Extended Abstracts of the 2006 International Conference on Solid State Devices and Materials, Yokohama, 2006, pp. 784-785

P-11-2

Development of a Functional Chromosome Nano-dissection System Using Porous Anodic Alumina Pattern Chip and AFM Cantilever

Do-Kyun Kim[1], Masato Saito[1], Young-Soo Kwon[2] and Eiichi Tamiya[1]

[1]School of Materials Science, Japan Advanced Institute of Science & Technology,
1-1, Asahidai, Nomi, Ishikawa 923-1292, Japan
Phone; +81-761-51-1598 Fax: +81-761-51-1665 E-mail: dkim@jaist.ac.jp
[2] Department of Electrical Engineering, Dong-A University,
840, Hadan-2dong Saha-gu, Busan 604-714, Korea

1. Introduction

The dissected chromosomes can be used in various conventional applications, including establishing probes for fluorescence *in situ* hybridization (FISH), the generation of chromosome band-specific libraries and physical mapping for cytogenetic analysis [1]

In the present study, we developed a functional chromosome nano-dissection system that can dissect various chromosomes in one-chip by using the pattern chip with nano-scale porous anodic alumina. This chromosome nano-dissection system can dissect directly a specific chromosome into a nano-scale pore without AFM dissection process. Thus, we expect that are chromosome nanoarray chip is very suitable for arraying chromosome fragments without the recovery process with the possibility of direct immobilization of chromosome fragments into nano-scale pore of this chip.

2. Experimentals

For the preparation of the porous anodic alumina membrane for nano-scale pattern chip, we developed a two-step anodizing method [2].

In order to smooth the surface morphology, the aluminum sheets were mechanically polished with 6, 3, 1, and 0.25 μm diamond suspensions. After rinsing in acetone, ethanol and distilled water, the aluminum sheets were electrochemically polished in H_3PO_4 + H_2SO_4 + H_2O (8.5 : 1 : 0.5) + CrO_3 (35g/l) for 10 min at 70 ^oC. The first anodization was conducted under constant voltage (40 V) in a 0.3 M aqueous oxalic acid solution for 1 h. After the first step anodization, the generated the anodic alumina was removed by immersing the sample in a solution comprised of a mixture of phosphoric (1.8 wt%) and chromic acids (2 wt%). After removal of the anodic alumina, a textured pattern of concave was obtained on the surface of aluminum (Al) sheets. Then, the second anodization was carried out with the same conditions for 1 h as described above.

Metaphase chromosomes were prepared by dropping the cell suspension onto nano-scale pattern chip, and air-drying until use.

All AFM experiments were performed using a commercial AFM unit equipped with a calibrated 20- m xy-scan and 10- m z-scan range PZT-scanner. The chromosomes that were penetrated into the nano-pores on the pattern chip by pressing with a cantilever of AFM were dissected to nanometer size. The dissection was carried out in the following conditions: for the dissection of chromosome by AFM, the contact mode was used. The resonant frequency of the cantilever was 315 kHz and the scan speed was 0.01 μm/sec. The amplitude reference was kept in –0.5.

3. Results and Discussion

The Fig. 1 (a), (b) shows typical AFM topographic images with measurement sizes (1 μm and 200 nm) of pattern chip that was established by the two-step anodizing process. Diameters of the nano-pore and the cell were about 60 nm (1.2×10^{10} pores/cm^2) and 120 nm, respectively. The walls of about 45 nm in height were established in all sides regularly, so that the stability of chromosome fragments after the dissection was expected. The nano-pores could be seen clearly. Besides, the nano-pores of various sizes were also obtained using different input voltages.

Fig. 2 (a) shows a typical topographic image of human metaphase chromosomes that are recognized as several types of chromosomes. The image area is multiplied 25 by 25 μm. the dark-brown (umber) of the image shows the surface of pattern chip with nano-scale porous anodic alumina. Fig. 2 (b) shows a typical topographic image of human metaphase chromosomes that are recognized as one type of chromosomes in Fig. 2 (a). The candidate of chromosome in Fig. 2 (b) is parts of groups E or F and numbered as 16, 19 or 20 because they were about 4-5 μm in length and had a metacentric centromere. The cross-sectional AFM analysis corresponding to diagonal line (A and B in Fig. 2 (b)) is shown in Fig. 2 (c). The height information in non-contact mode is calibrated just as for the height information in the contact mode. We could confirm that the height of chromosome was about 104 nm before by pressing with the cantilevers of AFM.

The AFM images of a dissected chromosome that was penetrated into the nano-pores on the pattern chip by pressure with the cantilever of AFM are shown in Fig. 3 (a), (b). For the dissection of chromosome by pressing with the cantilevers of AFM, the contact mode was used. The height of chromosome, that was similar to the height of porous anodic alumina by measurement of AFM, was now about 46 nm after pressing with the cantilever of AFM. Thus, we observed that the dissection of a chromosome was achieved for further DNA and the protein nanoanalysis of the chromosome fragments, because the pressure that was applied on all surface of the pattern chip by the cantilever of AFM was enough to

dissect the chromosome.

