
P13 – Scanning Force Microscopy of Reassembled Collagen Fibrils and Natural Fibrils in Cortical Human Bone

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Bone is a nanocomposite of proteins and minerals. At the molecular length scale the soft organic matrix (type I collagen) is reinforced by a stiff inorganic component (hydroxylapatite). Our study is focused on cortical human bone that is mechanically grinded, polished, and chemically etched prior to imaging with tapping mode scanning force microscopy (SFM). For comparison we study collagen fibrils reassembled from purified collagen isolated from bovine hide. In both specimens we find individual collagen fibrils with the typical D-band showing a periodicity of 67 nm. Measurements in moist air led to a controlled swelling of the purified collagen fibrils. The swelling was found to be completely reversible upon reduction of the ambient humidity. In contrast, the bone samples display no swelling under the same conditions. Additionally, the indentation of the SFM tip into collagen fibrils as function of the amplitude set-point was probed during swelling. We also performed bimodal SFM measurements; here the amplitude and the phase of the second flexural eigenmode of the cantilever were used for imaging while the amplitude of the first eigenmode was used as feedback signal. We will compare the results obtained on reassembled and natural collagen fibrils embedded in cortical human bone.

Scanning force microscopy of collagen fibrils: reassembled and embedded in cortical human bone

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Preparation

Collagen

Purified collagen isolated from bovine hide, 30 µl buffer solution (L-Glycin/KCl) at pH 9.2) deposited on freshly cleaved mica, 2 µl collagen solution (PureCol™) injected into buffer solution for 60 min (Ref. [1]), transfer of collagen to cleaned mica substrate or plasma cleaned silicon substrate.

Native human bone

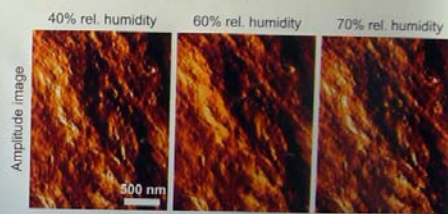
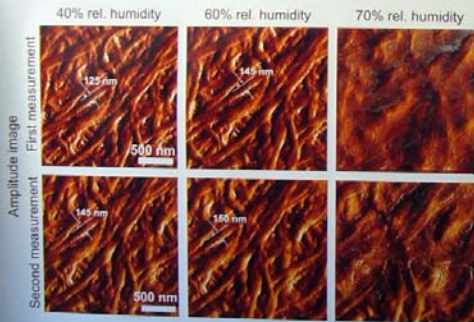
Specimens were cut (15 x 10 x 1 mm³) from a human femur, mechanically grinded and polished, etched with 1% formic acid for 10 s and rinsed with methanol or etched with RF plasma.

Measurements in moist air

Reassembled collagen

Embedded collagen in native human bone

Constant supply of moist air, stepwise increase of humidity; T = 26°C.



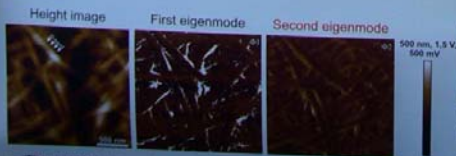
For reassembled collagen a swelling of the isolated fibrils can be observed; on the other hand for collagen embedded in bone no significant change is detectable.

Bimodal atomic force microscopy

Feedback: 1. eigenmode amplitude
 Imaging: 1. & 2. eigenmode phase Refs. [2,3,4]



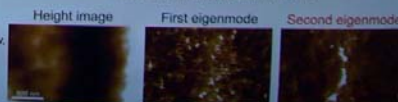
Collagen



The second eigenmode is less sensitive to height gradients than the first eigenmode; less feedback artefacts in phase image.



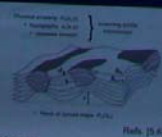
10 s etched with 1% HCOOH, flushed with CH₃OH.



After three plasma etching steps (air, 200 W, 500 m/min, 1 min); after each etching step, 1 d storage in ambient air.
 Better signal-to-noise ratio; more and sharper details.

Outlook

- Quantitative phase analysis
- Micromechanical information
- Material specific etching
- Additional information
- Volume image of human bone and collagen
- chemical or plasma etching



Acknowledgements

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