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Bio-Nanotechnology Through Microsystems

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1. Introduction

Integration of biochemical/chemical system on a chip, sometimes called μTAS (micro Total Analysis System) or Lab-on-a-Chip, is an emerging area of micro-technology, and is expected to bring about high-speed, low-inventory, low-cost, on-site diagnosis and synthesis of chemical substances. As a limiting case of the miniaturization, one may conceive a system that targets single molecule. Advantages of a single-molecule-based system is not only limited to its ultimate sensitivity: by being able to trace a molecule, one can make observations, say of biological/biochemical processes, with space resolution on real-time bases. This is in sharp contrast to existing biochemical methods where only statistical average in a solution is of concern. Amongst many biological molecules, DNA is the best candidate for single-molecular processes, not only because it is the basic drawing of the living things, but also it can be amplified easily. As many copies as you want can be made based on modifications made on single molecule.

The single-molecule-based system requires proper manipulation techniques. Recent advances in micro- and nano- technology, including a) micro-fabrication, b) electrostatic manipulation, c) laser tweezers, d) micro-positioning, e) scanning probe microscopy, together with f) molecular binding and patterning techniques, and g) visualization techniques such as fluorescence microscopy in combination with new fluorescent probes, have reached to such a stage that individual molecules can be manipulated by physical means. DNA having 3000 base-pairs (3kb) has the length of 1μm (0.34 μm/kb), which is well within the reach of recent microfabrication capabilities.

The authors have been engaged in the investigation of physical DNA molecular manipulation methods and its applications for more than 10 years. Some topics are presented in this paper.

2. Immobilization of DNA in microstructures

A DNA molecule in water makes Brownian motion, and its shape is like a random-coil fluctuating with time due to the thermal agitation (fig.1 a). In order to realize space-resolved observation and manipulation, the molecule must be immobilized onto a stationary phase somehow. We have developed an electrostatic method for this purpose. The

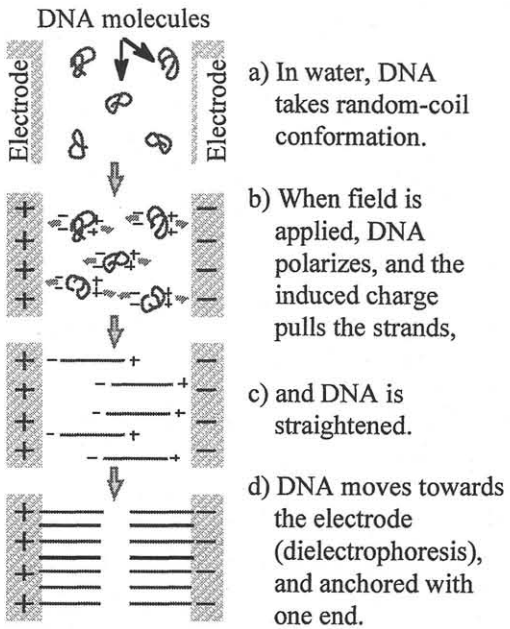

Fig.1 Electrostatic stretch-and-positioing of DNA

device used is just a pair of parallel electrodes on an insulating substrate. When the electrodes are energized to create high-intensity electrostatic field (c.a. 1MV/m, 1MHz), DNA polarizes (fig. b), and the molecule is stretched to a straight conformation (the phenomenon called electrostatic orientation, fig. c). DNA is pulled spontaneously into the high intensity field created at the edge of the electrodes (dielectrophoresis; DEP), until the molecular terminus touches the electrode (fig. d). When an active metal such as aluminum is used as the electrode material, the contact point is permanently fixed, presumably by a covalent bond. The stretching at this stage is reversible, but the DNA can be immobilized onto the substrate as stretched by various means including the use of electrostatic interactions or molecular linkers. Once DNA is stretch-and-positioned, one has an access to arbitrary position of any one of the molecules with the resolution determined by that of the tool, so that cutting, picking up, making chemical modifications etc. become possible. We call such molecular modification with spatial resolution "molecular surgery".

