Improved Nanodimensional Analysis by Morphological Models Generated from Atomic Force Microscope Images

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Statement of Purpose: Designing biomimetic bone substitutes requires accurate knowledge of the structure of bone. At the nanometer scale, bone is made of collagen fibrils reinforced with apatitic mineralites. The two dimensional molecular scale packing of collagen within a plane cutting a longitudinal section through a fibril is well described by the Hodge-Petruska D-staggered array. However, the three dimensional stacking of these planes along the radial direction of the fibril remains unknown. It is within the void spaces of this 3D structure that much of the bone mineralite likely resides.[1] Thus, better knowledge of the size and shape of bone mineralites will allow us to put constraints on the geometry of this radial order within a fibril. In particular, void spaces within a fibril must be equal to or larger than the size of intrafibrillar mineralites. For this reason, we seek to determine the size and shape of bone mineralites. This is difficult because the mineralites are extremely small and thin. Atomic force microscopy (AFM) has the potential to obtain the 3D structure of nanoscale objects. Unfortunately, AFM produces inaccurate approximations of the objects under study. The largest source of error is due to AFM tips being large compared to imaged objects resulting in images containing substantial unwanted information about the tip. Previously, we used morphological erosion to handle this problem. In this project, we improve upon erosion by using a morphological modeling technique[2] to remove this tip-broadening artifact.

Methods: Morphological modeling works by proposing a guessed model with several adjustable parameters. The initial guessed model is dilated with the experimentally determined AFM tip to produce a simulated image. The simulated image is subtracted from the experimental image to obtain a difference image. The root mean square (RMS) error of the difference image quantifies the goodness-of-fit and directs adjustment of the model's parameters using a genetic algorithm (Genetic Algorithm Direct Search Toolbox, Version 1.0.3, Mathworks, Inc., Natick, MA). A set of previously collected AFM images of mineralites isolated from 1-3 month old bovines were used.[3] Initial mineralite lengths, locations, and angles were obtained using the particle analysis command in Scion Image® (Scion Corporation®, Frederick, Maryland). The bone mineralite images were then modeled using a 3D box model and an ellipsoid model. The box model was chosen because previous transmission electron microscopy work showed bone mineralites with platelet shapes. The ellipsoid model was chosen because the raw AFM images had ellipsoidal shapes. Both the box and ellipsoid models have six parameters: length, width, thickness, x and y locations of the center, and angle between the length and fast-scan axis of the image. Model-model comparison was performed to ensure that the optimal model can be found using the genetic algorithm.

Results / Discussion: Histograms comparing mineralite dimensions obtained using erosion (Fig. 1) vs. morphological modeling (Fig's 2 & 3) are shown below. Within one standard deviation, the thicknesses of all methods are the same. Differences between the eroded and modeled thickness result from the modeled values being accurate averages through the instrument noise field while the eroded values were drawn from peaks in the noise. The lengths and widths determined using the box model are significantly smaller than those obtained using erosion. Assuming no undercut structures, the box model places a reasonable lower bound on mineralite size. There is no significant difference between the RMS errors of the box vs. ellipsoid models. Thus, utilizing only the AFM information, we can not distinguish which of these two models is superior; both fit the data equally well.

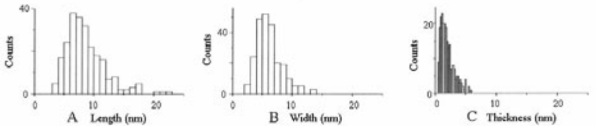

Figure 1: Dimensions from erosion: 9±3 nm (length) × 6±2 nm (width) × 2.0±1.2 nm (thickness) (N = 238).[3]

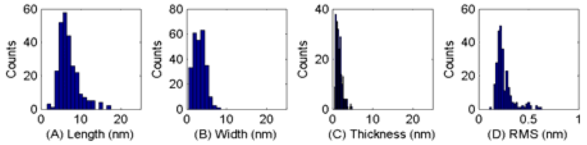

Figure 2: Dimensions from box model: 6.9±2.5 nm (length) × 3.2±1.5 nm (width) × 1.6±0.8 nm (thickness) (N = 261).

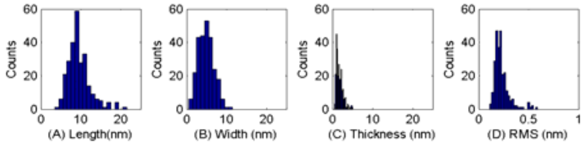

Figure 3: Dimensions from ellipsoid model: 9.5±2.8 nm (length) × 4.8±1.9 nm (width) × 1.7±0.8 nm (thickness) (N = 261).

Conclusions: Morphological modeling with appropriate models puts lower limits on mineralite dimensions. Since morphological erosion places upper limits on these dimensions, we now have better estimates of the range in which the size of young bovine mineralites fall: 6.9-9.0 nm (length) × 3.2-6.0 nm (width) × 1.6-2.0 nm (thickness). Morphological modeling removes most of the human element in making measurements from AFM images and generates characteristic dimensions using all the image data points instead of a few cross sections. This should improve agreement among analyses made by different investigators as well as the statistical significance of their results.

References:

1. Eppell SJ, et al., J Orthop Res. 2001;19:1027-1034.
2. Todd BA, et al., Biophys J. 2003;84:3982-3991.
3. Tong W, et al., Calcif Tissue Int. 2003;72:592-598.