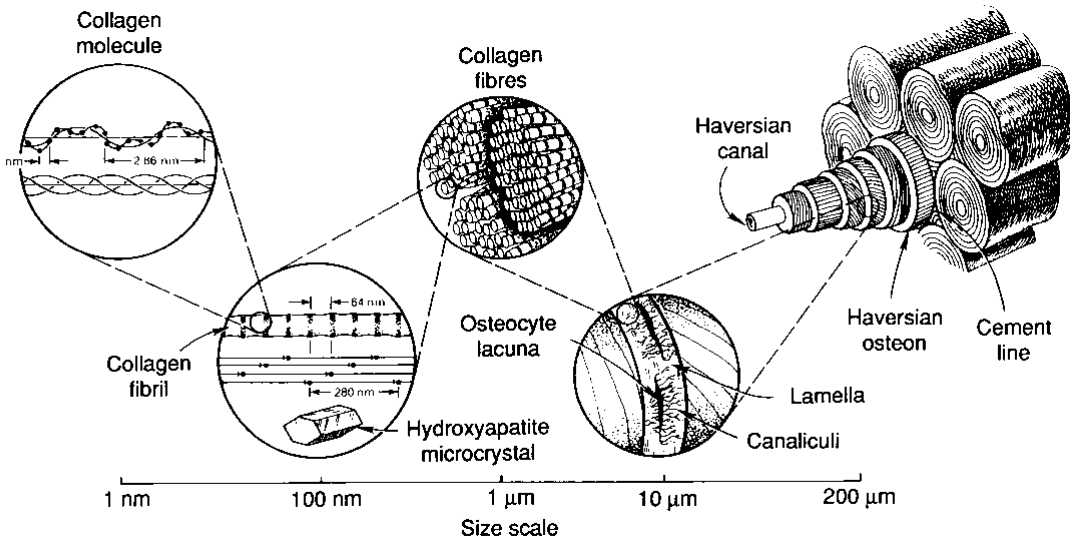

Figure 1-11: Ultrastructural length-scales of bone (from nm to μm) These features are in the range of length-scales for nanoindentation testing, from 1 nm to 10 μm [from Lakes, 1993]

At the organ (macro-) scale of whole bones (*e.g.* the thigh bone—femur) both types of bone (cortical and trabecular) are present, cortical bone forming a protective shell around the porous trabecular bone. Additional non-bone tissue components are present in whole bones, including the marrow, vasculature and nerves.

Bone's functions as a structural material are many and varied. Bones provide physical barrier protection to the brain (skull) and spinal cord, and most organs (ribcage). Long bones form the structural elements of the skeleton that allow for walking upright and other joint motions. In the jaw, teeth are firmly anchored into the bone.

1.2.2.3 Bone Phase Continuity

There has been some recent interest in the phase continuity of the different phases (organic, mineral) in bone, especially within the context of forming ultrastructural models of bone as a composite material. The original edition of Gray's Anatomy [Gray, 1901], page 1101 states:

> *Bone consists of an animal and an earthy part intimately combined together.*
>
> *The animal part may be obtained by immersing the bone for a considerable time in dilute mineral acid, after which process the bone comes out exactly the same shape as before, but perfectly flexible, so that a long bone (one of the ribs, for example) can easily be tied into a knot ...*
>
> *The earthy part may be obtained separate by calcination, by which the animal matter is completely burned out. The bone will still retain its original form, but it will be white and brittle, will have lost about one-third of its original weight, and will crumble down with the slightest force. The earthy matter confers on bone its hardness and rigidity, and the animal matter its tenacity.*

This issue of bone phase continuity will be explored further in Chapter 5.

1.2.2.4 Bone Healing and Remodeling

In either bone healing or de novo bone formation, human bone is produced in two steps: (1) the deposition of primary bone, and (2) the remodeling of the primary bone to form secondary bone.

Primary bone is deposited in two stages, the formation of a collagen network and

the subsequent mineralization of this collagen matrix. New osteoid becomes calcified 70% within days but full calcification beyond this takes months [Hayes, 1991]. In immature newly formed woven bone the average mineral size is smaller than in mature, fully mineralized bone [Kaplan et al, 1994].

Secondary bone is formed when primary bone is resorbed by osteoclast cells and new bone is deposited by osteoblast cells. The remodeling process creates longitudinal (tubular) channels with cylindrical lamellae (layers) of bone in a system called "Haversian canals" or "secondary osteons" [Hayes, 1991]. Secondary osteons are bounded by a cement line of highly mineralized tissue with little or no collagen content. Much of the structural complexity of bone at intermediate length-scales is due to the lamellar and osteonal structures.

