

BioNano/Micro System in Nanobiotechnology

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Abstract: The field of BioNano/Micro System applies both nanotechnology and MEMS (microelectromechanical systems) to solve biological and medical problems as well as biological structures and principles to solve engineering MEMS problems. In this paper, some technical efforts to develop nanobiotechnology, focusing on nanobiosensor, microfluidics and lab-on-a-chip are briefly reviewed. In order to ensure continued success of the BioNano/Micro System in the commercial arena, several key issues need our attention. One of the important things is to the understanding of Nature, biological sciences and to the application of well-establishing conventional technology platform and infrastructure for commercialization.

Keywords : BioMEMS, Microfluidics, Nanobiosensor, Nanobiotechnology

1. Introduction

Recently, biological sciences become “systems biology” or “systems bioengineering” [1], which means that “new biology” needs a systematic approach to study individual biological contents in biological systems. Experimental methods also need an engineering approach for high-throughput and high-contents analysis based on small quantity of biological samples [2].

Many efforts were tried to transform conventional biological works into lab-on-a-chip by combining microfluidics with nanotechnology [3, 4]. This is because microfluidics helps to minimize the time and cost associated with routine biological analysis while improving reproducibility. Controlling flow containing small volume of liquids in microfluidic channels, microfluidics can be used to speed up and simplify the sample preparation steps in lab-on-a-chip and to offer high-throughput, low-cost versions of traditional research methods. Microfluidics technology offers many potential advantages over conventional assay platform, including small sample volumes, short assay time, multiple assay, and automation [5].

Nanotechnology covers the science for exploring the materials and phenomena in the nanometer (atomic, molecular) scale [6], including the technology for manipulating and controlling the structure and components in the nanometer scale, thus inventing new materials, devices and systems [7]. It is expected that nanotechnology also offers researchers the chance to detect rare events or molecules that are present only at low concentrations and to investigate cellular behaviors inside of the cells without destroying them [8]. In this sense, nanobiotechnology is a key multidisciplinary research field for variety of sciences and engineering applications. Nanobiotechnology can be defined as the application of nanotechnology and microfluidics to life sciences, including two fundamental approaches. One is the application of nano-scaled tools to biological systems [9] and the other is the use of biological systems as templates in the development of novel nano-scaled products [10]. Nanobiotechnology provides new opportunities to transform wide areas of science and engineering thanks to new ways of combining nanotechnology and biotechnology. It not only can play a complementary role with respect to both of the aforementioned technology areas but also create novel synergy effects. Nanobiotechnology is the intersection of inorganic and organic engineering to solve critical problems in biology. These problems can be the creation of new drugs, drug delivery vehicles, diagnostics, sensors, assays, fluidics tools and

manufacturing processes for all of the above [11].

BioNano/Micro System applies both nanotechnology and MEMS (microelectromechanical systems) to solve biological and medical problems as well as biological structures and principles to solve engineering MEMS problems. The research includes multidisciplinary approaches including biotechnology, information technology, and nanotechnology and has flourished as a bio-convergence technology where the divisions between the fields of science will begin to break down [12]. In this paper, two major applications of “BioNano/Micro System” in nanobiotechnology will be described.

2. Fundamentals of Micro/Nanofabrication

There has been a noticeable growth in nano/micro system technology for biotechnology-related products, such as biosensor, biochip and microfluidic device, etc. [13]. Although MEMS has been stemmed from the integrated circuit (IC) technology, it is different with IC technology because of its miniaturized non-electrical (optical, thermofluidic and biochemical) components. Meanwhile, implementation of MEMS/NEMS to bio-related areas is known as BioMEMS/NEMS (Bio Micro/Nano Electro Mechanical Systems). Nowadays, it is getting a paradigm shift in the making, “MEMS become BioMEMS” [14]. This is the reason of distinctive natures and advantages resulting from the biological application of MEMS. On the basis of the needs for low cost, miniaturization, microfluidics and high throughput system, BioMEMS technology has been intensively developed not only to replace conventional methods and tools as an alternative but also to create a noble niche market as one of the most promising and commercializing MEMS applications in the biotechnology and biomedical industry. As an example, many researches of microfluidic devices are mainly focused on the development of tools for biochemistry [15], molecular and cell biology [16], and micro total analysis system (μ -TAS) [17], lab-on-a-chip [18], biomedical sensor [19], point-of-care (POC) diagnostics [20], DDS (drug delivery system) [21], and biomimetic device for therapeutic purpose [22].

