BONE: A NATURAL NANOCOMPOSITE

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Introduction

Bone is a composite material of a fibrous biopolymer (type-I collagen) and a mineral phase (carbonated hydroxyapatite) assembled into a complex, hierarchical structure. There are two types of bone, compact and cancellous (trabecular or spongy). Cancellous bone appears in the bony structure where high impact loads are generated such as the skull, vertebrae, ribs and the head of the femur. Compact bone consists of osteons, which are tubular structures composed of concentric lamellae that surround the blood vessel. Cancellous bone consists of a flat lamellar structure forming platelets and rods that assemble into a porous structure. Antler bone is similar to skeletal bone; however the entire antler consists of compact bone surrounding a cancellous bone interior. Amino acid analysis of bovine femur and antler bone show that the composition is identical.

Our goal was to investigate the structure and mechanical properties of the mineral and protein phases in bone by demineralization and deproteination. Compact bone and cancellous bone from bovine femur and elk antler (*Cervus elaphus canadensis*) were examined in this study. Structural features as well as mechanical properties of demineralized, deproteinated, and untreated samples were investigated to determine the effect of each component on the mechanical properties.

Experimental

Materials

Both compact bone and cancellous bone from bovine femur and antler were used for this study. Rectangles (5 mm × 5 mm × 7.5 mm) for compression testing and beams (30 mm × 3 mm × 2 mm) for three-point bending tests were cut using a diamond saw. Demineralization or deproteination of bone is accomplished by submerging a sample in 0.6N HCl or mild bleach, respectively.

Structural characterization

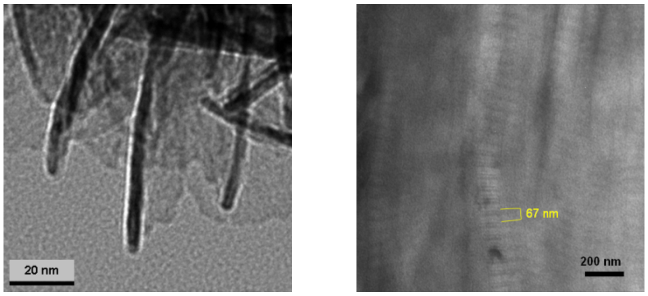
Optical microscopy, micro-computerized tomography (μ-CT), X-ray diffraction (XRD), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to examine the structure of samples before and after demineralization and deproteination.

Mechanical Testing

Compressive tests were conducted using a universal testing machine (Instron 3367 Dual Column Testing System) equipped with a 30 kN load cell. The crosshead speed was 0.05 mm/min, which corresponded to a strain rate of 1×10^{-4} sec^{-1}. Samples were tested at room temperature in the air-dried condition.

Results and Discussion

The morphology and size of the mineral crystallites is shown in a TEM micrograph (Fig. 1(a)). The crystallites have a platelet shape, with width and length ranging from 20 to 70 nm and thickness of ~4 nm. The mineral crystallites grow with a specific crystalline orientation; the *c*-axes of the crystals are well aligned with the fibril long axis (*c*-axis is the short direction). Fig. 1(b) is a TEM micrograph of collagen fibrils obtained from demineralized antler. Each fibril shows periodic striations of ~ 67 nm, which are due the staggered arrangement of collagen molecules. The collagen fibrils are ~ 100 nm in diameter with a preferred orientation along the long axis of bone. Bundles of mineralized collagen fibrils form fibers and further assemble into lamellae ~ 3-7 μm thick. Several layers of the lamellae arranged in concentric rings around the vascular channels and form an osteon in compact bone.



(a) (b)

Fig. 1 TEM micrographs of (a) mineral crystallites and (b) collagen fibrils in antler.

The XRD patterns show that the untreated and deproteinated (mineral only) samples contain nano-crystalline hydroxyapatite while the demineralized (protein only) samples appeared amorphous. μ-CT scans indicate that both the deminerlized and deproteinated bone samples appear identical at macro-scale. The structure of cancellous bone is anisotropic, with aligned channels directed parallel to the long axis of bone. Optical micrographs show that the concentric ring structure in the osteons was undisturbed after demineralization yet the pure mineral phase showed no such concentric rings – rather the mineral was evenly distributed around the central blood vessels. SEM studies show that the minerals were aligned in a coherent manner with collagen fibrils, forming a continuous network.

The mechanical properties of cancellous antler and bone obtained from the compressive tests are summarized in Table I. The compressive stress-strain curves of cancellous bovine femur in dry condition showed brittle failure with significant stress drop after the linear-elastic region while those of cancellous antler behave plastic collapse, with a plateau region after yield point. The modulus and strength of demineralized (100% protein) and deproteinated (100% mineral) samples are much lower than the values from the untreated samples.

