Analysis of the Mechanical Properties of Chromosomes in Air and Liquid by AFM

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***Abstract*—Chromosomes contain all the genomic information thus making the study of chromosomes practical and significant. Herein, the morphologies of chromosomes prepared with different methods were monitored by atomic force microscopy (AFM). Moreover, the mechanical properties of chromosomes in air and liquid were investigated quantitatively through AFM-based force spectroscopy. The differences of chromosome morphologies and mechanical properties caused by sample preparations indicated that the chromosome characterization in liquid was more meaningful to reveal the physiological characteristics of chromosomes.**

Keywords— atomic force microscopy, chromosome, morphology, mechanical properties

I. INTRODUCTION

Chromosomes are the carriers of genetic materials during cell divisions, where the structures of chromosomes play an important role in gene regulation [1-3]. In the process of cell divisions, DNA replication can be affected by abnormal or mutated chromosome structures, which will indirectly lead to a series of diseases in life [4, 5], such as Down syndrome [6] and malignant tumors [7-9]. In the metaphase of mitosis, chromatin is highly spiralized, shortened, and thickened to form chromosomes [10]. Metaphase chromosomes are usually used in the karyotype analysis to diagnose chromosomal diseases based on their morphological and number changes [11]. Therefore, the research of chromosome structures is one of the most important tasks to improve the quality of life.

In the past decades, researchers have used many analytical methods to study chromosomes, such as chromatin immunoprecipitation (ChIP), fluorescence in situ hybridization (FISH), single cell gel electrophoresis (SCGE) and sister chromatid exchange (SCE) [9, 12, 13]. The immunofluorescence method proves that the deletion of trichoplein (TpMs) will lead to wrong chromosome separation, DNA damage and chromosome instability in cancer cells [14]. Sabatini et al. identified the family pathogenic mutant genes by G banding and FISH analysis of the target region of chromosome 21 [6]. Although the cytogenetic methods can analyze chromosomes, they generally depend many biochemical reagents and the optical microscopy. The consumes of biochemical reagents are not only complicated, but also have certain impacts on people and the environment. In addition, due to the diffraction limit of optical microscopy, the structures of chromosome cannot be observed with nanometer resolution [15-20].

Nowadays, AFM is used in the field of biological sample imaging with the spatial resolution of 0.02 nm and the force resolution of about 10 pN. Compared with transmission electron microscopy (TEM) and scanning electron microscopy (SEM), the sample preparation for AFM detection is simple [21, 22]. Furthermore, the AFM quantitative mapping of multiple parameters provides the mechanical information of biological samples in liquid environments [23-25].

Atomic force microscopy (AFM) has made remarkable achievements in the nanoscale observation and manipulation of chromosomes [26, 27]. Tamayo et al. [28] studied the morphology of chromosome before and after dehydration by AFM, and further obtained the compression mode of chromatin. Fan et al. [29] used AFM to investigate the ultrastructural organization of G1-phase premature condensed chromosomes (PCC) and proved that the chromatin fiber volume changed during mitosis. Hoshi et al. [30] applied AFM to image human metaphase chromosomes in liquid, and the results suggested that the method could be used to study the effect of chemical treatments on chromosomes in relation to the structural changes. In addition, Bucchianico et al. [26] used AFM as a tool for manipulating single chromosomes to generate individual cell specific genetic probes. Recently, an automatic image analysis method, QuantAFM (Quantification of AFM images), was used to extract the geometric features of filamentary structures from AFM images and made a detailed statistical analysis of the structural features of mononucleosome [20]. Although a lot of research has been done where the AFM acted as a powerful tool for chromosome characterization and manipulation, there is still a need to pay more attention to the mechanical properties of chromosomes.

In this work, we prepared the chromosome samples for AFM mapping in air and liquid environments, respectively. The quantitative images of chromosome structures were obtained by the AFM-based characterization. The mechanical properties of chromosomes in air and liquid environments were analyzed. And the statistical analysis of the mechanical properties in different parts of the chromosome provides a new method for studying chromosomal structures.

