

Nano-Morphology of Cartilage in Hydrated and Dehydrated Conditions Revealed by Atomic Force Microscopy

Peizhi Zhu* and Ming Fang

Department of Chemistry, University of Michigan, Ann Arbor, MI 48109, USA

Abstract

In this study, Atomic Force Microscopy (AFM) was used to examine nano-scale structure difference in superficial and intermediate layers of bovine cartilage under dehydrated and hydrated conditions. The nanostructure of cartilage was quantitatively described using collagen fibril D-periodic spacing, fibril diameter, and surface roughness. Cartilage collagen fiber distribution was inhomogeneous in dehydrated condition but more homogeneous in hydrated condition. AFM images showed that the cartilage in dehydrated condition has higher roughness than that in hydrated condition. This study indicates that water is an important factor in maintaining the structure of extracellular matrix of cartilage. The loss of water can directly result in a rougher structure both on surface and bulk of articular cartilage. The morphology change of hydration-dehydration processes in cartilage is reversible.

Keywords: Cartilage; Collagen; Dehydration; Atomic force microscopy (AFM)

Introduction

The multi-component and highly hierarchical structure of cartilage is designed to adapt to the specialized functional requirements, including frictionless motion and load bearing at the joints [1,2]. Water has been reported to play a major role in controlling the dynamics of structural conformation of macromolecules and the function of cartilage and bone tissue [3-5]. In recent years, AFM has been used in studying nanostructure and nanomechanical properties of cartilage. Chan *et al.* [6] investigated the mechanism of friction in the boundary lubrication regime of articular cartilage using AFM. Stolz and coworkers [7,8] have shown that indentation AFM can detect aging and osteoarthritis induced morphological and nanomechanical changes in cartilage. To the best of our knowledge, no studies have specifically investigated the morphological differences of cartilage between hydrated and dehydrated conditions. Even though it is acknowledged that water plays a vital role in maintaining the structure and functions of cartilage, little is known at the submicron levels about how water affects the structure of cartilage. Therefore, in this study we used AFM imaging to address the morphological changes at nano-scale level in cartilage under hydrated and dehydrated conditions.

Bovine articular cartilage samples were harvested from a healthy young cow (about 6 months old) in a USDA-approved local slaughter house (Boyers Meat Processing, Canton, MI, USA) within 3 hours of death. The cartilage samples were immediately cut into about 2 mm x 2 mm square shape, and then embedded in Tissue-Tek optimal cutting temperature (OCT) solution (Sakura Finetek Inc., Torrance, CA, USA) and frozen at -20°C until use. Next, 10 µm thin-sections of cartilage were obtained using Microm HM550 Cryostat (Thermo Scientific Inc., Walldorf, Germany) and transferred onto Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA, USA). Sections that were at 30 µm and 300 µm depth from the articular cartilage surface were used in this study to represent superficial and intermediate layer of cartilage. The cartilage sections were rinsed with PBS buffer for 3 minutes and kept at -20°C prior to AFM study.

A Dimension Icon AFM (Bruker AXS, Santa Barbara, CA, USA) was used to image the dehydrated and hydrated samples. Scanasyt Fluid + probes (Bruker probes, nominal tip radius 2 nm, force constant 0.7 N/m) and scanasyt peak force tapping mode were used in both

dry and wet conditions. To image in water, a few droplets of ultrapure water were added to the 10 µm cartilage section. A droplet of water was also added to the AFM fluid cell in order to prevent air bubbles from forming. The specimens were soaked in water for 15 minutes before imaging. Water instead of PBS was used for fluid imaging, because 1X PBS buffer resulted in an unfavorable tip-sample interaction and consequently blurred images. Line scan rates were set at 1 Hz or lower at 512 lines per frame. Image analyses and measurements were performed using SPIP software (V5.0.8, Image Metrology; Horsholm, Denmark). Collagen fibril D-spacings were measured using 2D fast fourier transform (FFT) toolkit of SPIP software, detailed description and validation can be found in previous studies [9,10]. 3D topography images were generated from Nanoscope Analysis software (Bruker AXS, Santa Barbara, CA, USA). Surface roughness measurements were carried out using Nanoscope Analysis software too.