Table I. Mechanical properties of cancellous antler and bone.

	Cancellous Antler			Cancellous Bovine Femur		
	Untreated	Demineralized	Deproteinated	Untreated	Demineralized	Deproteinated
ρ (g/cm^3)	0.40	0.29	0.19	0.43	0.32	0.25
E (GPa)	0.50	0.13	0.09	0.68	0.14	0.05
σ (MPa)	10.3	2.7	0.9	12.7	3.0	0.5

The mechanical properties of cancellous antler and bone are modeled by the Gibson-Ashby [1] constitutive equations for cellular solids:

$$\frac{E^*}{E_s} = C_1\left(\frac{\rho^*}{\rho_s}\right)^n \qquad (1)$$

$$\frac{\sigma^*}{\sigma_s} = C_2\left(\frac{\rho^*}{\rho_s}\right)^m \qquad (2)$$

Here E^* and E_s are the elastic modulus, σ^* and σ_s are the compressive strength, and ρ^* and ρ_s are the apparent density of cellular and solid materials, respectively. Both relative elastic modulus and compressive strength of cancellous bones increase with relative density, as shown in Fig. 2.

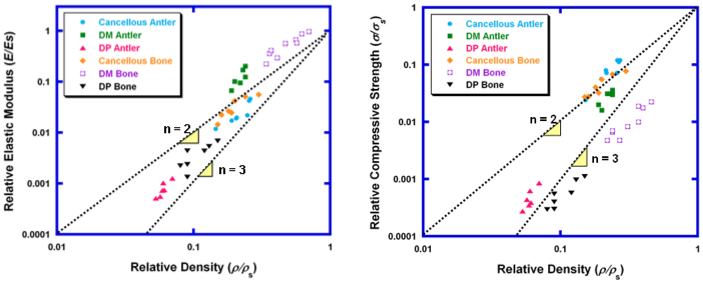
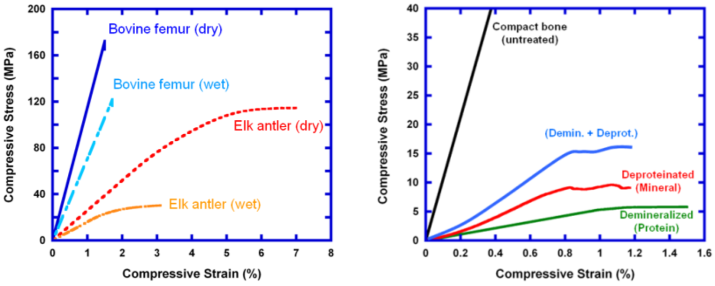


Fig. 2 Relative elastic modulus and compressive strength as a function of relative density.

Table II. Mechanical properties of compact antler and bone.

	Compact Antler			Compact Bovine Femur		
	Untreated	Demineralized	Deproteinated	Untreated	Demineralized	Deproteinated
ρ (g/cm^3)	1.40	0.90	0.82	1.99	1.02	1.55
E (GPa)	3.7	0.46	0.83	13.9	0.74	0.98
σ (MPa)	116	3.2	8.9	168	6.8	17.2

The mechanical properties of untreated, demineralized, and deproteinated compact antler and bone are shown in Table II. Both elastic modulus and strength of compact bovine femur bone are much higher than that of the antler. However, antler can undergo significant amount of plastic deformation without fracture. The compressive stress-strain curves of dry and rehydrated antler and bone is shown in Fig. 3(a). Rehydration has a significant effect – the water is thought to plasticize the collagen matrix [2]. The mechanical properties of pure protein and mineral phases of bone are much weaker than untreated one. Fig. 3(b) showed that the weighted sum of the stress-strain curve for demineralized and deproteinated bone was far lower than that of the untreated bone, indicating a strong synergistic effect between the two phases.



(a) (b)

Fig. 3 (a) Compressive stress-strain curves of compact bone and antler; (b) synergistic effect between the mineral and protein phases in bone.

Conclusions

Bone is a natural nano-composite of mineral crystallites and type-I collagen fibrils. The structure and mechanical properties of the mineral and protein constituents of bovine femur and antler were investigated and compared. Both the mineral and protein phases appeared identical at the macro-scale. The minerals were aligned in a coherent manner, forming a continuous network. The compressive mechanical properties of cancellous bone (untreated, demineralized and deproteinated) can be modeled as cellular solids. The compressive mechanical properties of pure protein and pure mineral samples are much weaker than original bone. The sum of the contributions from mineral and protein phases was far lower than that of the untreated bone, indicating a strong synergetic effect between the two phases. Hydration has a significant effect by lowering the elastic modulus and yield strength.

References

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Acknowledgment

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