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 about 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. Scanasyst Fluid + probes (Bruker probes, nominal tip radius 2 nm, force constant 0.7 N/m) and scanasyst 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 distinguishably 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 ± 2 lines per band are indicated by black arrows in Figure 1b and 2b.

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

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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 µm; 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 µm 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-spacing 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.

In summary, we applied AFM to study bovine articular 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.

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References