Bone formation and remodeling is particularly important within the context of bone healing, as occurs after implantation of a metal implant into bone for dental or medical reconstruction. The biomechanics of metal implants into bone have been considered extensively, particularly for large orthopaedic implants, as used for joint replacement in the hip or knee [Huiskes, 1991]. Bone-implant interfaces in the jawbone, associated with dental implants, have also been considered [Geng et al, 2001]. Bone remodels extensively *in vivo* in the absence of major changes in local stress conditions, and also undergoes a process of adaptive remodeling in response to a change in local stress conditions as occurs when a metal implant is embedded in the bone matrix.

1.2.2.5 Mechanical Behavior of Bone

At the macrostructural scale, there is a large difference between the mechanical response of cortical and trabecular bone due to the large differences in relative density associated with the macro-scale porosity of trabecular bone (Figure 1-12). Until recently, a subject of some debate was whether the mechanical properties, particularly the elastic modulus, of cortical and trabecular bone were similar at fundamental material length-scales. Difficulties associated with small scale mechanical testing of fundamental

structures, such as an individual trabecular strut, had clouded the issue. However, it has recently been demonstrated using both nanoindentation and ultrasound attenuation techniques that the elastic modulus of cortical and trabecular bone is indeed approximately the same [Turner et al, 1999]. Therefore the material bone can be considered as a single entity, regardless of its form or porosity at the organ scale.

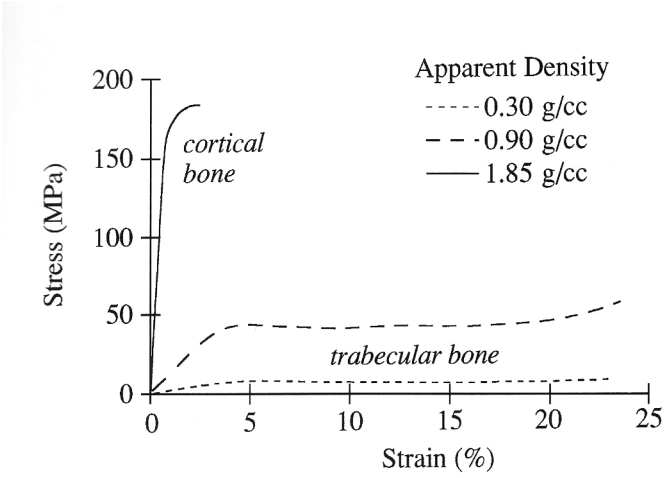

Figure 1-12: Stress-strain (σ-ε) responses for cortical and trabecular bone. The samples have different apparent densities due to porosity, particularly in trabecular bone. [from Kaplan et al, 1994]

Consistent with the mechanical behavior of other biological tissues, bone does demonstrate time-dependent mechanical behavior due to the presence of the hydrated protein phase [Sasaki and Enyo, 1995]. This time-dependent mechanical response is demonstrated in differences in the stress-strain response (Figure 1-13) for different loading rates, illustrating increases in the apparent elastic modulus with increased strain rate. The time-dependent mechanical response of bone has been shown to be highly dependent on the water content [Sasaki and Enyo, 1995; Yamashita et al, 2001], leading some to characterize the time-dependence as poroelastic instead of viscoelastic [Cowin, 1999].

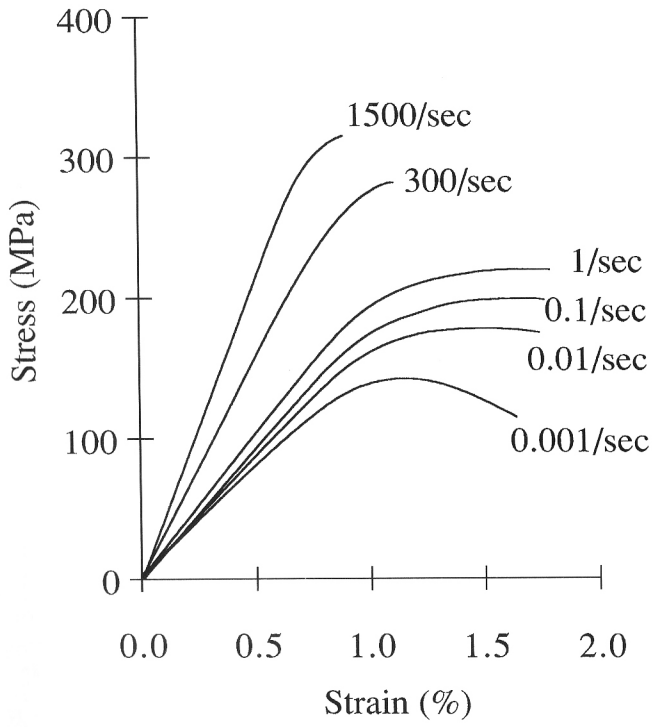

Figure 1-13: Strain-rate effects on bone mechanical stress-strain (σ-ε) responses. Both the apparent failure strength (σ_{max}) and elastic modulus (*E*) are affected by rate. [from Kaplan et al, 1994]

