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Development of DNA chip nanoarray by Fluidic Self-assembly method for Detection of DNA Hybridization

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1. Introduction

In this paper, we report a new approach for DNA chip nanoarray fabrication. Multifunctional DNA chip nanoarray was fabricated by immobilizing many kinds of biomaterials on nano-transducers (nano-particles). DNA chip nanoarray was prepared by randomly distributing a mixture of the nano-particles on a nano-scale pattern chip (porous anodic alumina (PAA)) containing thousands of nm-scale sites. The nano-particles occupied a different sites from site to site. The nano-particles were arranged on the nano-scale pattern chip by the random fluidic self-assembly (RFSA) method.

2. Experimentals

Fabrication of nano-scale pattern chip. For the preparation of the nano-scale pattern chip with porous anodic alumina, we developed a two-step anodizing method[1]. The aluminum sheets (Aldrich 99.999%, 25 ×65×0.5 mm) were degreased in acetone, and then annealed at 480℃ for 40 min to remove mechanical stresses and recrystallize. Subsequently, to smooth the surface morphology, the aluminum sheets were mechanically polished with 6, 3, 1, and 0.25 ㎛ diamond suspensions. After rinsing in acetone, ethanol and distilled water the aluminum sheets was electrochemical polishing in $H_3PO_4 + H_2SO_4 + H_2O$ (8.5 : 1 : 0.5) + CrO_3 (35g/l) for 10 min at 70℃.

The first anodizing was conducted under constant voltage (40 V) in a 0.3 M aqueous oxalic acid solution[2] for 5hours. The anode temperature was kept constant 10℃ and the electrolyte was vigorously stirred during anodizing in order to maintain temperature and electrolyte concentration uniformity. The details of the mechanism of the ordered formation of the holes in the textured aluminum (Al) are not clear at present, it is thought that each convex can induce the independent formation of a hole due to its geometrical effect. After the first step anodizing 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 anodic alumina a textured pattern of concave was obtained on the surface of aluminum (Al) sheets. Then the second anodizing was carried out under it again with same first anodizing conditions for 1hours.

Immobilization of probe DNA on nano-particles. All nano-particles (with diameter of 60nm) used in this study were fabricated by CRL Co. in UK. The nano-particles having the activated carboxyl groups were immersed in the aqueous buffer solution (pH7.9, 10mM Tris-HCl, 0.2M NaCl) of avidin (100μg in 1mL) for 1hour. The avidin was immobilized on the surface of nano-particles after rinsing with aqueous solution several times. The nano-particles coated avidins were immersed into an aqueous solution (1mL) of ethanol-amine (1M) for 30min, so as to deactivate the carboxyl group as β-hydroxyethylamide. The nano-particles coated avidins were immersed into 1mL of the aqueous buffer solution (pH7.9, 10mM Tris-HCl, 0.2M NaCl) of biotinylated oligonucleotides at 25 °C for 1hour, the nano-particle was picked up to control the immobilization amount. The biotinylated DNA strands were found to bind to one out of four binding sites of the avidin molecules. The immobilized amount of biotin-DNA was controlled by the immersion time in DNA solution.

Arrangement of nano-particles on nano-scale pattern. All nano-particles (with diameter of 60nm) used in this study were fabricated by CRL Co. in UK. We dropped suspension (Ethanol 90% + Distilled water 10%) with 1μl concentration that was contained nano-particles having both various immobilized DNAs onto the nano-scale pattern chip. Then, nano-particle group was subsided to the nano-scale pattern chip using RFSA by gravity, and nano-particle group were arranged in each nano-pore on the nano-scale pattern chip randomly. An integration type DNA chip nanoarray was able to fabricate simply, stably, high-throughput and cost-effectively by this process. The nano-scale pattern chip and the DNA chip nanoarray were then imaged using commercial atomic force microarray (AFM).

3. Results and Discussion

To obtain an ordered hexagonal array of the pores on nano-scale pattern chip a double oxidation step method has been developed, which uses oxalic acid (HOOC-COOH) as oxidizing agent and an oxidation voltage 40V. An analogous two-step method was tested which in principle has the advantage of higher oxidation rate and lower voltage operation values[3].

