

Variations in bone mineral properties with age and disease

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Keywords: Biomineralization, Bone Mineral, Mineral Analysis, X-ray Diffraction, Infrared Microspectroscopy

The mineral component of bone has two primary functions – mechanical and homeostatic. Bone apatite crystals reinforce the collagenous matrix giving it strength and rigidity, while also serving as a storage repository for Ca, Mg, and inorganic phosphate ions. To serve these purposes, these bone mineral crystals have a relatively narrow range of sizes (~ 100 Å), however these sizes, and the properties of the mineral in general, differ with tissue site as well as with age in health and disease. The mineral properties that vary include mineral composition, crystallite size and perfection, and crystallite orientation.

Recent evidence¹ suggests that while bone mineral density (BMD) is correlated with bone strength and fracture risk², it is not a sufficient predictor of whole bone mechanical properties. Currey suggested a series of equations relating measures of bone strength to parameters including geometry, architecture, and mineral content³. Similar components were found to contribute to both cortical and cancellous bone strength⁴. The equations were not perfect predictors of bone strength, and an additional unidentified term had to be added.

Here, I would like to suggest that not only the amount of mineral, but also the properties of the mineral, as defined above, must be considered as factors contributing to bone mechanical strength. These properties, also referred to as bone quality⁵ are generally referred to as intrinsic bone material properties. One example of how crystal properties can alter bone strength can be seen in the brittle bones of animals and people with osteogenesis imperfecta (OI). In OI not only is the matrix defective, but the crystals are abnormally small, have abnormal compositions, and may be found outside the collagen matrix⁶. To validate the hypothesis that mineral properties as well as mineral content affect bone mechanical properties it will be critical to know both the

mineral and mechanical properties of bones in health and disease. In this presentation I will review the methods for determining mineral crystal properties, selecting a few examples from studies of man and animals, discuss the limitations of these methods, and indicate how they support the hypothesis that bone mineral crystal properties are important contributors to bone mechanical strength.

Methods for determining bone mineral crystal size and perfection and orientation have different spatial resolution and requirements for sample preparation. The “gold standard” is wide-angle X-ray diffraction (XRD). XRD requires tissue homogenization, hence the spatial resolution is moot, however XRD shows the only mineral phase in bone is a poorly crystalline apatite whose particle size and perfection (crystallinity) increases with tissue and individual age throughout most of life⁷. Diffraction with synchrotron radiation provides similar information but, by contrast, has a spatial resolution of ~ 7 μm , and shows the mineral crystals adjacent to the Haversian canal are less crystalline than those distant from the canal⁸. Small angle X-ray scattering provides information on crystal volume and orientation, but assumptions must be made about shape, at approximately 100 μm spatial resolution⁹. Transmission electron microscopy (TEM) can have nm resolution and combined with selected area diffraction or dark field analysis can provide information on crystal phase and crystal orientation, but to characterize the mineral distribution in a bone hundreds of sections must be evaluated. Also it is a destructive technique in that the specimen may not be re-used. Preparation of bone for TEM requires many precautions to prevent acid dissolution of mineral crystals, and to prevent matrix interference¹⁰. When done under these conditions, mineral crystals have been shown to be thin plates, and to have similar shapes in a variety of species. Scanning electron microscopy (SEM) also has μm spatial resolution, requires the same nonaqueous tissue preparation, and provides information on crystal shape and agglomeration¹¹. Tomographic electron microscopy provides 3-dimensional views of mineral distribution, but less detailed insight into crystal size¹². These studies using independent techniques show the mineral crys-

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Accepted 1 August 2002

tals are aligned with the collagen matrix, but the alignment is not perfect. Atomic force microscopic studies of isolated bone crystals give three-dimensional information on crystal sizes which agree well with information calculated from XRD¹³ and with earlier suggestions of age-dependent changes in crystal width but not breadth from electron paramagnetic resonance¹⁴. Most of these methods, however, lack the spatial resolution needed to describe the distribution of mineral properties in bone.

Recently, data from spectroscopic methods (IR, Raman, NMR), coupled with microscopy and image analysis, have been correlated with XRD studies of model compounds. These correlations have been applied to the analysis of human and animal bones at 7-20 μm spatial resolution^{6,15-17}. While these techniques do not provide the direct phase identification given by XRD, the spatial resolution enables testing of hypotheses relating the variation in bone mineral properties to those in mechanical strength. The advantage of the IR and Raman microspectroscopy is that they provide information on mineral amount, crystallinity, and composition (carbonate and acid phosphate substitutions) and matrix chemistry (protein, lipids, proteoglycans) in a non-destructive manner at high spatial resolution.

In general, when processing artifacts are avoided in the aging healthy animal, it has been shown that crystals become larger in length and breadth, but not thickness. Most extreme changes in mineral crystal properties occur during embryonic development and then in the aging skeleton. With age crystals become more stoichiometric (fewer substitutions) and more oriented. Changes in carbonate and acid phosphate contents vary with species and tissue site, although this may be related to method of data presentation.

Not all diseased bone mineral resembles aging bone mineral, although this is the case in most tissues from humans and animal models of osteoporosis and osteomalacia, but certainly not osteopetrosis¹⁸ or hypophosphatemic rickets¹⁹. Gender, nutrition, genetics, and environmental factors also affect bone mineral properties. Nonetheless supporting the underlying hypothesis, in most cases where mechanical properties have been correlated with mineral properties^{5,11,16,20-22}, the hypothesis is validated.

For example, a study of growth hormone effects in the dwarf rat⁵ mineral density was most highly correlated with longitudinal and shear modulus, mineral crystal width and porosity correlated best with Poisson's ratio. In a rat model of osteoporosis, the antiresorptive drug tiludronate was shown to increase bone mineral content, mechanical strength, and width of crystals as measured by XRD¹¹. Correlating mechanical data from four-point bending tests with micro Raman analysis of bone mineral properties showed that the age-related increase in crystallinity was associated with decreased deformation to failure, i.e., increased brittleness¹⁶. In contrast, the increased crystallinity following fluoridation of 10-week-old bones was associated with increased deformation, i.e., increased ductility, perhaps due to the altered mineral composition¹⁶. In several transgenic

mice models to be discussed, altered torsional bone strength has been related to changes in mineral content, mineral quality, and matrix properties. It should be noted that because these mineral properties are not uniformly distributed within a given bone^{23,24}, correlations might have to be confirmed by mechanical tests that evaluate strength at specific sites.

Acknowledgment

Supported by NIH grants DE04141 and AR041325.

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