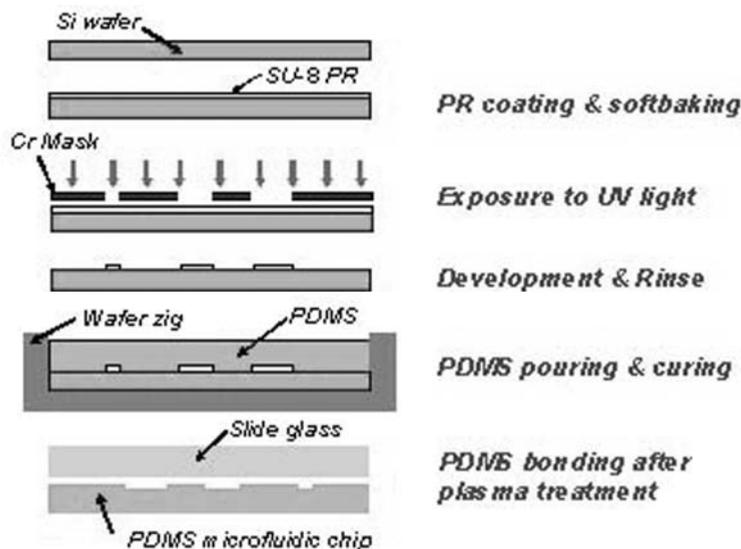


Fig. 1 : Microfabrication of the microfluidic device.

In general, silicon (Si) or glass micromachining is a well established process because both Si and glass can be isotropically /

anisotropically etched using either wet (chemical) etching or plasma (etching) techniques. Disadvantages of these micromachining include incompatibility with wide range of biological reagents and expensive fabrication process. Therefore, plastic micromachining is the area of great interest for biosystems application. Especially, polymer replication technologies such as hot embossing, injection molding and casting processes are widely used for the microfabrication of some microfluidic devices [23]. Due to their thermoplastic properties, several polymer candidates such as polycarbonate (PC) and polymethylmethacrylate (PMMA) are used for micromolding process. Poly(dimethylsiloxane) (PDMS), an elastomeric polymer, is an another good material especially for biological applications as well as (multilayer) soft lithography [24, 25]. Most of the microfluidic device can be easily fabricated by conventional PDMS molding processes (Fig. 1). To make a molding master, the negative photoresist (SU 8) is patterned on Si wafer by photolithography. The coating speed and time determines the thickness of the microchannel. After the patterning, the prepared mixture of PDMS is degassed under the vacuum, poured onto the mold and cured for 30 min at 100°C on the hot plate. The cured PDMS is peeled from the mold and rinsed in the ethanol. Inlet and outlet holes are punched before the PDMS rinsing step. The rinsed PDMS and slide glass were dried in the dry oven at 80°C and treated by air plasma (200 mTorr, 200 W) using an expanded plasma cleaner for 20 s. Then, the PDMS and slide glass were bonded immediately. Fig. 2 shows a photograph of the microfabricated device for cell sorting [26]. Recently, multilayer soft lithography for elastomeric pump and valve was developed by multilayer bonding of silicone elastomer: two-component addition-cure silicone rubber where the bottom layer has an excess of one of the components, whereas the upper layer has an excess of the other [25]. Fluidigm formed to commercialize this technology and to offer the foundry services.