In Figures 1-12 and 1-13 it is interesting to note that the stress-strain response of bone is initially approximately linear, and thus at small strains a linear elastic modulus can be reasonably defined. In sharp contrast, the stress-strain response of collagenous soft tissues, including demineralized bone (Figure 1-14), are highly nonlinear, with the apparent modulus increasing with increasing strain levels.

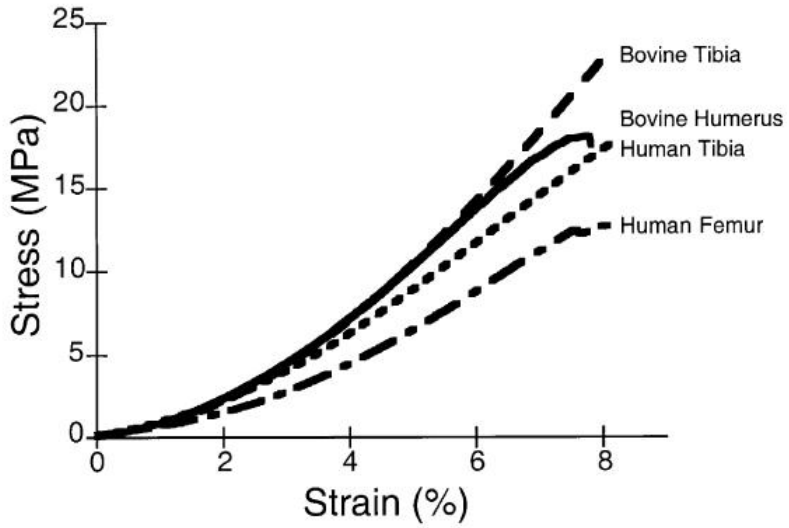

Figure 1-14: Stress-strain (σ-ε) responses of demineralized bone [from Catanese et al, 1999]. The response shapes and magnitude differ substantially from those of whole bones (as shown in Figure 1-13).

The mechanical behavior of bone is complicated compared to a homogeneous engineering material. Due to the local variations in bone composition, the measured elastic modulus of bone is dependent on the local concentration of mineral in a manner that is poorly understood [Katz, 1971]. Also, due to both the intermediate structural inhomogeneity (e.g. the tubular nature of the osteonal system) and also the ultrastructure-level local orientation variations of the plate-like minerals, bone behavior is not isotropic but has properties that depend on local orientation (*e.g.* is "anisotropic"). In femoral cortical bone there is a substantial difference between the elastic modulus values in the longitudinal (E_L =17 GPa) and transverse (E_T = 11.5 GPa) directions relative to the long bone axis [Hayes, 1991]. These two important factors in determining the local mechanical response of bone, the composition and arrangement of mineral at the ultrastructural scale, will be considered at length in the current work.

1.2.3 Mineralized Tooth Tissues

Aside from bone, important mineralized tissues in the normal human body are located in the teeth. (Other mineralized materials in the body are frequently associated with pathology, such as the calcification of arteries in atherosclerosis or the calcification of breast tissue in tumor formation.) A tooth has a visible crown, and extended roots anchored firmly into the bone of the jaw (Figure 1-15). Teeth are roughly a three-layered structure of tissues with extremely different compositions and mechanical responses. The external enamel layer is extremely dense and stiff, related to the wear-resistance required for chewing (mastication). The enamel layer covers a bone-like intermediate dentin layer. The most internal layer of teeth is the cellular and soft pulp. Composition and structure of the mineralized dentin and enamel tissues will next be discussed in further detail.