II. Method and Experiment

A. Chromosome Sample Preparation in Air

HaCat cells were purchased from the Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences (IBCB) (Shanghai, China), and identified. HaCat cells were cultured in the DMEM medium (Gibco Life Technologies, USA) together with the fetal bovine serum (FBS) (10% v/v), penicillin (100 U/mL) and streptomycin (100 U/mL) in a cell culture incubator (Yamato, Japan) at 37°C and 5% CO_2. The HaCat cells after cultivation were harvested in metaphase by adding colchicine (Sangon Biotech, China) to the culture medium at the final concentration of 0.05 μg/ml for 1 h. The cell suspension was then exposed to 75 mM KCl for 30 min at 37 °C and fixed with the Carnoy's solution (methanol–acetic acid 3:1 v/v). The chromosome spreads were then formed by dropping the cell suspension onto the glass slide [31].

B. Chromosome Sample Preparation in Liquid

In the preparation of liquid sample, the colchicine was added to the culture medium at the final concentration of 0.06 μg/ml for 12 h, which was different from the concentration and time in air. The cells were centrifuged for 10 min at 4℃, resuspended as a pellet in 10 ml of the culture medium for 20 min at 4℃, centrifuged again for 10 min, and then exposed to 75 mM KCl for l5 min. The cells were finally collected by centrifugation and isolated using the hexylene glycol method [21].

C. Measurements

To investigate the morphologies and mechanical properties of chromosome samples prepared by different methods, an AFM (JPK, Nano Wizard 3, Germany) was used. The images were sensed by a MLCT probe (Bruker, the spring constant of 0.07 N/m) and acquired in the quantitatively imaging (QI) mode. By fitting the Hertz model, the mechanical parameters of chromosomes were calculated.

III. Results and Discussions

A. AFM Mapping of Chromosomes

In this work, we analyzed the mechanical properties of chromosomes in air and liquid environments by AFM (Fig. 1). After extracted by different methods, the chromosomes in the air or buffer were then measured by AFM to analyze the chromosome surface morphology, height, adhesion force and Young's modulus.

B. Chromosomal Morphologies in the Air

In order to study the morphologies of chromosomes in the air (Fig. 2A) and in the buffer (Fig. 2B), the chromosomes prepared with different methods were observed by AFM, respectively. Then two cross-section lines were chosen (distances a-b and c-d) from the AFM images to show the structural differences.

The process of natural drying and dehydration changed the surface of the chromosomes, presenting the loss of obvious surface structures and the decreased average height to 125 nm. The maximum disparity between the peak and valley of the chromosome surface in the air was 38 nm. The possible reasons could be the cover of a protein film produced by the standard cytogenetic preparation [32] and structural shrinkage caused by dehydration. Therefore, the ultrastructure of chromosomes was unavailable with altered structures.

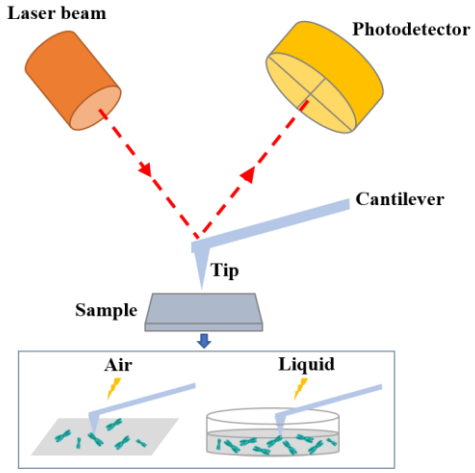

Fig. 1. Schematic diagram of chromosomes detection by AFM.

C. Chromosomal Morphologies in Liquid

Compared with the results in air, the chromosomes in liquid were much closer to their physiological states and can be observed by AFM (Fig. 2B). The height image shows the obvious surface fluctuations caused by the original spiral structure which is further quantitatively characterized by the cross-section analysis. Specifically, the surface of chromatids in liquid is shown by the alternating presence of dark and bright bands. The average height of chromosome is 580 nm, which is much larger than that in air. And the peak-valley disparity is 400 nm. The characteristics are more obvious than dried chromosomes and do not require any treatment.

