NanoWell Array-based Digital BioChip Platform

Tomoji Kawai and HeaYeon.Lee
Institute of Scientific and Industrial Research
Osaka University
8-1 Mihogaoka, Ibaraki, Osaka 567-0047
Japan

Abstract: - We describe the nanometrics geometry of a well-oriented nanowell (NW) array derived from nanofabrication technology which can easily be employed for digital detection with a high S/N ratio, miniaturization, integrated assays and single molecule analysis. In this geometry, the depth and width of the nanosized well can be adjusted to allow for only one or a few biomolecules to enter inside the nanowell and become attached with the self-assembled modified gold surface.

Key-Words: Nanowell (NW) array, biochip, Digital, DNA, Specific binding, Bio-information Analysis

1 Introduction

In recent years, a new paradigm of nanobiochip combining miniaturization and integration has been exploited in areas such as combinational chemistry, biotechnology, functional genomics, proteomics and clinical diagnostics [1-11]. The new intelligent analysis systems with high signal-to-noise ratios, self-organization and various plate formats allow for automated high-throughput screening, drug delivery systems and single molecule analysis. In particular, single molecule assays are of considerable interest in the area of diagnostic and therapeutic sensors. The notion of automation, miniaturization and integration requires the fabrication of appropriately designed nanometrics for high sensitivity homogenous assays.

2 Problem Formulation

The general analytical tools have been restricted by random array of probe molecule and the formation of protein-protein cluster, resulting in hgh background signal. Nanoarrays consist of an arrangement of probe biomolecules immobilized at high density and high specificity onto a chemically modified surface. Employment of nanoarrays for the development of nanobiosensors has attracted a great deal of attention [12-16]. Various affinity-binding assisted analytical tools have been developed based on nanowires, nanoparticles, nanotubes, or microcantilevers. However, there is no simple nanometrics geometry which can be commonly used for high selectivity and high sensitivity nanobiochip applications.

3 Problem Solution

As the solving strategies, we present a nanowell (NW) array, whose nanometrics geometry can be easily fabricated using the current nanolithography technologies and applied for numerous applications.

In this geometry, most areas of the Au electrode were covered with the blocking layer, and only a nanosized gold surface becomes exposed to the open space above each NW. The depth and width of the nanosized well can be adjusted to allow for only one or a few biomolecules to enter inside the NW and become attached with the self-assembled modified gold surface. The number of NW arrays can be controlled during the fabrication and can be quantified using an assay signal.

Fig. 1 shows an AFM and a schematic image of immobilized ssDNA using streptavidin-biotin interaction onto the NW array. Although there are also bright spots on the resist layer, these DNA/streptavidin complexes do not contribute to the electrochemical signals. Few probe ssDNA molecules, bound by streptavidin–biotin, can be localized and hybridized with complementary DNA inside each 100 nm NW.

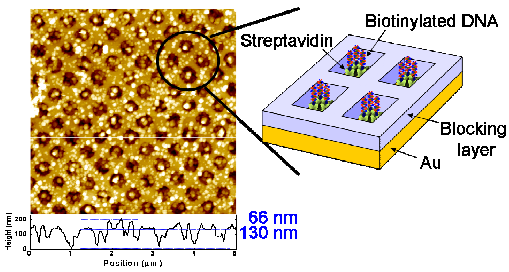

Figure 1. An AFM image of probe ssDNA immobilized by streptavidin-biotin on an nanowell array. Schematic diagram of the nanowell array geometry designed to minimize the nonspecific binding or aggregation of biomaterials.

There are several advantages of the NW array chip, including an increase in the S/N ratio through restricted aggregation, well-oriented immobilization of probing biomaterials, an integrated assay involving various methods, and an easy way of fabrication compatible with nanofabrication technology. Additionally, the NW array-biochip is a critical factor for precious assays due to the high S/N ratio and quantification of the amplified electric signal controlling the number of NW arrays in the near future.

To demonstrate the usefulness of this nanometrics geometry, we also present results of electrochemical DNA with the specificity of assay of biomaterials located inside NWs with a small amount. Figure 2 shows electrochemical analysis of label-free DNA on a 100 nm nanowell array (20 μm in total diameter) and an electrode 200 μm in diameter. Electrochemical measurement were carried out in K_3FeCN_6 solutions. Compared to micro-size electrode, the electrochemical responses on NW electrode were significantly enhanced about 100 times with electrochemically active area for the binding event of streptavidin to the biotinylated DNA. A substantial decrease in redox current density was found with the addition of target DNA without non-specific binding or aggregation. It is envisioned that these features could make the molded NW electrode presented here especially useful for various biological assays involving electrochemical detections.

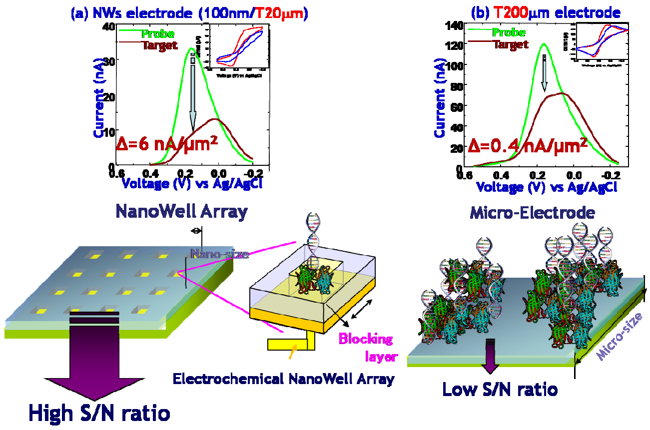

Figure 2. Electrochemical assay of DNA protocol on (a) a 100 nm nanowell array (20 μm in total diameter) and (b) an electrode 200 μm in diameter. Square-wave voltammogram and cyclic voltammograms were carried out in K_3FeCN_6 solutions at a scan rate of 100 mV/ s: after immobilization of the probe DNA molecules: the green lines and after hybridization of the target DNA molecules: the red lines

4 Conclusion

Optimal metrics of digital biochip is crucial in the miniaturization and integration of biochip platforms for use in a clinical point-of-care system. Nanofabrication technologies should be useful for developing highly sensitive, reproducible nanobiochip. There is necessary in order to improve conventional bioassay performance in terms of a higher signal-to-noise ratio (S/N), smaller reaction volumes, and larger numbers of assay sites. Using this premise, we demonstrate the use of nanowell array metrics derived from nanofabrication technology, where the nano-size within the array permits the attachment of only one or a few biomolecules to an exposed, nanosized gold dot inside each nanowell. This nanoarray is fabricated using current nanolithography technology. We also have reported a NW-based biochip platform for electrochemical detecting about DNA, lipid vesicle, antibody-antigen interaction [14,15]. When we applied this array to electrochemical DNA detection, we obtained a two-orders-of-magnitude enhancement in sensitivity. This NW array could be applied to numerous other integrated digital biochip and biosensors system.

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