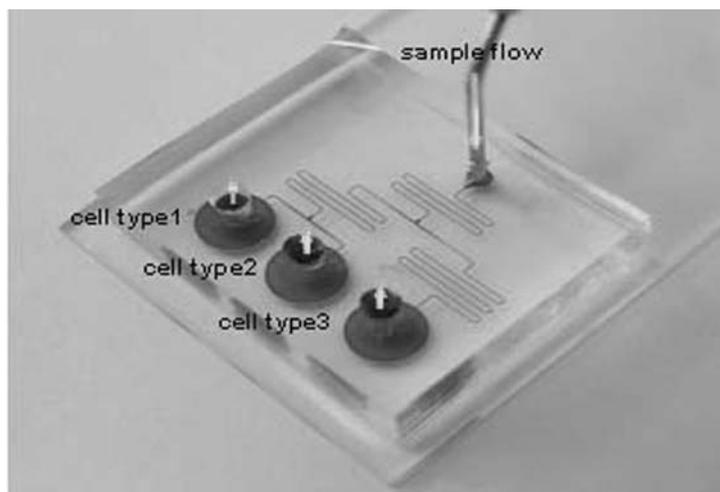


Fig. 2 : Photograph of typical PDMS chip for separation purpose. Red line shows the microchannel with a width of 60 μm .

3. Application areas of BioNano/Micro System

3.1. Nanobiosensor

At present, the development of robust, sensitive and high-throughput biosensor is one of the major issues in the area of nanobiotechnology [27-30]. Until now, some technical achievements for biological detection have been reported such as a diffusion-based immunoassay [31] and nanoparticle-based protein assays [32]. The use of nanoparticles in biological detection enhances the signal sensitivity of sensor due to the various electronic and optical properties as a consequence of their dimensions [29]. The biological detection system for nanoscale devices should be provided for simpler, one step, and homogeneous assays.

The assay platform for lab-on-a-chip also ensures compatibility with miniaturization.

To understand life science and its application for engineering purpose, it is very important to detect biomolecules that are present only at low concentrations. Besides the method for providing biological information, biological detection technology leads to industrial applications of disease diagnostics and predictive, preventative medicine. Systems biology also needs new high throughput screening tools and methods to compare multiple analytes in biological samples simultaneously. Therefore, recent progress on miniaturized bioanalytical systems has meaningful in supporting genomics, proteomics, and drug discovery processes. Because they reduced overall cost of the diagnostics, sensors and drugs, and added the value for high throughput screening of pharmaceuticals in the biotechnology industry. Miniaturization and array concept should be considered as an analytical tool for multiplexed analytes, resulting from introduction of forthcoming nanotechnology.

Recently, an electrical detection technology based on carbon nanotube [33], silicon wire [34], and chip-based cantilever [35, 36] has been developed for high-sensitive biomolecular detection. Silicon nanowires with a diameter of 10 nm have been used for ultrasensitive protein detection [34]. When the analyte molecules bind to the nanowires, there will be a detectable electrical signal. It was also reported that structure change from the protein-protein interaction induce an electrical signal between electrode and redox recycling species of conjugated protein. As an example, maltose-binding protein can be utilized for the electrochemical sensor based on this scheme [37].

In addition, semiconductor quantum dots such as cadmium selenide were exploited for bio-labeling, such as detection reagents for microscopy, DNA chip, flow cytometry and immunoassays [38]. These nanocrystals have a good ability to show simple excitation enabling for simultaneous multicolor, multi-component assays. Since the bead based technology based on the quantum dots also enables multiplexed assays, it can be useful to support the platform technology for proteomics, genotyping, and gene expression. Suspension arrays using nanocrystals is a good example for high throughput screening applications of bead-based approach [39]. Moreover, microbead-based assays have several advantages over the flat microarray, such as no washing steps, multiplexed assay using an encoded microbead, amplified signal due to large surface-to-volume ratio and short assay time because of the freely moveable microbeads in mediums [40, 41]. Another potential area for quantum dots will be molecular imaging. This technology can be used for live cell imaging and in vivo imaging [42].

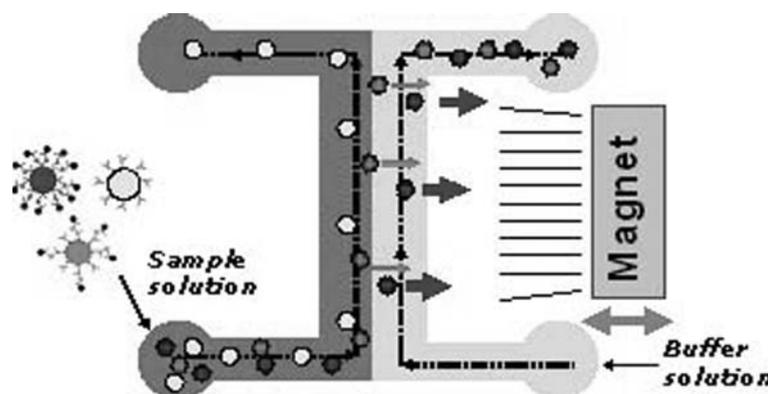


Fig. 3 : Magnetic force-based multiplexed immunoassay scheme using superparamagnetic nanoparticles in microfluidic channel.

