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Nanoscale Fabrication of Biomolecular Layer and Its Application to Biodevices

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Abstract Biodevices composed of biomolecular layer have been developed in various fields such as medical diagnosis, pharmaceutical screening, electronic device, photonic device, environmental pollution detection device, and etc. The biomolecules such as protein, DNA and pigment, and cells have been used to construct the biodevices such as biomolecular diode, biostorage device, bioelectroluminescence device, protein chip, DNA chip, and cell chip. Substantial interest has focused upon thin film fabrication or the formation of biomaterials mono- or multi-layers on the solid surfaces to construct the biodevices. Based on the development of nanotechnology, nanoscale fabrication technology for biofilm has been emerged and applied to biodevices due to the various advantages such as high density immobilization and orientation control of immobilized biomolecules. This review described the nanoscale fabrication of biomolecular film and its application to bioelectronic devices and biochips.

Keywords: nanobiotechnology, biochip, bioelectronic device, protein chip, DNA chip, cell chip

INTRODUCTION

Biodevices, based on biomimetics, have emerged as a breakthrough with great potential for generating new concepts and technologies for the development of next generation electronics [1-4]. The main concept was inspired from the fact that individual biomolecules, especially proteins, could be used as the basic unit of an electronic device, so that the integration scale of the device could be increased by several orders of magnitude. The fervor related to biomolecule based electronics began in the early 1980s, and research groups related to the topics have subsequently emerged in the United States, Japan and Europe. The experts in the various fields, such as life science, physics, chemistry, chemical engineering, electronics and computer science, have begun collaboration on these fascinating research subjects, and opened the field of new hybrid technology. Biomolecule based electronics are not a single pathway of technological development, and are not in direct competition with any existing research projects in the race to develop a specific technological artifact. The difficulties of producing biomolecular electronic devices based on the biomimetics have certainly been acknowledged by all those involved, but the ideas and technologies of the life sciences and related fields have been actively employed, as shown in Fig. 1, to step to the next stage in the development of new functional devices.

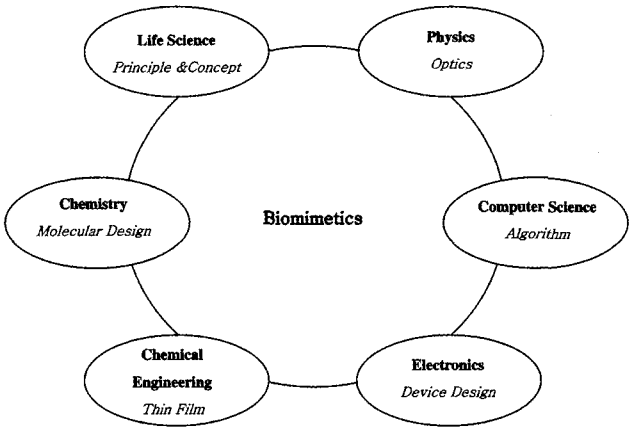

Fig. 1. Technologies based on biomimetics.

A fundamental application would be for biosensors. Since the development of the enzyme based biosensor for glucose detection was first described by Clark in 1962, there has been an impressive proliferation of applications involving a wide variety of target molecules [5-8]. One of the most remarkable advancement in the history of biosensors was the DNA probe for genetic information diagnosis. With the great strides in genetics and related fields, dramatic progress has been made in DNA and

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RNA sensors. From the initial stage, it is now commercially available in the form of a chip. Nowadays, biosensors are one of the main streams in biomimetics and/or biomolecular electronics.

Another possible candidate to be realized in the near future would be biodevices or bioelectronics. In the late 1970s, ideas concerning biodevices and biochips emerged, which were essentially related to the construction of electronic devices using biological molecules, such as proteins, as the smaller functional unit. By appropriately aligning such molecules, it was thought that extremely small devices could be built, where the constituents of the device could be protein complexes, molecular wires and interface molecules, etc. It was first reported that artificial molecular diodes were fabricated by mimicking the electron transfer mechanisms in biological systems, such as a photosynthetic reaction center [9].