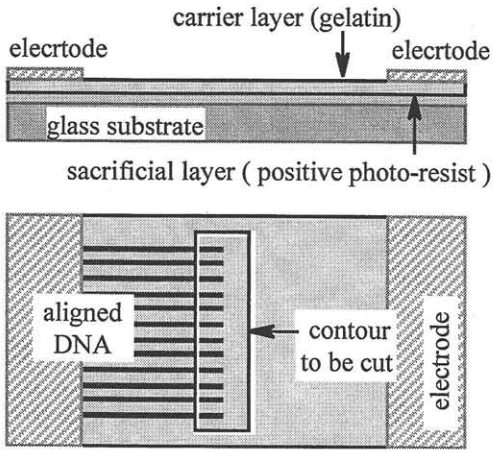

Fig.2 DNA analysis based on molecular surgery

3. DNA analysis based on molecular surgery

In conventional sequencing, the original DNA is cut into fragments, each fragment analyzed, and the total sequence is re-constructed using overlaps between the fragments. If one can pick up arbitrary portion of a DNA strand and analyze, such reconstruction process will become unnecessary. We have developed molecular-surgery based DNA analysis method as shown in fig.2. The device consists of a glass substrate, onto which a sacrificial layer, a carrier layer, and a pair of electrodes are deposited. DNA strands are immobilized on the carrier layer, and then the strands, together with the carrier layer is cut with an AFM stylus in a square shape as shown in the figure. Then, the sacrificial layer is dissolved, and DNA adhering on the square strip of carrier layer is recovered on a membrane filter, put into a test tube to be amplified by PCR. Successful cut and recovery of a desired portion of DNA has been demonstrated [1].

4. Molecular surgery with enzyme-immobilized particle

DNA cutting with predetermined molecular structure can be done with the use of enzymes. If the enzyme is immobilized onto a microparticle, and pressed against a stretch-and-positioned DNA as shown in fig.3, the enzymatic reaction occurs only at the contact point, and DNA is cut with

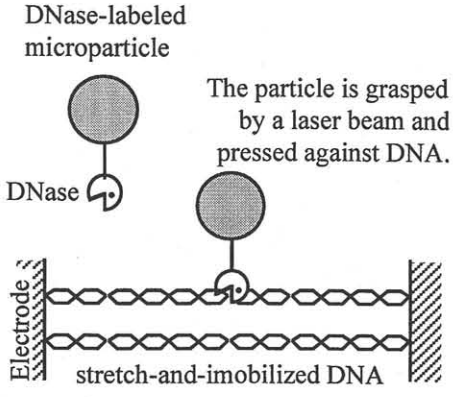

Fig.3 Molecular surgery with enzyme-immobilized particle

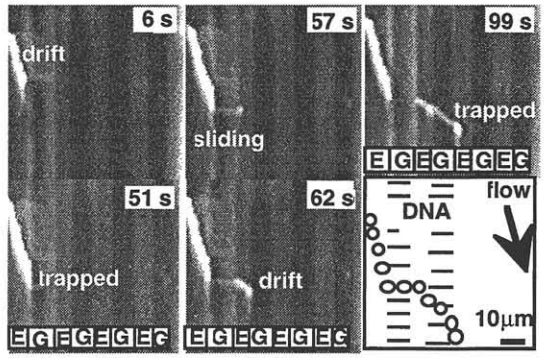

Fig.4 Sliding of EcoRI along DNA

the molecular structure determined by nature of the enzyme. The experiments show that, when DNaseI is used DNA can be cut instantaneously, while the cutting in accordance with the restriction map is observed when restriction enzymes are used [2].

5. Observation of DNA-protein interaction

DNA enzymes, such as restriction enzymes, DNA and RNA polymerases, bind with the DNA strand, find a specific sequence, and then start reactions. Using stretch-and-immobilized DNA strands, together with fluorescence-loabeled enzymes, we can observe the dynamic motion of the enzyme on real-time basis. Our motivation to visualize such interactions is twofold: 1) optical mapping of specific sequences (application-oriented), and 2) investigation of the dynamic process of the interaction (basic-research oriented). Fig.4 is the trajectory of a restriction enzyme interacting with DNA observed under a fluorescence microscope [3].

Acknowledgments

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