In our group, the magnetic force-based immunoassay was devised first and successfully applied to detect the rabbit IgG as the

model analyte of microfluidic sandwich immunoassay [43]. Fig. 3 shows the immunoassay scheme based on the magnetic force in the PDMS microfluidic device using superparamagnetic 50-nm nanoparticles as a label and fluorescent 1- μm polystyrene beads as a solid support. The superparamagnetic nanoparticles and polystyrene beads are immobilized with specific antibodies. When target analytes react with the polystyrene beads and superparamagnetic nanoparticles simultaneously, the superparamagnetic nanoparticles can be attached onto the microbeads by the antigen-antibody complex. Only the microbeads conjugated with superparamagnetic nanoparticles by analytes consequently move to the high gradient magnetic fields under the specific applied magnetic field. In this study, the movements of microbeads conjugated with magnetic nanoparticles were demonstrated by magnetic field gradients. High magnetic field gradients using micro electromagnets could be applied to this detection method for high sensitivity and lower detection limit. In addition, the multiplexed immunoassay using an encoded microbead which is immobilized with a certain antibody could be possible using this detection principle. This detection scheme is underway to develop an integrated μ -TAS device.

3.2. Micro/Nano Fluidics and Lab-on-a-chip

In microchannels in which either width or height is less than 200 μm , an aqueous flow is generally laminar, not turbulent. At this point, only diffusion is an efficient process for mixing the dissolved contents of two or more fluids, and thus the particles in microfluidic channels can be separated by diffusion according to their size. Microfluidics cover the technologies and devices capable of controlling and transferring tiny quantities of liquids, and allow biological assays to be integrated and accomplished on a small scale [44]. Microfluidics are the science of designing and manufacturing devices and processes for manipulation of extremely small volumes of liquid, and promises to minimize the time and cost associated with routine biological analysis. This technology is regarded as the first wave of micro-laboratories (referred to as μ -TAS, micro total analysis systems) and the next generation of drug discovery tools [45].

Micro/nano fluidics is a key technology for commercialization of μ -TAS or lab-on-a-chip (LOC). μ -TAS was developed by efforts to integrate many analytical functions with a single device or unit that contains the sampling unit, microfluidic channels, detection, and controller unit. Therefore, LOC means that μ -TAS is realized at a chip scale. LOC is a kind of chemical microprocessor made by integrating many kinds of apparatus on a chip having a dimension of several centimeters being made of glass, silicone or plastic, and allows automated experiments to be conducted with high speed, high efficiency and low cost.

Until now, many researches on LOC have aimed to develop an on-chip system where biological and chemical analyses are accompanied [46]. Especially, previous research has been focused on the downsizing because the analytical performance can be improved at the chip scale. For example, on-chip capillary electrophoresis has distinct advantages, such as brief analysis time [47] and considerable high separation efficiencies [48], in comparison to conventional capillary and slab-gel formats. In addition, microreactor has provided the improved environment for biochemical synthesis in terms of speed, productivity and controllability [49]. These advantages of miniaturization result in the evolution of small devices for wide applications [50, 51]. However, former chips have still several limitations because they have accompanied by analytical procedures without on-chip pretreatment. In order to develop the fully integrated LOC, miniaturization and integration technology of microfluidic devices could contribute their functions to work together on a chip scale. However, most LOC developed can be still used to provide a specific function such as sample pretreatment, separation, dilution, mixing, reaction and detection.