In order to visualize dehydration induced morphological changes on sub-micron scale, we combined tissue cryo-sectioning and AFM imaging, recommended by Graham and coworkers for high resolution soft tissue imaging with minimum sample preparation [11]. No fixation or chemical staining was used, which avoids tissue denaturation that often accompanies sample preparations of other imaging techniques. Superficial articular bovine cartilage (at 30 µm depth) imaged in water and air both showed a meshwork organization of bovine cartilage (Figure 1 and 2). The streaky artifacts in fluid imaging were likely caused by AFM tip scanning through loosely bound thin fibrils (Figure 1a and 2a). Two populations of fibrils with distinctively different diameters were found in fluid AFM images. The ~180 nm thick fibrils are indicated by white arrows and ~20 nm thin fibrils are indicated by black arrows in Figure 1b and 2b. Short segments of type II collagen fibrils with distinguishable D-banding patterns and roughly 180 ±

*Corresponding author: Peizhi Zhu, Department of Chemistry, 930 N. University Ave. Ann Arbor, MI, USA, E-mail: pzzhu@umich.edu

Received May 29, 2012; Accepted July 10, 2012; Published July 13, 2012

Citation: Zhu P, Fang M (2012) Nano-Morphology of Cartilage in Hydrated and Dehydrated Conditions Revealed by Atomic Force Microscopy. J Phys Chem Biophys 2:106. doi:10.4172/2161-0398.1000106

Copyright: © 2012 Zhu P, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

50 nm diameters were found both in water and air. In water fibril, D-spacing was measured 67.9 ± 1.2 nm (averaged from fibrils indicated by white arrows in Figure 1(b)). In dehydrated state, fibril D-spacing was measured 65.8 ± 0.8 nm (average from fibrils indicated by arrows in Figure 1(e)), in agreement with literature reports [12]. The sub-fibrillar structure of ~100-200 nm thick type II collagen fibrils has been described by Antipova and coworkers [12]. While in type I collagen based connective tissues, fibril bundles can have an axial length over 100 μ m; type II collagens in cartilage form a random 3D meshwork, which explains their appearance as short segments when observed on a flat surface. Both type I and type II exhibit similar D-periodic banding patterns [12].

The majority of type II collagen fibrils have average diameter of 180 ± 50 nm in both water and air imaging conditions; however, in water a significant portion of fibrils were found with much smaller diameters (20 ± 10 nm) (black arrows in Figure 1(b) to 2(b)). Although no D-banding patterns were found along these fibrils, we suspect they are also type II collagen fibrils. Distinctively thin fibrils were noted before and it was suggested that thin fibrils may serve as a precursor for fibrillogenesis [13]. The sub-fibrillar structure of ~20 nm thin-fibrils was described by Holmes et al. as a “10+4” arrangement of microfibrils, which contains a core of four microfibrils and a ring of ten surrounding microfibrils [13]. These 4 nm thin microfibril are constructed by five tropocollagens in the equatorial plane [13,14]. In our study, these thin fibrils were not as prevalent when dehydrated (Figure 1(d) to 1(f)), which prompt us to speculate that 20 nm thin fibrils may partially account for volumetric swelling of cartilage in water. As seen in Table 1, roughness analysis showed average roughness in water is 40 ± 10 nm, average roughness in dehydrated state is 10 ± 1 nm. The three-fold increase in roughness could be caused by swelling of microfibrils and bulk matrix in water. Nano-morphology of 300 μ m deep-layer articular cartilage is shown in Figure 3. Short segments of type II collagen fibrils were visible both in water and air conditions, however the D-spacings with could only be analyzed in the dehydrated state. Type II collagen fibril D-banding patterns are 65.6 nm, 66.9 nm and 68.2 nm in Figure 3e. In addition, we also noticed 20-nm thin fibrils disappearing when tissue is dehydrated.

