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Atomic Force Microscopy Study of Human Dentin Collagen Fibrils

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Abstract

Objective: The aim of this study is to observe human dentin collagen fibrils with Atomic force microscopy (AFM) in air. **Methods:** Freshly extracted caries-free human third molars were collected and stored in normal saline at℃4 until prepared. The midcoronal dentin specimens were sectioned and wet polished through a series of SiC papers, then the specimens were etched with 10%(w/w) citric acid for 15s and further treated with an aqueous solution of 10vol% NaOCl as deproteinizing agent for 120s to remove the non-collagenous proteins. Subsequently, the topography of dentin specimens was measured with AFM in air. **Results:** Images of dentin collagen fibrils were observed. Dentin collagen fibrils interweaved into a network in the intertubular dentin, and a cross-banding periodicity along collagen fibrils was about 67nm. **Conclusions:** Clear images of dentin collagen fibrils were obtained with AFM and the detailed structures of dentin collagen fibrils were measured accurately. AFM is a useful tool for real-time observation of dentin collagen fibrils.

Keywords

Dentin, collagen fibrils, atomic force microscopy (AFM)

Dentin constitutes the main body of teeth, which is mainly composed of dentin tubules, odontoblast processes and intercellular matrix. The mineralized dentin cell stroma is rich in fine collagen fibers. It is the main organic component of dentin and provides the strength and elasticity of the scaffold of dentin structure. This special structural characteristic of dentin determines its influence on the development of caries, the formation and aging of dentin bonding. It is of great significance to study the real state of collagen fibers in dentin. AFM technology is one of the important tools in nanoscience research. It is a direct surface morphology detection technology. Since it came out in the 1980s, it has been applied in many fields and played a great role in the research of biomaterials. Its advantage is that the sample does not need special pretreatment and can be observed directly in air or liquid environment [1]. There is no relevant report on the observation of dentin collagen fibers by AFM in China. In this paper, AFM was used to study the imaging of collagen fibers in dentin.

1. Materials And Methods

1.1 preparation and treatment of dentin samples

With the informed consent of the patient, fresh third molars (aged 20 ~ 28 years, regardless of gender) that need to be removed due to orthodontics were taken, the periodontal ligament was removed, and stored in 4 ℃ normal saline before use. Cut the dentin at a certain part of the crown perpendicular to the long axis of the tooth to make a flat dentin sheet with uniform thickness. The observation surface shall be wet polished with silicon carbide abrasive

paper in the order of 600 mesh, 800 mesh, 1000 mesh, 1500 mesh, 2000 mesh, 2500 mesh, 3000 mesh and 5000 mesh, etched with 10% (mass ratio) citric acid for 15 seconds, then treated with 10% (volume ratio) NaOCl solution for 120 seconds, washed with double distilled water, and AFM observation shall be carried out immediately. The observed samples were treated with 10% (volume ratio) NaOCl solution for 4 minutes and 10 minutes respectively, washed with double distilled water, and observed by AFM respectively.

1.2 AFM imaging observation

AFM (Veeco / Di, nanoscope IV a, USA) was used to scan dentin samples. The samples were dried with nitrogen before scanning, fixed, and scanned in tapping mode. The surface morphology changes of the same dentin sample before and after acid etching and treated with NaOCl solution were observed by AFM. The acquired image data are processed and analyzed by AFM's own software.