NANOSCALE BIOMOLECULAR DEVICES

Biomolecular Diode based on the Photosynthesis

The transfer of an electron from one side of a molecule to the other, or between molecules, is one of the most fundamental and ubiquitous processes in biological systems [10]. The control and exploitation of this process in organized molecular systems are major proposition for molecular electronics and bioelectronics [11]. Progress in molecular electronic devices engineering is still rather modest, due to problems associated with the elucidation and effective control of such structures and interactions at the nano level.

Photoinduced electron transport processes in nature, such as photoelectric conversion and long-range electron transfer in photosynthetic organisms, are known to occur, not only very efficiently, but also unidirectionally, guided by molecular functional groups [10,12]. The concepts for the development of new functional electronic devices can be inspired from biological systems, such as the electron transfer chain or the photosynthetic reaction center. By mimicking the organization of the functional molecules in a biological photosynthetic system, artificial bioelectronic devices can be realized. In the initial process of photosynthesis, a biological electron transfer system, photoelectric conversion occurs, followed by long-range electron transfer, which takes place very efficiently in one direction through the biomolecules [13]. The specific energy and electron transfer take place on a molecular scale, due to the redox potential difference and electron transfer property of the functional molecules; especially the electron acceptor, sensitizer and electron donor [9].

Molecular films, fabricated by appropriate techniques, can be used as model systems for the corresponding photosynthetic reaction center in the biological system. In recent years, substantial interest has focused on thin film fabrication or the formation of biomaterials mono- and multi-layers on solid surfaces [14,15].

Based on these techniques, various artificial biomolecular devices have been fabricated to mimic the electron transport function of biological photosynthesis. Isoda *et al.* investigated a biomolecular photodiode composed of flavin-porphyrin hetero LB films and its optical and electrical characteristics [16]. They use flavin and porphyrin as a sensitizer (S) and an electron acceptor (A), respectively. Fujihira *et al.* investigated an electrochemical photodiode that consisted of the Langmuir-Blodgett (LB) films of three functional biomolecules or an aligned triad on the electrode, which worked in electrolyte solution [17]. Investigations of electron transfer between the electrode and the excited dye molecules were also carried out, in which ferrocene; pyrene and viologen were used as the electron donor (D), S and A units, respectively. The metal/insulator/metal (MIM) structured device, consisting of hetero-type LB films of D, S and A, was fabricated and the photoinduced electron transfer investigated [17]. Recently, a biomolecular photodiode composed of electron D/S/Relay (R)/A type 4 component MIM devices has been investigated [18]. Development of a biomolecular photodiode is important in the area of molecular electronics, as it can be applied to molecular memory devices due to its photoswitching and rectifying characteristics. Fig. 2 shows schematic structure of MIM device, and photoswitching property of biomolecular photodiode.

A biomolecular photodiode, consisting of LB films of ferrocene, flavin, viologen and TCNQ as the D, S, R and A units, respectively, was designed based on the photoinduced electron transport in a natural system [19]. By using two acceptor molecules (R and A), the time for the separated charge state ($A^-/R/S/D^+$) can be sustained longer than that of the A^-/S^+ hetero system. Charge recombination from R and A, to the ground state S, can be reduced due to the fast electron transport from S^* to A, via R, and the increased distance between S and A in the presence of R. Based on these effects, the molecular photodiode composed of D/S/R/A hetero LB films is expected to show better diode and switching properties than those of S/A and D/S/A hetero LB films. By adding the D molecules, backward electron transport of excited S can be reduced, and by the addition of the R molecules, charge recombination from A to ground state S can be reduced. Choi *et al.* investigated biomolecular photodiodes using green fluorescent protein (GFP) and cytochrome *c* [20,21]. Recently, researchers have investigated the nano-scale diode using scanning probe microscopy (SPM). Cui *et al.* investigated the scanning tunneling spectroscopy (STS) based current-voltage (I-V) measurement, and measured the single molecular conductivity of the organic SA layer [22]. Khomutov *et al.* investigated the single molecular conductivity of cytochrome *c* LB

