Mineral content is a poor predictor of mechanical properties in single bone structural units of human cortical bone

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Introduction There is increasing evidence for the role of the intrinsic bone tissue quality in skeletal fragility and the associated medical treatments. A current method for assessment of bone quality at the tissue level involves quantification of the local distribution of mineral content that is recognized as an important predictor of bone stiffness at the macroscopic level (Currey 1988). However, it remains unclear how mineralization affects mechanical properties at the tissue level. The purpose of this preliminary study was to evaluate the correlation between mineralization and mechanical properties of human osteonal and interstitial bone at the level of single bone structural units (BSU).

Methods Two slices of compact bone were cut perpendicularly to the long axis of the proximal femoral diaphysis (i.e. perpendicular to the Haversian channels) of an 86 year old female. Fourty-three osteons (single BSU) and seven regions of interstitial bone were identified in the posterior cortex of both slices. Mean degree of mineralisation of bone (MDMB) was determined by quantitative microradiography (Meunier and Boivin 1997, Boivin et al. 2000). MDMB was evaluated for each BSU excluding the vascular channels and represents therefore an average value for the matrix including the lacunar-canalicular porosity. Based on MDMB, the clinically used bone mineral content BMC can be calculated by correcting the effect of the vascular pores by

\[ \text{BMC} = \text{MDMB} \left(1 - V_{\text{vasc}}\right) \]  

Here \( V_{\text{vasc}} \) represents the volume fraction of the vascular channels, that was estimated to be 0.033.

Based on BMC the tissue density \( \rho_{\text{tissue}} \) can be calculated employing the relation

\[ \rho_{\text{tissue}} = \rho_o + \frac{\rho_m - \rho_o}{\rho_m V_{\text{matrix}}} \text{BMC} \]  

Here \( \rho_o=1.46\text{g/cm}^3 \) represents the density of osteoid, \( \rho_m=2.8\text{g/cm}^3 \) the density of the pure mineral phase and \( V_{\text{matrix}} \) of the matrix that was assumed to be 0.95 respectively. Indentation modulus and hardness of each BSU/region (after 24 hours drying at 50°C) were measured by nanoindentation using 5 tests at 0.9 micrometer depth. The indentation modulus represents the direct output of the indentation experiment when the deformation of the diamond tip (with known elastic constants) is subtracted,

\[ E_{\text{ind}} = \frac{E_{\text{Young}}}{1 - v^2} \]  

1 The bone structural unit (BSU) represents the end result of a remodeling cycle; in cortical bone, it constitutes a haversian system (or cortical osteon), and in cancellous bone, it is a wall or "packet" of bone or trabecular osteon (Eriksen et al. 1994).
which combines the Young's modulus $E_{\text{Young}}$ and the Poisson ratio $\nu$ for an isotropic material (Hengsberger et al. 2001). Hardness is defined by

$$ H = \frac{P(h_{\text{max}})}{A(c)(h_{\text{max}})} $$

and represents the mean pressure the material can resist. Here $P(h_{\text{max}})$ is the applied load and $A(c)(h_{\text{max}})$ the actual contact area between the diamond indenter and the material at maximum depth (Oliver et al. 1992).

The longitudinal wave modulus of the selected regions was characterized by scanning acoustic microscopy (SAM) at 50MHz under fully wet conditions. While scanning, the local impedance $Z$ of the material (back-reflected signal) and the sound velocity $v$ (transmitted signal) within the material are measured independently which allows for quantification of the longitudinal wave modulus with $C = Z v$.

For an isotropic material, the elasticity constant obtained by SAM depends on both Young's modulus and Poisson ratio (Briggs 1992)

$$ C = E_{\text{Young}} \left( \frac{1 - \nu}{(1 - 2\nu)(1 + \nu)} \right) $$

**Results** Each BSU exhibited an individual degree and a homogenous distribution of MDMB ranging from 1.38 to 1.71 g mineral/cm$^3$ with a relatively high mean value (± standard deviation) of 1.55±0.085 g mineral/cm$^3$. Indentation modulus ranged from 8.46 to 26.8 GPa with an average (± stdeva) of 19.5±4.6 GPa. Results for hardness varied between 0.29 and 1.28 GPa with a mean (± stdeva) of 0.77±0.17 GPa.

Indentation modulus and hardness were also homogeneous within single BSU but varied with high statistical significance between BSU (p<0.0001). Hardness showed some correlation with indentation modulus ($R^2=0.4$). Both indentation modulus ($R^2=0.38$) and hardness ($R^2=0.35$) showed a correlation with tissue density. The following power function was found for indentation modulus (see Figure 1 left)

$$ E_{\text{ind}} = 124GPa \left( \frac{\rho_{\text{tissue}}}{\rho_{\text{m}}} \right)^{8.1} $$

**Figure 1** Power function fit between indentation modulus and $\rho_{\text{tissue}}/\rho_{\text{m}}$ (left) and between indentation modulus and the longitudinal wave modulus obtained by SAM (right)
However, indentation modulus of distinct BSUs with the same average tissue density could show differences as high as 50%.

Longitudinal wave modulus obtained by SAM varied between 26.3 GPa and 52.7 GPa with a mean and standard deviation of 38.8±8.3 GPa and showed a moderate correlation with indentation modulus ($R^2=0.68$, see Figure 1 right) and with tissue density ($R^2=0.7$).

**Discussion** The high significance of BSU as a statistical factor in both indentation modulus and hardness confirms that beside representing the basic structural unit of the bone matrix and the associated remodeling process, the BSU represents also the homogeneous brick element of the bone construct in terms of mechanical properties.

Indentation modulus was about a factor two lower and correlated moderately with longitudinal wave modulus (SAM). Taking into account that the indentations were done on the dried sample and the acoustic measurements under fully wet conditions may even increase this factor. Setting equation (5) into (3) and assuming a Poisson ratio of $\nu=0.3$ shows that $C=1.23*E_{ind}$ that does not sufficiently explain this difference. It is probable that the viscoelastic behavior of the collagen fibers plays a major role at the strain rates applied by acoustic measurements.

All mechanical properties, hardness, indentation modulus and longitudinal wave modulus showed a correlation with MDMB (or the associated tissue density). Indentation modulus showed to be a power function of relative tissue density. The values of the unmineralized matrix and of the pure mineral phase of the evaluated power law in equation (5) correspond to $\rho_{tissue}=1.46$ g/cm$^3$ and $\rho_{tissue}=\rho_m=2.8$ g/cm$^3$ that gives $E_{ind}=0.64$ GPa and $E_{ind}=124$ GPa respectively. This asymptotic behavior fits quite well with typical modulus values for soft tissue and apatite crystals.

However MDMB explains only 38-70% of the variation of the SAM-modulus and indentation modulus and may not serve as a sufficient predictor of the mechanical properties at the level of single BSU. This finding implies that ultrastructural properties like the spatial arrangement of collagen fibers (Martin et al.,1989) and hydroxyapatite platelets also play an essentiel role in determining the mechanical properties of the BSU measured by nanoindentation. Further studies are needed to clarify this point.

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