2. Results

(1) The scanning images of AFM morphology of dentin samples before and after acid etching are shown in Fig. 1a and Fig. 1b respectively. The AFM images before acid etching clearly showed dentin tubules, peritubular dentin and inter tubular dentin (Fig. 1a); The AFM image after acid etching showed that the dentin tubules became thicker at 10 μ m × ten μ No collagen fibers were found in the dentin between the tubes in the scanning range of M (Fig. 1b); And in 2.5 μ m × two point five μ Within the scanning range of M, the structure of collagen fibers can be seen faintly (indicated by the arrow in Fig. 1c). The scanning image of AFM after acid etching and then treated with NaOCl solution for 120 seconds is shown in Fig. 1D. It can be seen that collagen fibers are intertwined in the dentin between tubes, and collagen fibers are surrounded around the dentin tubules and their lateral branches. Figure 1E shows the scanning range 2 μ m × two μ. The characteristic periodic transverse striations of collagen fibers were clearly visible, the collagen fiber network collapsed in the air, and the collagen fibers were close to each other; Fig. 1F is a mixed mode three-dimensional image corresponding to Fig. 1E.

(2) In Fig. 2a, the scanning range is 600 nm × 600 nm, the section of collagen fiber along the long axis was analyzed by using the software of AFM. In the section, the surface of collagen fiber showed a regular corrugated shape, and the height between peak and trough was about 4 nm; The horizontal distance of five consecutive cycles was 339.8 nm, and the calculated periodic structure width of collagen fibers was about 67 nm (Fig. 2b).

(3) Figure 3A shows the height of AFM surface morphology of dentin samples treated with NaOCl solution for 4 minutes. There are still periodic transverse lines of collagen fibers on the surface of dentin. Fig. 3b is the height map of AFM surface morphology of dentin samples treated with NaOCl solution for 10 minutes. The dentin surface is uneven, and there is no characteristic collagen fiber periodic transverse structure.

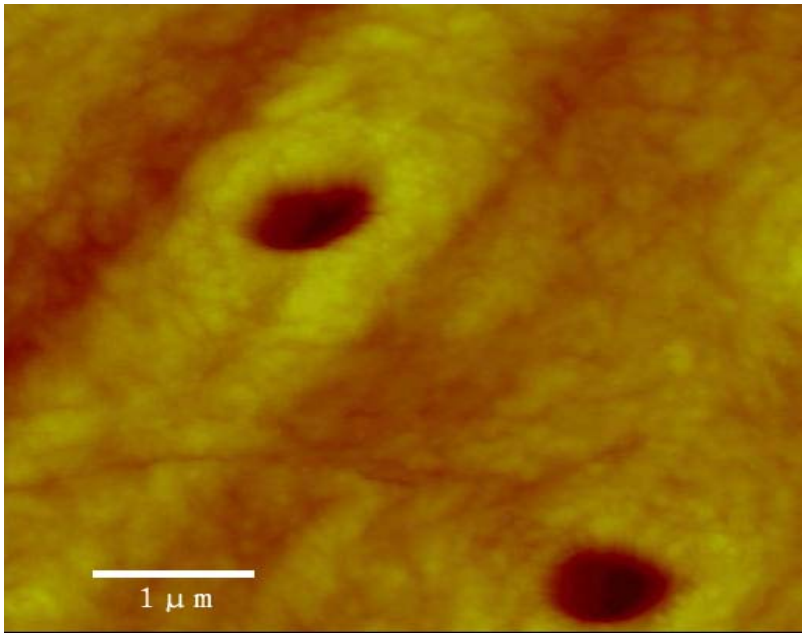

Figure 1A. (10μm×10μm)The AFM images before dentin etching clearly showed dentin tubules, peritubular dentin and inter tubular dentin.