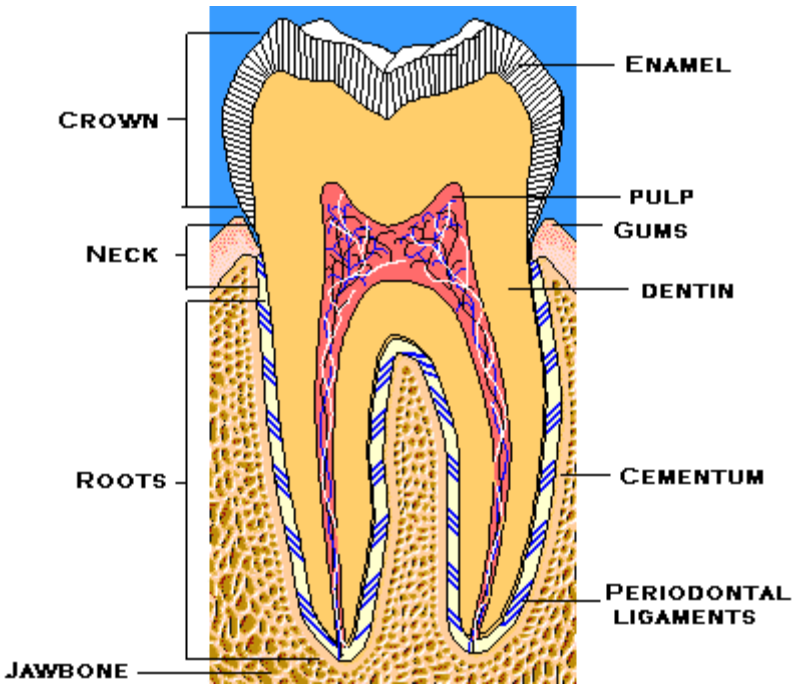

Figure 1-15: Cross-section of a tooth with the root embedded in the jaw bone [from www.tooth.net/info/Anotomyoftooth.htm, accessed 7/30/2003]

1.2.3.1 Enamel

The enamel matrix is composed mainly of mineral (> 90% by volume) with smaller amounts of water and organic phases [Sharawy and Yeager, 1991; Currey, 2002]. The mineral crystals are quite large compared to bone or dentin, about 25 nm thick, 100 nm in width, and 500 nm to tens of microns in length, thus demonstrating extremely large mineral particle aspect ratios [Currey, 2002]. The mineral crystals fuse laterally to form prisms, the basic structural unit of enamel, and there is very little protein located within a prism. The protein in enamel is concentrated at the inter-prism boundaries, in which there are also contained some small mineral crystals [Currey, 2002]. The enamel layer is at most about 2 mm thick. The enamel rods (prisms) are on average 4 μm in diameter [Sharawy and Yeager, 1991]. As in other mineralized tissues, the organic matrix is deposited first by the cells (ameloblasts) and then mineralized. However, in contrast to other mineralized tissues, during mineralization of enamel a substantial portion of the organic matrix (including water) is removed. Enamel is in fact similar in behavior to engineering ceramic materials and demonstrates an approximately time-independent mechanical response. Enamel meets the bone-like dentin in a transitional zone called the dentin-enamel junction (DEJ, Figure 1-16).

Figure 1-16: The dentin-enamel junction (DEJ) is a transition region of finite size between the hard, stiff enamel and the bone-like dentin [from Sharawy and Yeager, 1991].

1.2.3.2 Dentin

The dentin matrix is similar in composition to bone, with far less mineral than enamel, around 60% by volume [Sharawy and Yeager, 1991]. Dentin mineral is deposited in spherical heaps of crystals, called calcospherites, which fuse when they grow to relatively large sizes, ~10 μm in diameter [Currey, 2002]. The dentin mineral crystals are comparable in size to those in bone (3 nm thick and 100 nm in length) but substantially smaller than those found in enamel [Avery, 1991].

There are no cells in dentin, only elongated cell processes from odontoblast cells contained in the pulp. These cell processes are called dentinal tubules (Figure 1-17)and the dentin immediately outside the tubules is more mineralized by about 9% compared to the intertubular dentin over a region 0.4-0.75 μm from the tubule-dentin boundary [Avery, 1991] . It is unclear if this local mineralization difference is mechanically significant [Kinney et al, 1996]. The tubules are about 1 μm in diameter at the DEJ and increase to about 3 μm diameter near the pulp [Avery, 1991]. Also, in stark contrast to bone, dentin does not undergo remodeling. However, because of the similarities in composition, the mechanical behavior of dentin is similar to that of bone, and it is somewhat time-dependent.

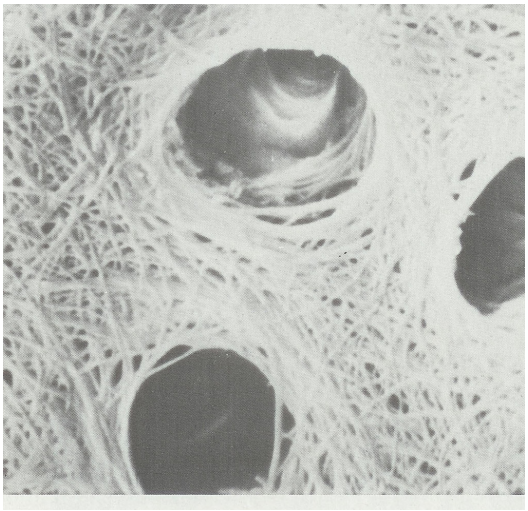

Figure 1-17: Collagen network around the dentinal tubules. [from Avery, 1991].