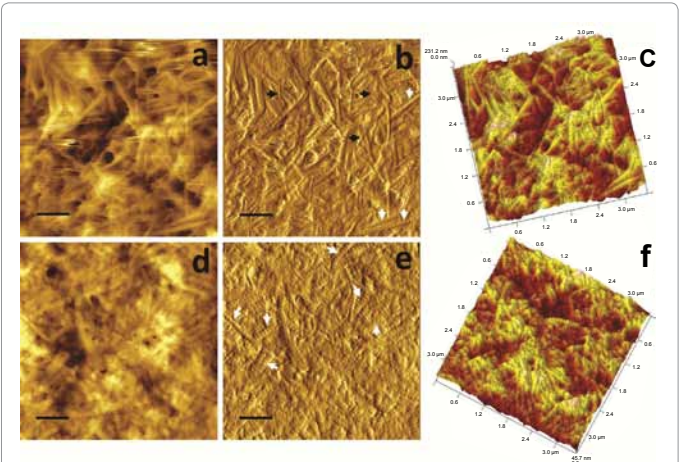
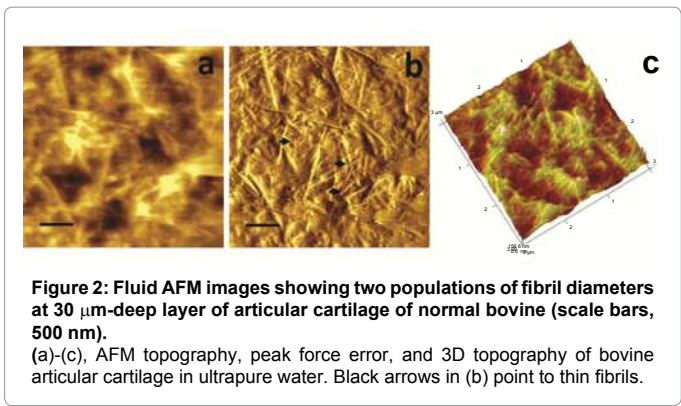


Figure 1: Nano-morphology of 30 μ m-deep layer articular cartilage of normal bovine (scale bars, 500 nm). (a)-(c) AFM topography, peak force error, and 3D topography of bovine articular cartilage in ultrapure water. (d)-(f) AFM topography, peak force error, and 3D topography of dehydrated bovine articular cartilage sections. White arrows in (b) and (e) point to thick fibrils with visible D-spacings which were recruited in the D-spacing analysis. Black arrows in (b) point to thin fibrils.



Physical Matrix	Fluid condition	Dehydrated condition
Type II Collagen Fibril D-spacing	67.9 ± 1.2 nm	65.8 ± 0.8 nm
Type II Collagen Fibril Diameter	180 ± 30 nm, 20 ± 10 nm	180 ± 50 nm
Average Roughness	9.93 ± 1.10 nm	41.0 ± 11.8 nm
Surface Area	$12.1 \mu\text{m}^2$	$20.2 \mu\text{m}^2$

Table 1: Morphological parameters measured from AFM images. All measurements were performed on 3.5 μ m size AFM images.

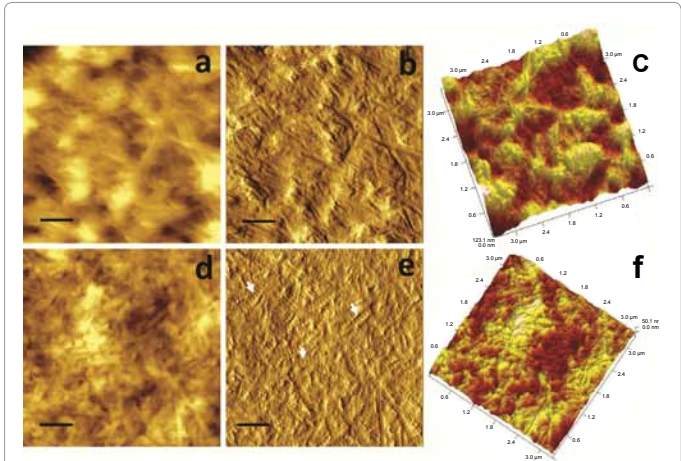


Figure 3: Nano-morphology of 300- μ m deep layer articular cartilage of normal bovine (scale bars, 500 nm). (a)-(c) AFM topography, peak force error, and 3D topography of bovine articular cartilage in ultrapure water. (d)-(f) AFM topography, peak force error, and 3D topography of articular cartilage sections imaged in air. Arrows in (e) point to fibrils with visible D-spacings which were recruited in the D-spacing analysis.