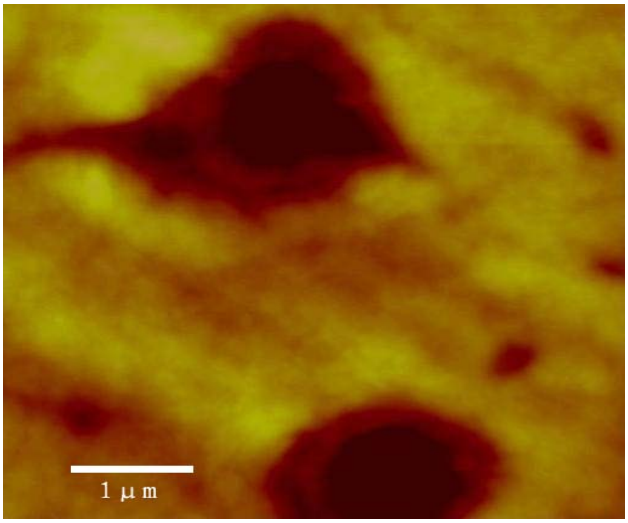

Figure 1B. (10μm×10μm) The AFM images after dentin etching showed that the dentin tubules became thicker and the collateral tubules were visible due to the removal of the smear layer.

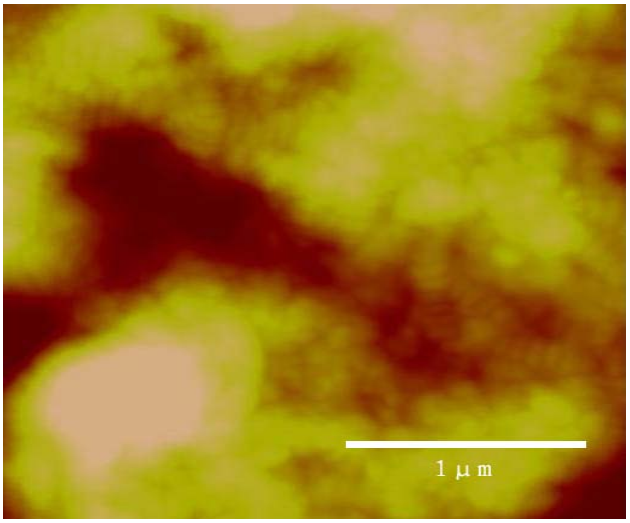

Figure 1C. (2.5μm×2.5μm) In this scanning range, the structure of collagen fibers can be seen faintly in the dentin after acid etching (indicated by the arrow).

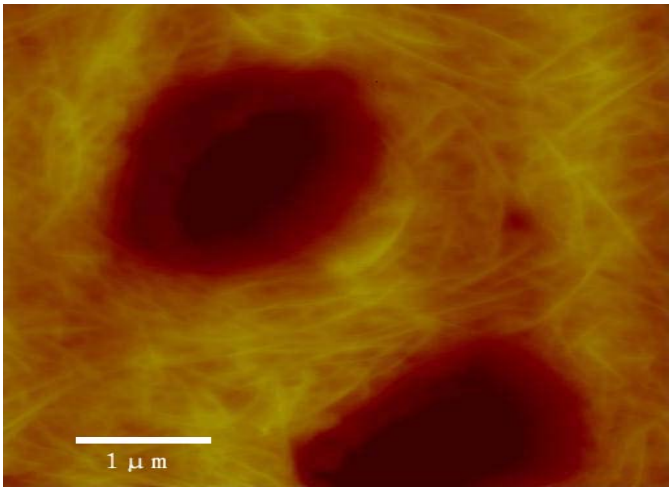

Figure 1D. (10μm×10μm) The AFM morphology treated with NaOCl solution after dentin etching shows that the collagen fibers in the dentin between the tubes are intertwined into a network.

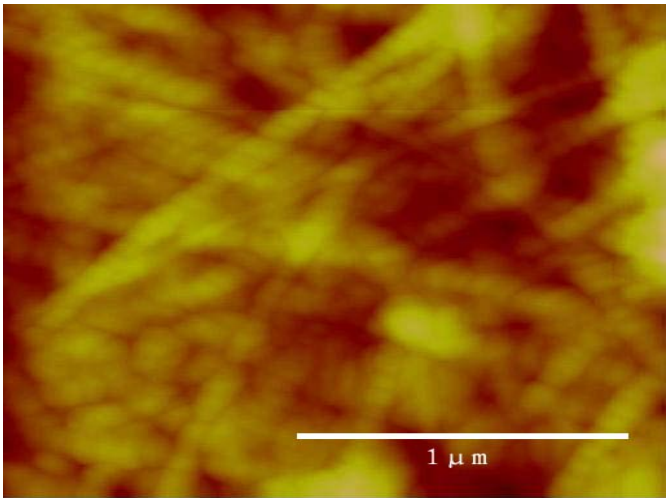

Figure 1E. (2μm×2μm) The characteristic periodic transverse striations of collagen fibers were clearly visible.