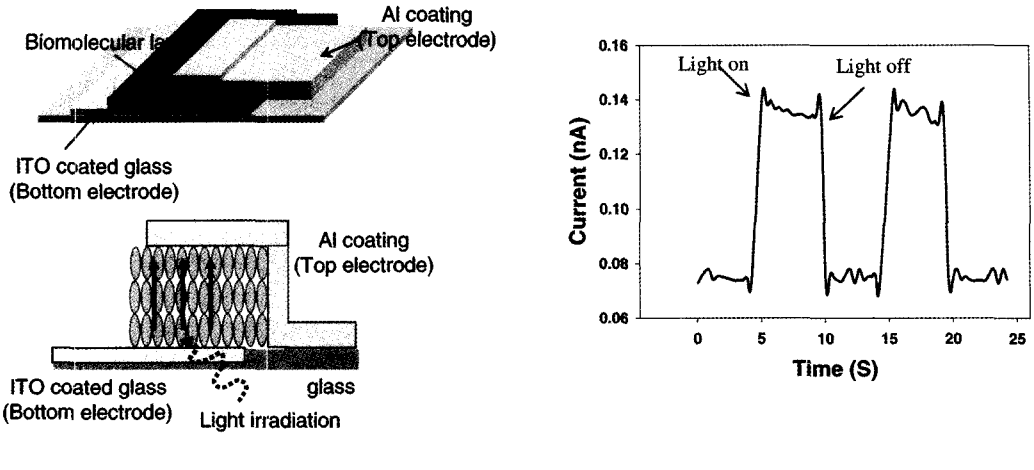

Fig. 2. (a) Schematic structure of MIM device, and (b) Photoswitching property of biomolecular device.

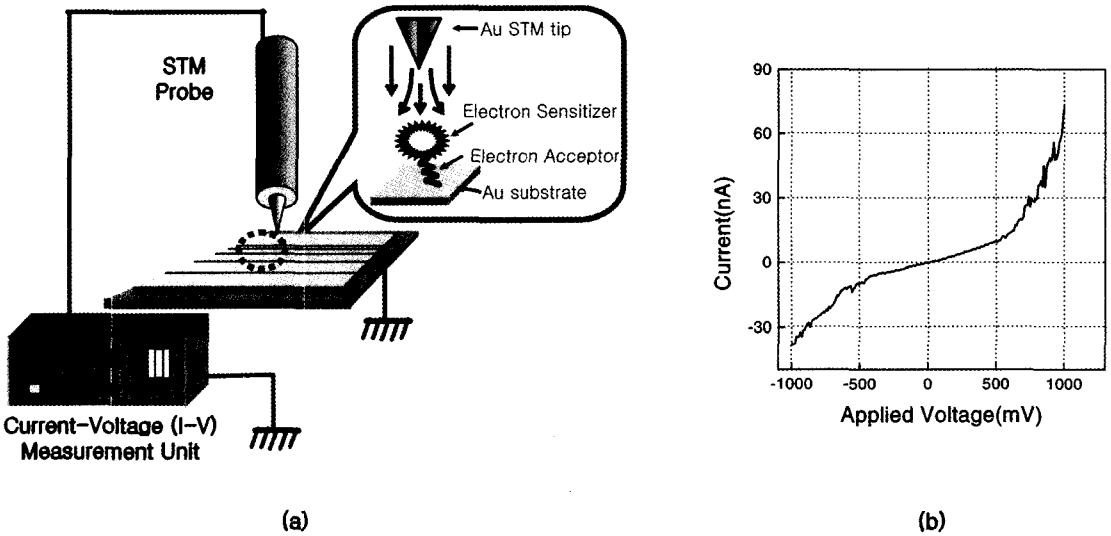

Fig. 3. (a) Experimental set-up for I-V measurement of biodevice, and (b) Rectifying property of biomolecular diode.

layer by STS based I-V measurement [23]. In our recent research, the biomolecular diode consisting of a chlorophyll *a* and ferredoxin heterolayer was investigated by STS based I-V characteristics. Fig. 3 shows experimental set-up for STS based I-V measurement of biodevice, and rectifying property of biomolecular diode.