In summary, we applied AFM to study bovine articulate cartilage under dehydrated and hydrated conditions. The morphological differences between dehydration and hydration are reversible. The characteristic collagen D-spacing decreases with dehydration. Thin fibrils with 20 nm diameter were found in cartilage under hydrated condition but not obvious under dehydration conditions. Dehydrated surface roughness of cartilage is multifold that of hydrated surface. This study indicates that water plays an important role in maintaining the structure of extracellular matrix of cartilage.

Acknowledgements

The authors would like to thank Electron Microbeam Analysis Laboratory at University of Michigan for technical assistance with the Bruker Dimension Icon AFM. The authors acknowledge funding by NIH grant R01 AR056657.

References

1. Sophia Fox AJ, Bedi A, Rodeo SA (2009) The Basic Science of Articular Cartilage: Structure, Composition, and Function. *Sports Health: A Multidisciplinary Approach* 1: 461-468.
2. Cohen NP, Foster RJ, Mow VC (1998) Composition and dynamics of articular cartilage: structure, function, and maintaining healthy state. *J Orthop Sports Phys Ther* 28: 203-215.
3. Shapiro EM, Borthakur A, Kaufman JH, Leigh JS, Reddy R (2001) Water distribution patterns inside bovine articular cartilage as visualized by ¹H magnetic resonance imaging. *Osteoarthritis Cartilage* 9: 533-538.
4. Xu J, Zhu P, Morris MD, Ramamoorthy A (2011) Solid-state NMR spectroscopy provides atomic-level insights into the dehydration of cartilage. *J Phys Chem B* 115: 9948-9954.
5. Zhu P, Xu J, Sahar N, Morris MD, Kohn DH, et al. (2009) Time-resolved dehydration-induced structural changes in an intact bovine cortical bone revealed by solid-state NMR spectroscopy. *J Am Chem Soc* 131: 17064-17065.
6. Chan SM, Neu CP, Duraine G, Komvopoulos K, Reddi AH (2010) Atomic force microscope investigation of the boundary-lubricant layer in articular cartilage. *Osteoarthritis Cartilage* 18: 956-963.
7. Loparic M, Wirz D, Daniels AU, Raiteri R, Vanlandingham MR, et al. (2010) Micro- and nanomechanical analysis of articular cartilage by indentation-type atomic force microscopy: validation with a gel-microfiber composite. *Biophys J* 98: 2731-2740.
8. Stolz M, Gottardi R, Raiteri R, Miot S, Martin I, et al. (2009) Early detection of aging cartilage and osteoarthritis in mice and patient samples using atomic force microscopy. *Nat Nanotechnol* 4: 186-192.
9. Wallace JM, Chen Q, Fang M, Erickson B, Orr BG, et al. (2010) Type I collagen exists as a distribution of nanoscale morphologies in teeth, bones, and tendons. *Langmuir* 26: 7349-7354.
10. Wallace JM, Erickson B, Les CM, Orr BG, Banaszak Holl MM (2010) Distribution of type I collagen morphologies in bone: relation to estrogen depletion. *Bone* 46: 1349-1354.
11. Graham HK, Hodson NW, Hoyland JA, Millward-Sadler SJ, Garrod D, et al. (2010) Tissue section AFM: In situ ultrastructural imaging of native biomolecules. *Matrix Biol* 29: 254-260.
12. Antipova O, Orgel JP (2010) In situ D-periodic molecular structure of type II collagen. *J Biol Chem* 285: 7087-7096.
13. Holmes DF, Kadler KE (2006) The 10+4 microfibril structure of thin cartilage fibrils. *Proc Natl Acad Sci U S A* 103: 17249-17254.
14. Smith JW (1968) Molecular pattern in native collagen. *Nature* 219: 157-158.