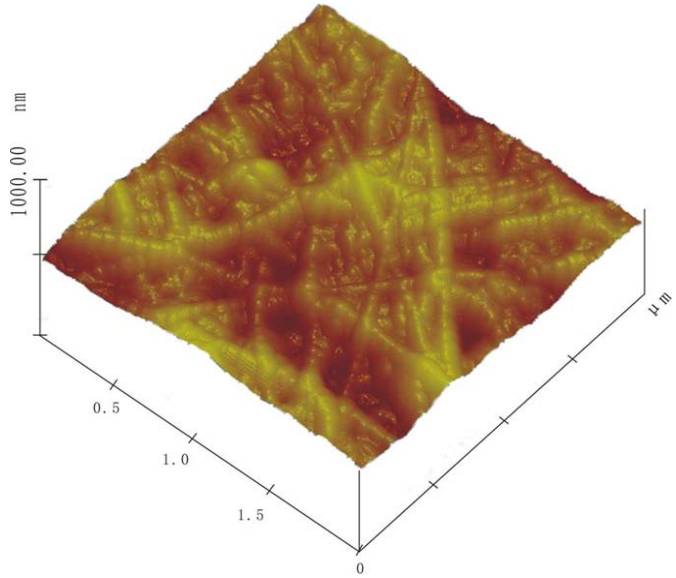

Figure 1F. (2μm×2μm) It is a mixed mode three-dimensional image corresponding to Fig. 1E.

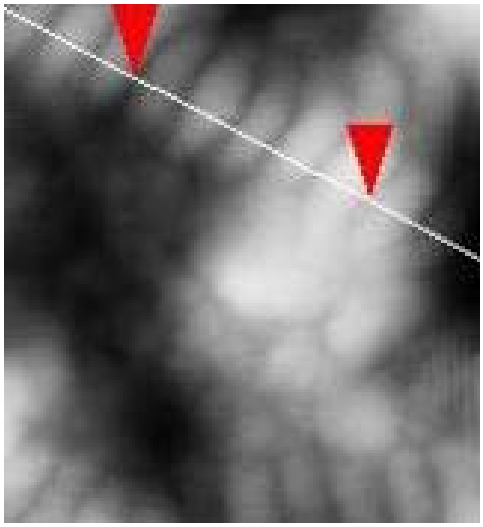

Figure 2A. (600nm×600nm) The location of the collagen fibers cut during the section analysis.

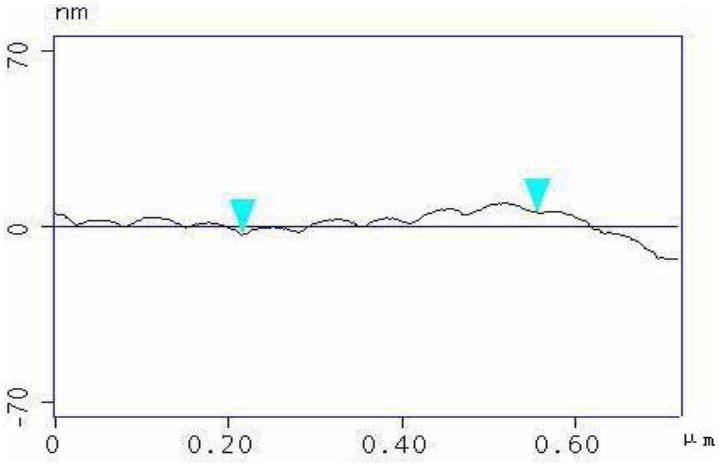

Figure 2B. In the sectional view, the surface morphology of collagen fibers showed periodic ripple shape, and the width of periodic structure was about 67 nm.