Biomolecular Storage Devices

Since 1989, various concepts for molecular information storage have been proposed. In 1989, Hopfield *et al.* proposed the concept for the shift register memory, and Choi *et al.* investigated the shift register memory using the biomolecular hetero LB layer [24, 25]. In 1991, Saito *et al.* proposed the fractal memory concept, and Choi *et al.* also investigated the fractal memory function of a biomolecular photodiode [26-30]. Lindsey *et al.* investigated a molecular approach for information storage [31,32]. The basic principle of molecular information storage is to store charge in oxidation states of redox-molecules that are immobilized on a metallic surface. The advantages of the proposed molecular information storage are the molecular properties and dimensions, improved charge-retention times, multiple bits storage and low operating power. Molecular information storage enables the storage of tera bits of memory. Roth *et al.* also investigated the charge storage of an organic molecular monolayer using a 100 μm sized micro electrode in an electrolyte solution [30-35]. Redox-active biomolecules have charged states at various potentials. Application of a reducing potential

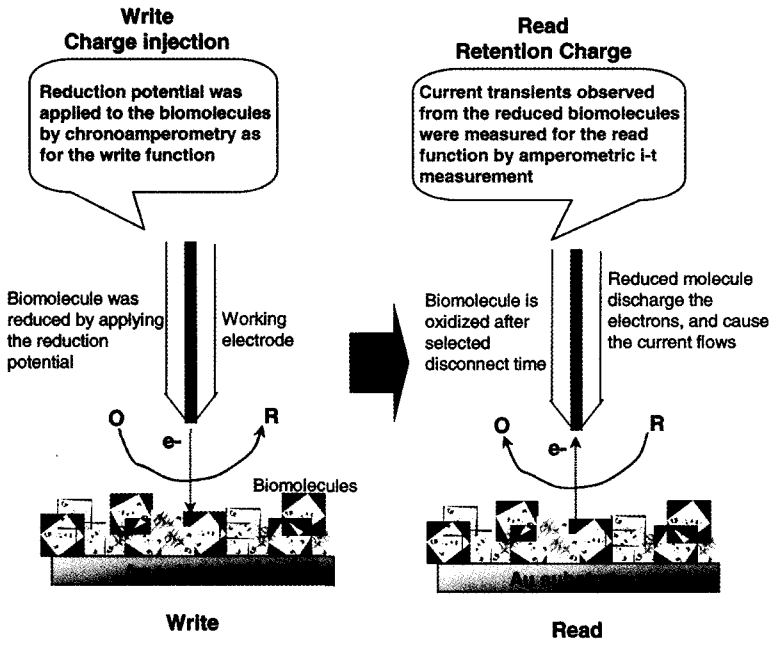

Fig. 4. Fundamental concept of biomolecular information storage.

causes the biomolecules to obtain electrons, resulting in a negatively charged monolayer. When an oxidizing potential is applied, electron-transfer returns the molecules to the neutral state [36]. In our recent study, biomolecular information storage was investigated by applying a reduction potential as a write function, and then measuring the stored reducing charge as a read function. The write function of biomolecular information storage was investigated by applying a reduction potential to the biomolecular layer by a chronomaperometry (CA) measurement. The charge-retention characteristics of the biomolecular layers were determined using open circuit potential amperometry (OCPA). The details of the write (CA)-read (OCPA) experiment, and its use in the measurement of the retention charge of a biomolecular layer, are shown in Fig. 4. The biomolecular information storage experiment was performed as follows. First, the biomolecular layer was reduced by the application of a reduction potential. Next, the applied potential was disconnected from the electrode. During the disconnection time, the electrochemical cell relaxed to the OCP, after which, the applied potential was changed to match the determined OCP. The counter electrode was reconnected, and the resulting current monitored as the biomolecular layer was oxidized (because the OCP is at an oxidizing potential). The intensity of the observed current was proportional to the number of molecules that remained reduced while the counter electrode was disconnected. The charge retention was measured by changing the disconnect time, so that all the initially reduced biomolecules decayed back to the neutral state. In biomolecular information storage, the charge retention time of the reduced state is around 100 sec. Thus, applying a reducing potential every 90 sec is necessary to preserve the reduced (writing) state.