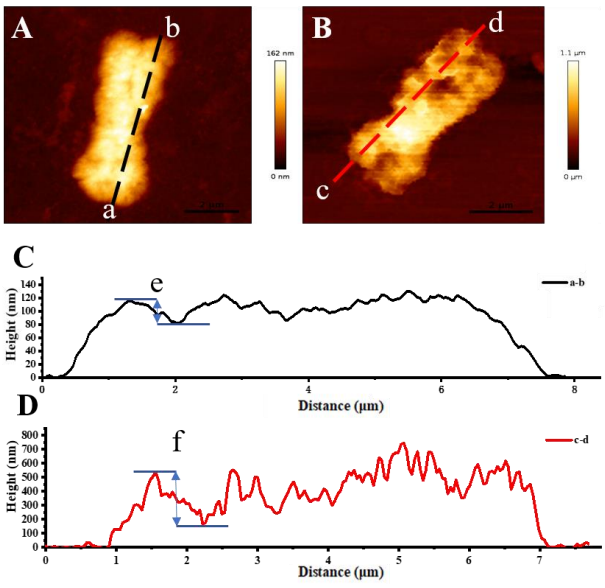

Fig. 2. AFM images of the chromosomes in the air (A) and in the buffer (B). The corresponding height profiles (C) and (D).

D. Mechanical Properties of the Chromosome in Liquid

AFM allows an in-situ topographical imaging of the chromosome in liquid and analyzing of the mechanical properties by force spectroscopy. This provides the new methods for the chromosome structure analysis.

We tested the mechanical properties of the dried chromosomes as shown in Fig. 3, and the results showed no analytical significance because the mechanical properties of the chromosomes were almost the same as the base of the glass slide. Thus, the chromosomes in liquid were mechanically detected and analyzed statistically.

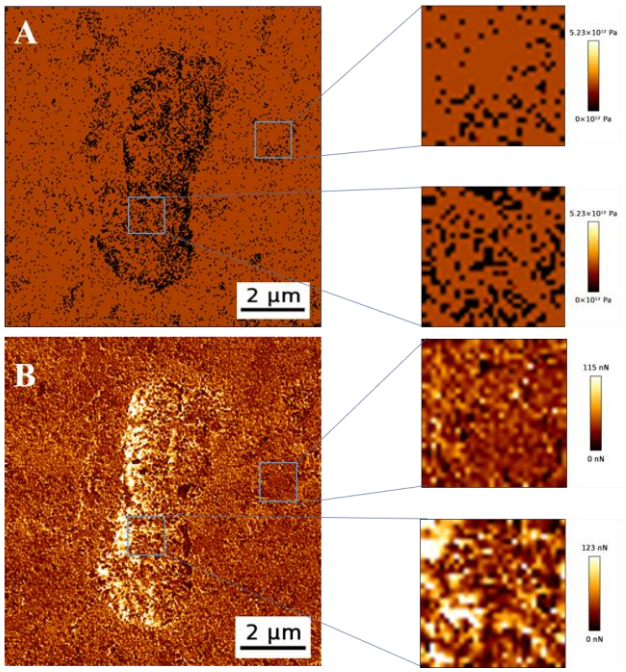

Fig.3. The adhesion image (A) and Young's modulus image (B) of dried chromosome.

Height. Figs. 4A&B showed the AFM height image and the statistical analyses of the chromosomes in the liquid environment, respectively. As shown in Fig. 4A, the chosen chromosome was visualized and showed the high chromatin density in the axial regions. The topography of the chromosome with rough surface structures indicated its high compaction level.

Forty points from each of ten chromosomes were selected to investigate the differences between different areas of interest, such as the centromeres (a), and sister chromatids intermediate (b), chromatids (c) and marginal (d) areas. The charts in Fig. 4B show the height distributions and the average data of different areas. The results show that the chromatid area is the highest while the margin area is the lowest. The height of the centromere area is higher than the sister chromatids intermediate. The quantitative trend of the height is consistent with the result of the chromosome topography images. The height distribution may be due to the different entanglements with the chromatin and the content of genes.