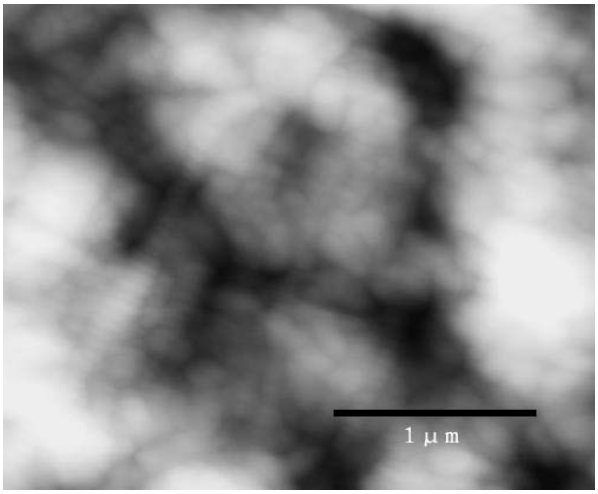

Figure 3A. (3μm×3μm) The AFM images of the dentin samples treated with NaOCl solution for 4 minutes showed that there were still periodic transverse striations of collagen fibers on the dentin surface.

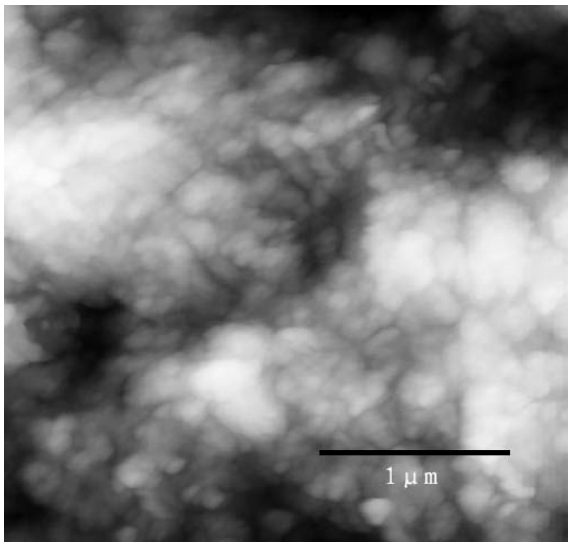

Figure 3B. (3μm×3μm) The AFM images of the dentin samples after acid etching treated with NaOCl solution for 10 minutes showed that the dentin surface was uneven and no characteristic collagen fiber periodic transverse striation structure was found.

3. Discussion

The content of organic components in dentin is high, accounting for 30% by volume, of which 91% ~ 92% are collagen, mainly type I collagen and 8% ~ 9% are non collagen matrix. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are the traditional methods to study dentin collagen fibers, but the samples need to be pretreated, such as gold spraying, dehydration, vacuum pumping and dyeing, which is easy to destroy the surface structure of the samples. Especially for the imaging scanning of collagen fibers, because collagen fibers are organic matter, the above treatment causes dehydration of collagen fibers and collapse of fiber network, which can not truly reflect their morphological characteristics, and the samples after the above treatment are not suitable for follow-up research. AFM does not need the above special treatment, and carries out real-time and real-time observation on the sample [1], reflecting the real morphology of the sample surface. Observation in natural air or liquid environment can obtain the real structure of the sample surface, and continuous observation and imaging of the same sample can be carried out, which is the unique advantage of AFM.