The Figure 1 (a), (b) shows typical AFM topographic image as to measurement size (1μm and 200nm) of nano-scale pattern chip that was established by the two-step anodizing process [40V, 1h, 0.3 M $C_2O_4H_2$, 10℃]. Diameters of the nano-pore and the cell are about 60nm (1.2×10^{10} pores/cm^2) and 120nm, respectively. The

wall of 45nm in height is established in all sides regularly, and the stability of the particles after the arrangement is expected. The nano-pores could be seen clearly. Besides, the nano-pores of various sizes were also obtained using changing of input voltage. However, a demonstration of the difficulties of measuring the real shape of the pores, even at their upper part, is given in Figure 1 (c), (d) where cross-sectional AFM analysis corresponded to the diagonal line (A and B) are shown. The line profile is formed by a set of cones instead of the expected cylinder shaped internal pore. This deviation of expected shape results from the inability of the probe tip to go inside the pores due to its conical geometry with a relatively low aspect ratio. Following of Figure 1 (d), it is evident the very short penetration of the tips into the nano-pores when the expected deepnesses of nano-pores are several micrometer.

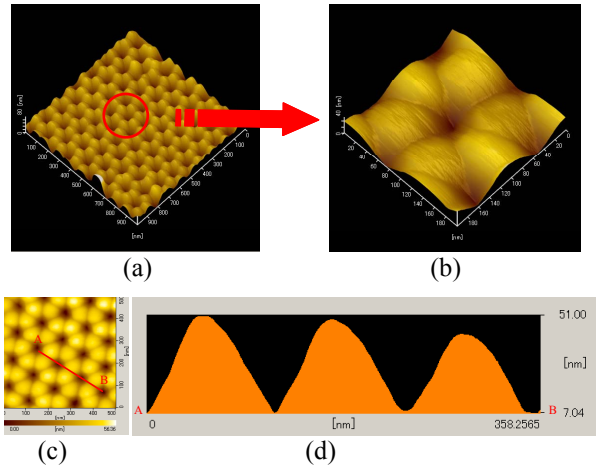

Fig 1. AFM images of nano-scale pattern chip obtained with two-step anodization method using oxalic acid. (a) typical AFM topographic image as to measurement size (1μm) (b) typical AFM topographic image of central area on Fig. 1 (a) as to measurement size (200nm) (c) typical 1-dimensional AFM image with the diagonal line A and B (measurement size 500nm) (d) cross-sectional AFM analysis corresponded to diagonal line (A and B in Fig. 1 (c))

The integration type DNA chip nanoarray that nano-particles are arranged on the nano-pores of nano-scale pattern chip using RFSA by gravity with water-pattern sites for assembly in a suspension was fabricated. The Figure 2 (a) shows the typical AFM topographic image as to measurement size (3μm) of DNA chip nanoarry that was established by RFSA using gravity. The arrows are indicated nano-particle group that were arranged in each nano-pore on the nano-scale pattern chip randomly. However, the probability that the nano-particles arrange in the nano-pores of nano- scale pattern chip was about 5~10%. It seems clear that nano-particle group were not arranged perfectly in each nano-pore on the nano-scale pattern chip because of low concentration of suspension with 1μl. Instead of that, the number of nano-particle was increased by a higher concentration of suspension that was dropped each nano-pore on the nano-scale pattern chip. The Figure 2 (b) shows typical AFM topographic image of central area (with two nano-particles) in Figure 2 (a) as to measurement size (500nm). The nano-particles were arranged firmly and 3-dimensionally due to the surrounding wall (about 45nm). However, although the diameter of all nano-particles used in this study was 60nm, the diameter of nano-particles was about 80nm in Figure 2 (b) because DNAs were immobilized on the surface of nano-particles.

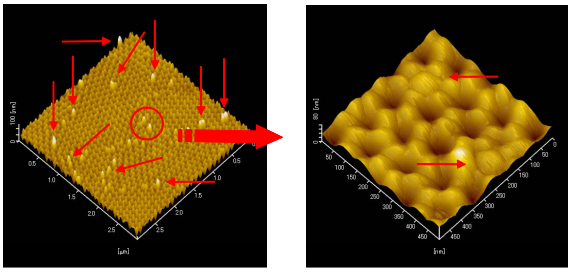

Fig 2. AFM images of DNA chip nanoarray that nano-particles are arranged on the nano-pores of nano-scale pattern chip using RFSA by gravity. (a) typical AFM topographic image as to measurement size (3μm) of DNA chip nanoarry (arrows indicate nano- particle group). (b) typical AFM topographic image of central area (with two nano-particles) in Figure 2 (a) as to measurement size (500nm).

4. Conclusion

We have used the RFSA technique based on the chip pattern of hydrophobic self-assembly layers to assemble microfabricated particles onto the chip pattern. Advantages of this method are process simplicity, wide applicability and stability. This method can be applicable as a new fabrication technology to develop an integration type biosensor microarray. We are presently extending this work to various biosensor microarrays.

Acknowledgments

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