## MODULUS LAGS INCREASING MINERALIZATION IN DEVELOPING EQUINE DENTAL ENAMEL

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The microstructural tissue organization and relative composition of organic, mineral, and water phases highly influence the nanomechanical properties of mineralized tissues. How these factors influence material properties at the ultrastructural and tissue level are studied using the developmental stages of equine tooth enamel. The tissue is formed by ameloblasts, the innermost layer of cells of the epithelial 'enamel organ', which secrete a noncollagenous protein matrix that begins to mineralize within minutes. This level of mineralization initially rises rapidly where the enamel crystals increase in diameter. The completion of enamel mineralization occurs long after the full thickness of the tissue has formed at any one developmental level on the tooth [1-5]. The result is a developing tissue with 'graded' mineralization.

An equine second mandibular molar (2-year-old) was embedded in PMMA, sectioned longitudinally, polished, carbon coated, imaged in qBSE (Zeiss DSM962 with Kontron external control computer). The second molar crown is still developing at this age. qBSE calibration standards used were monobrominated, monoiodinated and tetrabrominated dimethacrylates, as described by Banerjee and Boyde [6] and Kierdorf et al [7]. The entire enamel area of the sample, from the very youngest and least well calcified tissue to the most mature was imaged (nominal 36×: F.W. = 2475 microns) and montaged. Nanoindentation testing (UMIS2000, partial unloading, spherical tip with  $R = 2.5 \mu m$ , max load 10 - 20 mN) included two long arrays (columns of 5 indents x 25  $\mu m$ , 0.5 mm spacing) in the enamel (19 × 0.1 mm) and dentine (14.5 × 0.1 mm) to measure indentation modulus (*E*) [8], starting 3 mm from the growing tip and extended towards the occlusal surface.

The increase in modulus with enamel maturation lagged increasing mineral content and provided insight into the developmental biology of the enamel. The overall increase in the magnitude of the modulus with enamel maturation ranged from <20 GPa to >80 GPa while dentine maintained a near constant level of mineralization and modulus (~20 GPa). This sample provides a tissue that presents an endogenous, wide range in mineralization and contributes to our knowledge of how ultrastructural organization contributes to mechanical properties.

## **References:**

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