A new visual investigation into nanogold-based genechip assay by atomic force and scanning tunneling microscope

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Conformations of surface atoms in various stages of nanogold-based genechip testing are scanned by the atomic force and scanning tunneling microscope. We intuitively observe the process and differences in probe combination, nucleic acid hybridization, and silver staining, which might be useful to validate the assay method of genechip. We hope to use this technology to make the other invisible chemical or biochemical reaction become visible and convincible in the future.

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The detectable signals of biological samples marked with nanogold instead of fluorescence and isotope materials can be effectively enlarged in genechip assays and well documented[1,2]. Genechip assays based on nanogold technology have more potential benefits than the other methods[3−5]. Atomic force and scanning tunneling microscope (AFM-STM) works through the mechanisms of atomic force microscope combined with scanning tunneling microscope. It can be used to observe conformations of surface atoms and widely used in nano-biology and nano-medicines[6−8]. For example, it can be used to directly assay nucleus, proteins, cells, antibody, antigen, microorganisms, and genechips. In this letter, we investigate the conformations of surface atoms in various stages of nanogold-based genechip testing scanned by the AFM-STM.

Genechips were prepared according to the previous methods[9,10]. In brief, probe of staphylococcus aureus (ATCC 25923) was designed as 5'-tta gta gta ccg aag ctg gtc at-3' modified with the radical ($-NH_2$) in 5'-end (Takara, Dalian). The radical ($-NH_2$) in probe was chemically combined with the radical (–CHO) of formylphenyl slides (CEL Associates Inc., Poland). Through this chemical bond, probe can be fixed on formylphenyl slides.

Primers for polymerase chain reaction (PCR) were designed as follows. Sense: 5'-gtc ggt aca cga tat tct tca cg-3'; anti-sense: 5'-ctc tcg tat gac cag ctt cgg tac-3', whose 5'-end was lingered with the radical (–SH) (Takara, Dalian). PCR products were amplified by using DNA fragment of Staphylococcus aureus (ATCC, USA) as templates. The radical (–SH) in the amplified fragments was combined with the Monomaleimido-nanogold particles (diameter d=1.4 nm) (Nanoprobes Inc., USA) by covalent bond[9,10].

The amplified DNA fragments marked with nanogold particles were hybridized with the probes fixed on slides. Nanogold particles can be combined with silver (Nanoprobes Inc., USA) by metal-phase response. Silver aggregated on nanogold particles and formed a bigger brown inclusion, which made the signals of hybridization enlarged[9,10].

AFM-STM (IPC-205BJ, Chongqing University, Chongqing) was used to scan surface conformation in various stages of nanogold-based genechip testing, including the surface atomic configuration of formylphenyl slide before the probes were covered, that of genechip after the probes were covered, that of genechip after the target nucleic acids were hybridizated, and that of genechip after silver was stained. The results are shown in Fig. 1.

The surface atoms of genechip slide (formylphenyl glass) were in a regular porous-arrangement (Fig. 1(a)). After the probes were combined, the arrangement of surface atoms in genechip slides became irregular (Fig. 1(b)). After hybridization with the target nucleic acid marked with nanogold particles, surface atoms in genechip slides were in a relatively regular cable-like arrangement (Fig. 1(c)). However, after silver was stained, the surface atoms showed a larger protuberant mass (Fig. 1(d)).

To our knowledge, it is the first study to intuitively observe the state of the chemical response in genechip by AFM-STM. STM is an instrument for atom size surface analysis working on the principles of tunnel effect through assaying the Fermi-energy graded electronic cloud density. However, the detected samples must be conductor or

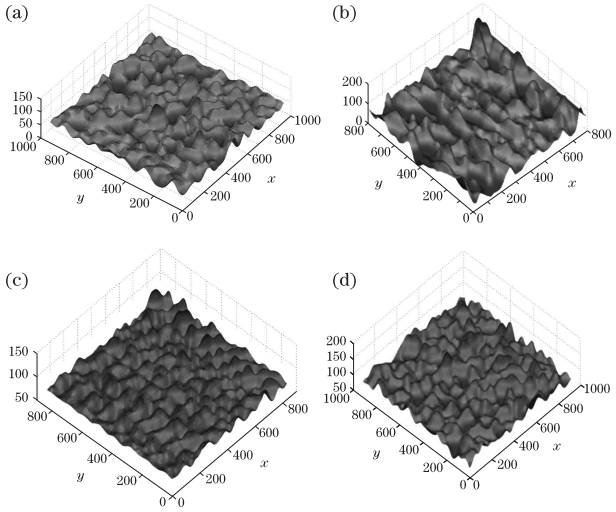

Fig. 1. AFM scanograms of surface conformation in various stages of nanogold-based genechip testing. (a) Conformation scanogram of surface atomic configuration of formylphenyl slide before combined with the probe (8.1×8.1 (nm)); (b) that of genechip after combined with the probe (7×7 (nm)); (c) that of genechip after hybridizatation with target nucleic acid (7.1×7.1 (nm)); (d) that of genechip after stained by the silver staining (8.1×8.1 (nm)).

semiconductor, which limits its applications. Fortunately AFM based on STM[11−13] can be used to observe the surface conformation of non-conductor samples through the interaction between atoms. AFM is able to observe the atomic arrangement and electronic behavior on the surface of samples[14−16], which is widely applied to observe the structures of cell and biological molecules in real time.

The AFM-STM we used worked on the principles of both STM and AFM[6−8]. Surface conformation information was obtained through documenting the weak atomic force between probes and samples and the computational models. The surface atoms of gengchip slide (formylphenyl glass) were in a regular porous-arrangement before the probes were combined, because the genechip slide surface has been treated as smooth as possible at first. After the probes were covered, the atoms had different structures and distributions in genechip slides. So, the arrangement of surface atoms in genechip became irregular. After hybridization with the target nucleic acid marked in nanogold particles, the probes and target DNA formed two-strand structure. So, surface atoms in genechip slides displayed in a regular intense cross cable-like arrangement. Furthermore, lots of silver particles were combined with nanogold and formed a large mass. So, the surface atoms showed a larger protuberant mass structure with silver staining.

In conclusion, we intuitively observe the process and changes in probe combination, nucleic acid hybridization, and silver staining by AFM-STM, which might be useful to validate or trace genechip assay in a visual way. Also, we hope to use this technology or updated methods to make "blind" chemical or biochemical reaction become visible and convincible in the future.

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