4. Conclusion

The development of a novel chromosome dissection system is described that could dissect various chromosomes by using the pattern chip with nano-scale porous anodic alumina. We have proceeded as follows; the pattern chip with nano-scale porous anodic alumina was fabricated. Then, metaphase chromosomes were prepared by dropping the cell suspension onto pattern chip and air-drying until use. Finally, the chromosomes that after penetrated into the nano-pores on the pattern chip by pressing with a cantilever of AFM were dissected to nanometer size.

This technique will form the basis for the development of a new system called as ″Nano-scale chromosome dissection chip″ that arrays chromosomal fragments with nano-scale on a chip for DNA and protein nanoanalysis.

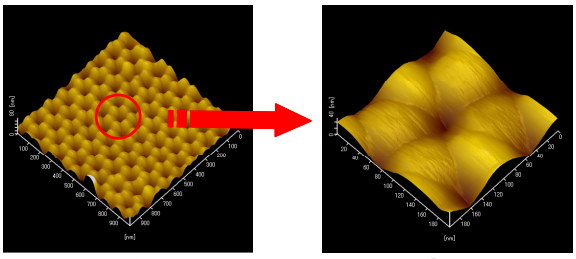
Acknowledgments

This work was supported by the Post-doctoral Fellowship Program of JAIST in Japan.

References

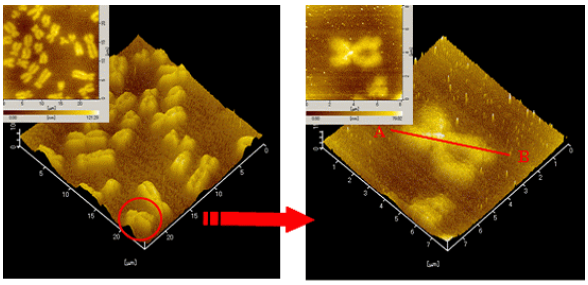
[1] G.P. Bates, B.J. Wainwright, R. Williamson, S. D. M. Brown, *Mol.Cell Biol.*, **6**, 3826-3830 (1986).

[2] H. Masuda and M. Satoh, *J.Appl. phys*, **35**, 126-129 (1996).

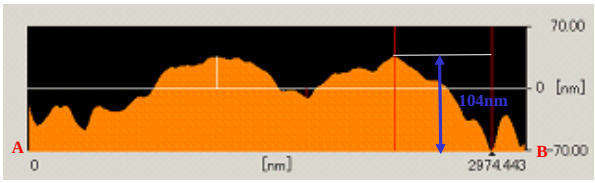


(a) (b)

Fig 1. AFM images of nano-scale pattern chip obtained with two-step anodization method using oxalic acid. (a) typical AFM topographic image as to measurement size (1 μm) (b) typical AFM topographic image of central area on Fig. 1 (a) as to measurement size (200 nm)

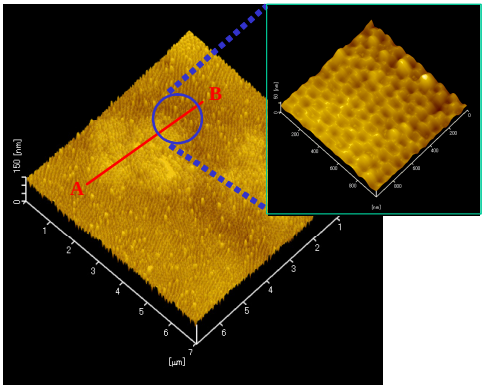


(a) (b)

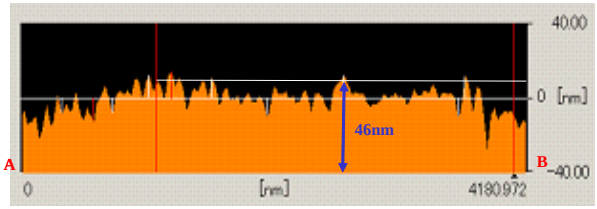


(c)

Fig. 2. Non-contact mode AFM image displaying topography of human metaphase chromosome on the pattern chip with nano-scale porous anodic alumina. (a) A typical topographic image of human metaphase chromosomes that are recognized as several types of chromosomes. (b) A typical topographic image of human metaphase chromosomes that are recognized as one type of chromosomes in Fig. 2 (a). (c) Cross-sectional AFM analysis corresponding to diagonal line (A and B in Fig. 2 (b)).



(a)



(b)

Fig. 3. AFM images of a dissected chromosome that was penetrated into the nano-pores on the pattern chip by pressure with the cantilever of AFM. (a) A typical AFM topographic image of dissected chromosome with the measurement sizes of 7 μm and 1 μm. (b) Cross-sectional AFM analysis corresponding to diagonal line (A and B in Fig. 3 (a)).