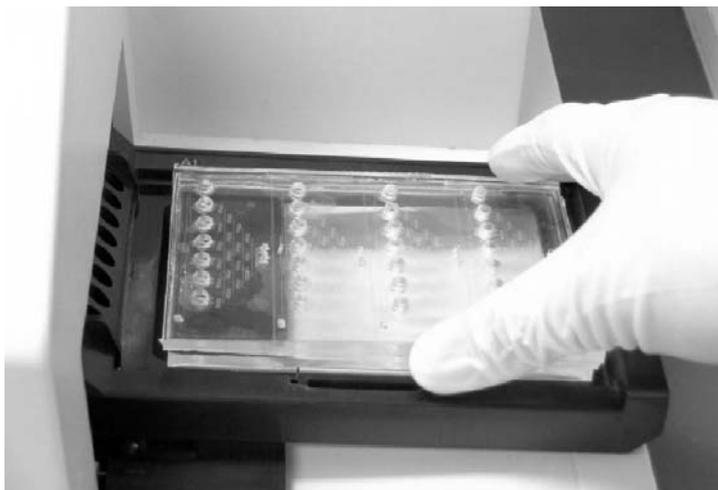


Fig. 4 : Photograph of the microplate reader compatible microfluidic device for enzyme assay. Single device has four enzyme assay modules and the reaction chambers are arranged to be compatible with a microplate reader. Actual size of the device is the same as a 96-well microtiter plate.

We recently report a novel platform technology of enzyme assay using microfluidic channel [52]. The device consists of microfluidic channel modules running four assay tests on a single device and each module comprises six reaction chambers linked with microfluidic channels. Overall dimension of the microfluidic device is the same size as a 96-well microtiter plate and 24 reaction chambers in device adapt to the conventional 96-well architecture to be detected by a microplate reader. Enzyme reactions are simultaneously occurred in six reaction chambers of each microfluidic assay module filled with different concentration of substrate solution generated by microfluidic channel network. This platform technology can provide the advantage to overcome the limitations of a conventional assay platform which requires a special detection instrument and tedious experimental procedures. This can be easily transferred to high-throughput assay with 384- or 1536-well microplate format.

Fluid manipulation technology is one of the important research areas to develop microfluidics and microfluidic devices due to the needs of efficient liquid pumping strategies. As a method to deliver infinitesimal amounts of solution in a microchannel, the most common method employs electric fields. The flow of solution can be controlled by using an electrokinetic force generated when a voltage is applied at both ends of the microchannel filled with the solution without using an additional pump or valve. The driving force of flow movement caused by electrophoretic flow, which results from the migration of charged species under the influence of electric field, and by electroosmotic flow (EOF) due to surface charge. This movement inside the microchannel can be used to separate the samples on the microchip [53-56]. However, if one or more channels are connected in a complicated manner, it is difficult to control the delivery of the solution. In addition, the electroosmotic flow is affected by the physical properties, such as acidity (pH), ionic strength and viscosity, of the solution to be delivered and microchannel surface condition.

As another method to deliver infinitesimal amounts of solution in a microchannel, several approaches were reported. They include the passive fluid phenomena (such as capillary wetting) [57], electrochemical processes for active control of liquids by the creation of gradients in surface pressure [58], conjugated materials [59], multilayer soft lithography [25], hydrogel valves capable of autonomous control of local flow [60], interfacial phenomena based on the surface-directed liquid flow inside microchannels [61], and colloidal devices [62].

Recently, an electrowetting technology based on the surface tension control has been demonstrated to actuate microdroplets [63].

Electrowetting refers to the modulation of the interfacial tension between a conducting liquid phase and a solid electrode, by the application of an electric field. Because the surface tension is a dominant force in microscale, its gradient can be used for dispensing, transport, merging, mixing, and splitting of liquid droplets.

3. CONCLUSION

In this paper, the fundamental concepts and some applications of BioNano/Micro Systems have been described. BioNano/Micro Systems will continue to advance in the future and disperse to other technological fields, resulting in a new devices and tools for niche market. Nature applies nanotechnology daily to grow the multifunctional cells and tissues of plants and animals from a single biological cell. It seems that a cell is a warehouse of nanoscale machines. Biology can teach the physical world of electronics, computing, materials science and manufacturing. It is expected that new paradigm for technology fusion results from the biotechnology-oriented information system or the biotechnology-motivated intelligent system.

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