1.2.3.3 Tooth Replacement via Dental Implants

Recent years have seen large increases in the number of persons having dental implants to replace one or more missing teeth. In this process, a metal stem is implanted into the jawbone, essentially replacing the tooth root, and a cosmetic prosthesis is attached onto the implant to replace the visible tooth (crown, Figure 1-18).

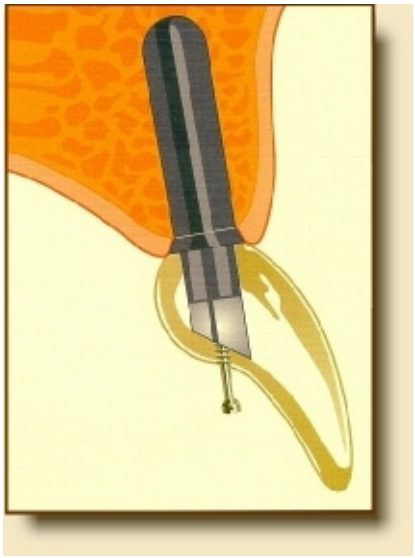

Figure 1-18: Dental implant anchored into bone [from http://www.oral-implant.com/ accessed 7/30/2003]

The failure or success of dental implants will be determined by the quality of bone near the implant [Sahin et al, 2002]. The bone quality is,in turn, determined in part by the mechanical loading on the dental implant, and the transmission of forces to bone across the bone-implant interface. Many studies involving finite element modeling of bone-implant systems have been performed, although most have assumed homogeneous bone mechanical behavior [Geng et al, 2001]. This assumption may be a primary problem in understanding bone-implant interactions and clinical success. The desired outcome is osseointegration, or intimate contact between the bone and the implant along the entire interface (Figure 1-19, left). Loss of bone along the interface (Figure 1-19,

right) is associated with loosening of the implant.

Figure 1-19: Osseointegration in dental implants: bone-implant interface with good ingrowth (left, image from the current work) and poor ingrowth (right, [from http://www.oral-implant.com/oral_implants.htm accessed 7/30/2003]).

The mechanical analysis of bone near dental-implant interfaces will be a major focus of this work, as will be described in the following section.

1.3 "Road Map" of this Work

This section is intended to orient the reader as to the organization of the remainder of this document. The next chapter (Chapter 2) provides an overview of the four fundamental mechanics concepts employed throughout: (1) indentation and contact mechanics; (2) viscoelasticity; (3) mechanics of composite materials; (4) finite element modeling. Following this introductory material is a chapter (Chapter 3) on elastic-plastic indentation experiments, beginning with critical analysis of elastic-plastic (Oliver-Pharr, [1992]) analysis, especially in compliant materials, and continuing with experimental results from nanoindentation tests performed on mineralized bone and tooth tissues. Next in Chapter 4 is an examination of time-dependent effects in indentation responses of mineralized tissues. Having established in Chapters 3 and 4 the key experimental result of dominant point-to-point variability in indentation responses, the remainder of the work incorporates modeling techniques to try and understand the observed experimental results. Key in this modeling analysis is an emphasis on reverse engineering: what can the observed mechanical response tell us about the underlying structure? In Chapter 5, fundamental models are constructed for mineralized tissues at ultrastructual length-scales, beginning first with homogeneous loading models to examine the basic composite mechanics and structure-properties linkages. In Chapter 6, I will return specifically to modeling of indentation testing of mineralized tissues, and to re-examination of the issue of variability in indentation responses within the composites modeling framework developed in Chapter 5. The final chapter (Chapter 7) begins with a demonstration of the implications for local property variability in bone near a dental implant interface. This is followed by a summary of work presented in this document, with emphasis on the original contributions made in this work. Opportunities are presented for future investigation of the topics discussed in the current work.

Two appendices accompany this work, concerning topics tangentially related to the primary theme of local-scale mechanical behavior of mineralized tissues. The first (Appendix A) is a mechanical analysis of biomimetic composite materials made from

gelatin and apatite. These composite materials are characterized by indentation testing, along with their component homogeneous phases, and examined within a composite materials framework. The second appendix (Appendix B) concerns the contact hardness, a parameter which is obtained from contact mechanical testing and frequently reported along with the elastic modulus, but which is poorly understood.