Biomolecular Electroluminescence Device

The organic based electroluminescent device (ED) is a light emitting diode (LED) which is based on carbon-based molecules instead of inorganic semiconductors. It is totally different from the inorganic semiconductors. According to research, the organic EL device is brighter, thinner, lighter, and faster than the normal liquid crystal LCD. They also need less power, higher contrast, look just as bright from all viewing angles, and low cost to produce than inorganic LCD. Also, according to research, biomolecular EL device has more advantage in efficiency and driving voltage aspect than organic EL device. Therefore, bio EL device would be applied to the next generation display which substitutes the organic and inorganic based EL.

Tajima *et al.* first reported a biomolecular electroluminescence (EL) device that used cytochrome *c* [37]. However, this EL device exhibited relatively low light intensity, and emitted light near the red end of the spectrum. Further, the cytochrome *c* based EL device could not be applied commercially due to its low physical and chemical stability in air. In our recent research, the bio EL device was fabricated using a biological pigment based heterolayer as the emitting layer, and reported its EL performance. The EL performance of a biomolecular heterolayer, which emits a blue light, remains to be investigated. In

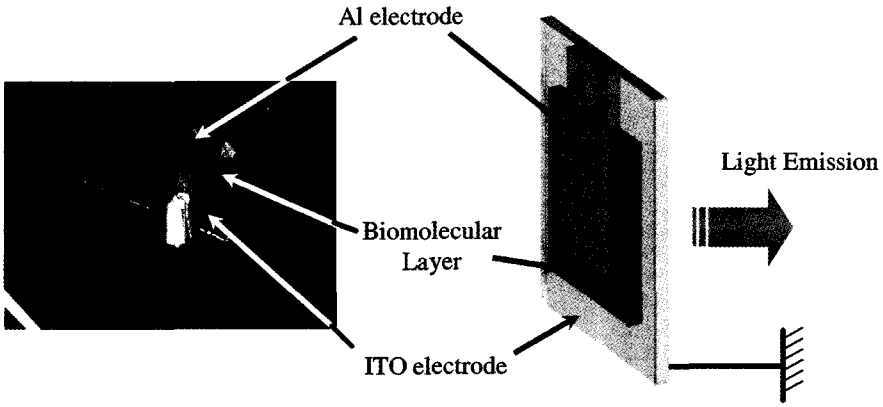

Fig. 5. Schematic structure of bioEL device.

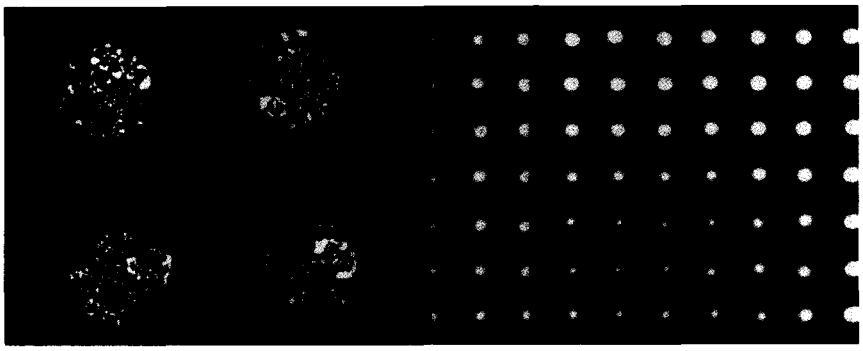

Fig. 6. Fluorescence image of DNA hybridized array.

our research, the external quantum efficiency (η ext) of the bio EL devices is same or slightly higher than organic based EL device. Fig. 5 shows a schematic structure of bio EL device consisting of biomolecular layer.

NANOSCALE BIOCHIP

DNA Chip

Owing to the advent of post genome age, the concern about functional genomics is hardly increased. The purpose of functional genomics is not only getting base sequence of genes but also getting information about function of each gene. The basic goal of functional genomics is that understand how to fabricate functional proteins, cells and more organs. The existing southern blot method using gel electrophoresis separating molecules on the basis of their size was exquisite but had much times. So, development of new analysis tools for DNA work is needed. DNA chip is a powerful and versatile tool in studying functional genomics. The basic principle of DNA chip is using DNA-DNA interaction and the results indicate specific sequences of nucleic acids or nucleic fragments for purposes of sequencing, resequencing, strain identification, gene mapping or monitoring gene expression [38]. Single-stranded DNA (ss-DNA) probe can form a double-stranded, base-paired hybrid with ssDNA if the probe sequence is the reverse complement of the target sequence. DNA chip has arrays of reagent (oligonucleotide, cDNA) on the surface of a small piece of glass or other substrate [39]. Number of immobilized DNA is usually several hundreds to ten thousand. Individual reagents in the array hybrid with specific molecules in sample applied to the array, and the specific binding (Fig. 6) provides information on the composition of the sample. In order to confirm the hybridization of target DNA with capture DNA, fluorescent materials were labeled and observed the fluorescence by florescence scanner. Affymetrix fabricated the oligonucleotide arrays for DNA chip based on the repeatable reaction of photosynthesis [40].