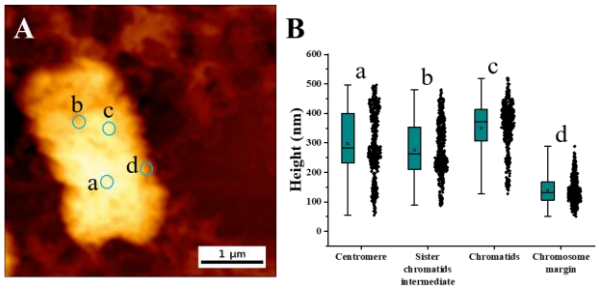

Fig. 4. Chromosomal height image (A) and statistical analysis of height (B).

Besides the morphology, the mechanical properties, adhesion and Young's modulus, could reflect chromosome microstructures.

Adhesion. The adhesion force is the measured interaction between the probe and the chromosome surface as shown in Fig. 5A. The darker areas in the picture correspond to less adhesion, and the lighter areas correspond to greater adhesion. The adhesion image shows the adhesive distribution caused by the different compositions on the chromosome. As shown in Fig. 5B, the margin area has the highest adhesion, while the chromatid area has the lowest adhesion. The average adhesion of the sister chromatids intermediate is higher than the centromere area. The chart in Fig. 5B shows the data distributions of the surface adhesions. Different ways of chromatin winding and compressing lead to different adhesions on the chromosome surfaces. The charts in Figure 5B show the data distribution of chromosome surface adhesion discontinuity. This phenomenon is due to different chromosome sizes, resulting in great changes in the surface adhesion.

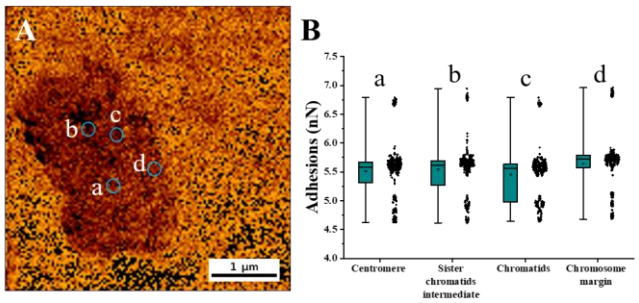

Fig. 5. Chromosomal adhesion image (A) and statistical analysis of adhesion (B).

Young's modulus. As shown in Fig. 6A, the Young's modulus distribution image was obtained. The darker area corresponds to a lower Young's modulus value, which represents a smaller hardness value. A statistical analysis for the Young's modulus was also performed. The chart in Fig. 6B shows the distribution of Young's moduli and their average values from different chromosomal areas. It can be seen that the chromatids and centromere areas are relatively harder, and the sister chromatids intermediate and margin areas are softer.

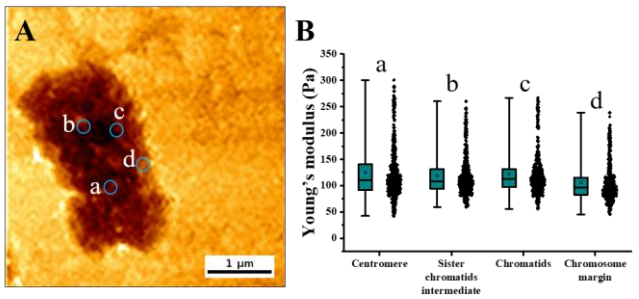

Fig. 6. Chromosomal Young's modulus image (A) and statistical analysis of Young's moduli (B).

Chromosomes are composed of DNA, RNA, histones and non-histone proteins in a special and complex winding manner. Thus, the smaller Young's moduli in the sister chromatids intermediate and margin areas are caused by the loose entanglement of chromatin. The magnitude of Young's modulus could reflect the tightness of chromosome

structure entanglements.

IV. Conclusion

In summary, besides the morphology measurements, the chromosomal mechanics, including the adhesion and Young's modulus, have been investigated by AFM. The AFM results and statistical analyses have illustrated that the structures of chromosomes can not only be reflected through high resolution images, but also can be quantitatively and mechanically characterized. Our experimental results are helpful for exploring high order structures of chromosomes.

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