There are mineral wrapped collagen fibers, and their characteristic collagen structure can not be observed [2]. The results showed that after acid etching, the characteristic transverse structure of collagen fibers could be observed by AFM scanning. However, due to the adhesion between non collagen components and collagen fibers, it interferes with the scanning imaging of collagen fibers by AFM. Collagen fibers can not be observed in large-scale scanning. Therefore, how to eliminate the interference of non collagen matrix components is very important to form a clear AFM scanning image of collagen fiber network. In this process, NaOCl solution is an ideal treatment agent to remove non collagen matrix components.

NaOCl solution is a common irrigator for root canal therapy and the main component of chemical caries removal drugs. NaOCl forms peroxide group ($O2^{-}$) in aqueous solution, induces single electron oxidation reaction, and breaks the long chain of protein peptides; At the same time, it can also chlorinate the protein terminal group to form n-chloramine and degrade [3], forming nonspecific protein hydrolysis. In the initial stage of NaOCl solution treatment, non collagen in dentin organic matrix is easy to be removed first, and with the extension of treatment time, the structure of collagen fibers will be destroyed finally [4]. The results of this experiment also reflect this point. However, compared with the relevant literature, it is worth discussing the concentration of NaOCl solution. Some studies believe that 3% NaOCl solution can more effectively expose the periodic structure of collagen fibers [5], while the concentration of NaOCl solution used in the study of Stefan habelitz et al. [6] is 6.5%. Both of them have typical collagen fiber structure when the treatment time of NaOCl solution is 100 ~ 120 seconds. The experiment was treated with 10% NaOCl solution for 120 seconds, and the experimental results were also ideal. However, Stefan habelitz et al. Pointed out in their research that the collagen fibers were completely removed when treated with 6.5% NaOCl solution for more than 240 seconds, while in this experiment, the collagen fiber structure was still visible after treated with NaOCl solution for 240 seconds. The reason for this difference may be related to the temperature, operation method and the concentration of effective NaOCl components in the stock solution for preparing NaOCl solution. In addition, the differences between different ethnic groups and different teeth may also affect the results.

Although collagen fibers can be observed after acid etching without polishing [7], ideal collagen fiber network imaging results cannot be formed. One thing is certain that the structure of collagen fibers will not be damaged after acid etching. Our assumption is that according to the characteristics of AFM, the dentin surface requires a certain flatness. After acid etching, the dentin surface demineralizes to form a thin layer of organic matrix, and then removes the non collagen components, leaving collagen fibers. The morphology of the dentin surface fluctuates little, which is conducive to AFM probe scanning imaging. From the experimental results, ideal AFM results of collagen fibers can be obtained under the conditions of polishing procedure and sample processing operation.

Collagen fibers are composed of finer fibrils or finer micro fibrils. Fibrils or micro fibrils are composed of procollagen molecules arranged in parallel and orderly. The transverse structure is caused by the stepped arrangement of procollagen molecules [8]. In different states (dry, wet, thermal crosslinking, etc.), the width of the periodic transverse structure of collagen fibers will change [6, 9]. In this experiment, the transverse structure width of collagen fiber obtained by section analysis is about 67 nm, suggesting that collagen fiber is not dehydrated. Therefore, in the experiment, AFM scanning must be carried out immediately after treatment to obtain the information of collagen fiber structure closest to nature. AFM collects the three-dimensional information of the sample surface and has accurate observation function. With the improvement of AFM probe tip preparation technology, the probe has higher sharpness and aspect ratio, and AFM can obtain more subtle structural information of the sample.

The structure of dentin is special, and the collagen fibers are closely related to dentin bonding repair, the devel-

opment of caries and the study of biomineralization. AFM real-time imaging observation of the structure, spatial arrangement and real state of collagen fibers is helpful to better understand the changes of the structure and function of collagen fibers caused by bonding acid etching, bonding aging or caries. In this experiment, only the collagen fibers in normal human dentin were measured, and the state of collagen fiber network in air was observed. The imaging observation of dentin collagen fibers by AFM provides a platform for subsequent related research.

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