However, DNA chip has some problems in detecting limits and analyzing to bind with target DNA. To overcome these problems, nano-technology has been applied. Nano-scale array can detect little quantity of target DNA and decrease error signals. Ultra sensitive nano-size DNA chip are fabricated using nano-size silicon wire [41]. In

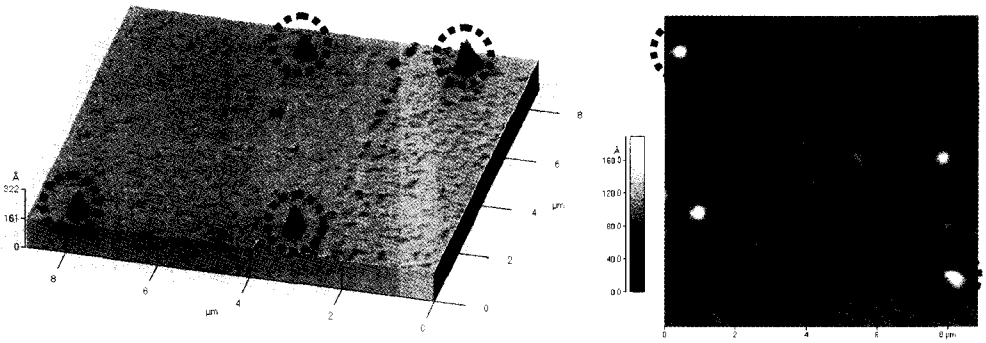

Fig. 7. 400 nm scan size AFM images of 2 by 2 DNA arrays on Au substrate by STM tip.

contrast existing optical method, nano-wire or nano tube can be used as a label free DNA chip. It can be used as a direct real-time electrical detection of target DNA. To fabricate DNA chip using multiple nano-electrodes, multiple and extreme sensitivity detection of DNA is possible.

In our recent research, possibility of nano size DNA array was investigated by STM based nano pattern formation. Fig. 7 shows a nano sized array by STM. The average size of immobilized DNA arrays is 400nm. DNA has negative charge. Thus, opposite charged STM tip can easily form DNA arrays. To amplify the signal sensitivity of nano-size DNA array, colloidal gold nano particles are used to amplify the electrical signal. Protein adsorption onto DNA immobilized Au substrate was investigated using the amine linker. This technique can be applied to fabricate the base plate for protein chip.

Protein Chip

With the increasing requirement of high throughput protein analyses such as diagnostics, drug screening, and environmental monitoring, protein chip is emerging as a new tool on behalf of traditional macroscopic technology such as 2-dimensional electrophoresis, mass spectroscopy (MS), capillary electrophoresis (CE), and enzyme-linked immunosorbent assay (ELISA) [42]. The protein chip, although analogous to DNA chip, faces much great challenges in terms of commercial product. This systematic analytical device for proteome study requires biological surface fabrication to retain the activity of immobilized protein, miniaturization of protein array, and detection technology with high sensitivity. The types of surfaces engineered for protein immobilization can be divided into two categories; the one is the physical adsorption onto surfaces through weak contact of proteins, and the other is utilizing covalent bonds between protein and surface which is preferred due to the molecular orientation, density control, and activity control [43]. Examples included the covalent immobilization using self-assembled monolayer [44], biotinlyated proteins onto streptavidin-coated surfaces [45], His-tagged proteins onto Ni2+-chelating surfaces [46].

Although the many protein arrays are created in accordance with the standard in the production of DNA chips, detection technology is a key parameter for protein chips because there is no equivalent polymerase chain reaction (PCR) for the amplification of proteins. At present, target proteins in protein chips are most sensitively detected by fluorescence, although labeling proteins with such as fluorophore, reduces the quantitative accuracy of assays because the label can change the way the molecule binds to other molecules [47]. For these reasons, label-free detection techniques such as surface plasmon resonance (SPR), and mass spectrometry (MS) are being developed as competitive candidates for microarray applications [48, 49]. SPR-based protein chips have been already showed up in a single-spot format by Biacore. In recent research, protein chip for the antigen detection was investigated. Fig. 8 show the schematic illustration and AFM images of protein G and antibody immobilization.

Despite few successes, protein chip market is still growing due to its attractive advantages. Ciphergen Biosystems, Zyomics, and Perkin Elmer, inc. are the leading business group for protein chip technology. Recently, nanotechnology is expected to be a key to overcome the current technological barriers of protein chip [50]. Many economic reports anticipate the significant growth of protein chip market. Consequently many venture business groups have been founded for the commercialization of protein chip technology.

Cell Chip

The understanding of modeling cell behavior using RNA or protein expression levels is impossible, because cell is much more complicated system than sum of its components. In addition, over the past few years, interest in biochemical experiment and analysis of living cells is increased for studying effects of drug and external stimuli on cell behavior. Thus, the cell chip is studied for diagnostic biochip such as DNA chip, cDNA chip and protein chip. Fig. 9 shows the schematic illustration of cell chip,

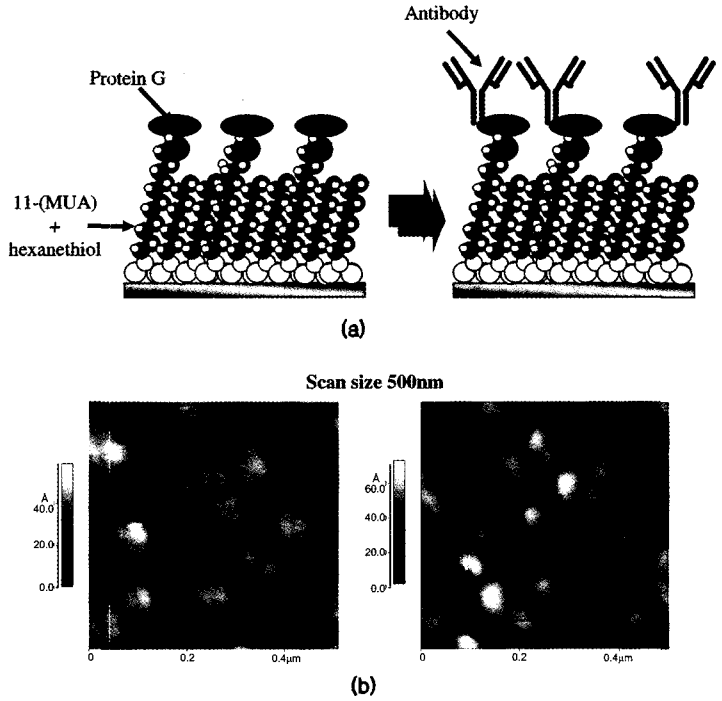

Fig. 8. (a) Schematic illustration of antibody immobilization on protein G layer, (b) 500 nm scan size AFM images of protein G and antibody layer.

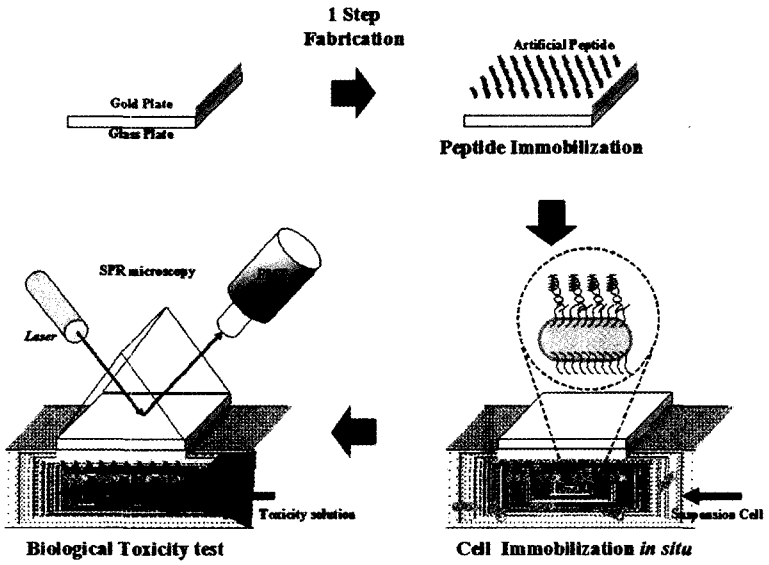

Fig. 9. Schematic illustration of cell chip fabrication, and detection process.

and detection process. In general, cell chip is classified two types. One is the microfludic device for analysis of living cells. For example, a microfluidic device consisting of an array of micro-injectiors integrated in a base flow channel has been fabricated. This device allows controlled application of drugs for cell cultures [51-53]. Another microfluidic device is electrically measured cell viability by detecting electrical resistance of a cell membrane after it is exposed to a toxic agent [54].

A cell microarray chip, fabricated through directly living cell immobilization on substrate, is developed another type of cell chip. Cell microarray chip is efficient and

conventional screening method because of monitoring of many samples at the same time. One of the cell microarrays for high throughput screening has been presented by Kapur *et. al.* [55]. *Ziauddin and Sabatini* have developed a cell-based microarray for identifying the cellular function of gene products [56]. Such cell microarry provides information about not only gene expression level but also consequences of gene expression using defined cDNA. Also, this transfected cell microarrays in high-throughput drug discovery has been applied by Sabatini *et. al.* [57]. Recently, cell based microarry is developed using frozen cell array [58], microcontact printing, and etc.

In our recent research, nanoscale fabrication of cell chip platform has been studied using two types of artificial peptides. One of the designed peptides is CRG 12 that is sequenced C-R-G-D-R-G-D-R-G-D-R-G-D. Another is CRG MAP which is sequenced C-R-G-D-R-G-D-R-G-D-R-G-D MAP (4 branched). The principle relies on the cell attachment receptor (integrin super family) on cell membranes. The integrins bind to short amino sequences (R-G-D) present in multiple component of the extracellular matrix, including collage, fibronectin, and lamin. The artificial peptides is formed the SA layer on Au surface because it have cystein (Cys, C) residues. The cell is immobilized on modified Au surface because R-G-D sequence is attached on cell membrane. In our research, cell chip for the biological toxicity test, which is the chip based in *situ* biological sensor for quantitative analysis, was investigated by using the SPR measurement.

As above explanation, the cell chip is powerful tool to analyze the physiology and functional information of viable biological system of living cells. From now on, cell chip can potentially develop the biopharmaceutical industry, genomics, and proteomics.

CONCLUSION

Biomolecular electronic devices have made some advances toward establishing strategies for the development of the molecular electronic device based on the photosynthesis. From our research, it is summarized that the proposed biomolecular electronic device, which mimics the biological photosynthesis, can be usefully applied in the future as new electronic devices, such as a molecular diode, molecular memory or EL device. Our next challenge is the real application and commercialization of the biomolecular electronic device. In our research, a basic biomolecular electronic device has been developed, and should now be extended to the construction of advanced electronic devices. These achievements lay the groundwork for further research on biomolecular electronics, which can help to overcome the limit of current electronic devices.

Throughout the research, we can acquire more scientific results that are the verification of scientific theory, application of various technological fields (biology, photonics, physics, and chemistry), and establishment of industrial base. Biodevices are not possible in common use and cannot make an industrial benefit immediately. However, considering present market demand and future prospect of electronics, Biodevice's share may grow rapidly in 21th century. If the biodevice could be early developed, it can secure monopolistic technological position in worldwide market. Ultimately, biodevices can establish new industry field coming from Biotechnology (BT) + Information technology (IT) + Nano technology (NT). Also, it is expected that the biodevices can contribute to stand the technological advanced country through the creation of high value industry which provide synergy and motive for society. The biodevice typically requires very short time from research to commercialization. Thus, research introduced above should be achieved at early time.

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