論 文 の 内 容 の 要 旨

論文題目　Study of integrated microelectrofluidic systems for miRNA profiling
(miRNA解析を目指したエレクトロニクス／マイクロ流体デバイス統合システムに関する研究)

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For over 30 years, molecular biosensing has relied on one of the most expensive but extraordinarily sensitive methods-fluorescence based optical analysis. Even though electrochemical methods became familiar with the development of pH electrode in early 1935 (*Kuhr, W. G, Nature Biotech.* ***18****, 1042 – 1043, 2000*) it did not draw much attention as a primary method of bimolecular detection since the electrochemical properties of nucleic acid remained unknown until late 1970. It is due to the blessings of some of the very important and significant technological advancement in recent times that biological events on suitable recognition surfaces could be possible to observe electrically and electrochemically. Many new techniques and ideas have been developed in the past 10 years to observe the naturally occurring biological phenomena such as hybridization of DNA-DNA or DNA-miRNA molecules and protein-protein interaction etc. Alternative to the conventional fluorescence based detection, recent development of CMOS based devices, SiNW field effect biosensor etc. seemed to be promising solutions. But the common issues such as high background noise caused by the electrochemical nature of detection associated with electrical biosensing still remained a challenge to meet. One can argue the solutions to such problem lies deep understanding in molecular level where the interactions are at a nanometer scale. Therefore, it is probably necessary to look beyond the detection techniques and device types, or to speak precisely, study of recognition surface, capture molecule binding with the surface and target capturing techniques might influence greatly the results that can help in high level sequencing. Study of probe structures and smaller but significant molecular binding effects such as coaxial stacking effect resulted in a sandwich hybridization technique has been researched by few but need to be well explored in order to take the present molecular sensing to the next level.

Here, we propose an integrated biosensing system assisted with microelectronic and microfluidic device technology and its application for the much needed study and experimental investigation of different molecular interactions and binding affinity on different recognition surfaces. We used the microfluidic and microelectronic platform with two recognition layers – gold and single-crystal gallium oxide having different electrochemical properties. With using distinct strategy for the observation of molecular interactions such as DNA-DNA and

DNA-miRNA on these surfaces, the understanding based on the results has helped in the development of complete standalone integrated microelectrofluidic system towards future point of care solution.

A complete biosensing system has several elements but the layer of recognition on which the biological events take place and signal transduction are the most fundamental part of the system. Furthermore, such kind of sensing mechanism heavily rely on the electrochemical solution, so facilitation of fluidic transport has also become an integral part for such system. With the advent of recent semiconductor technology and integrated circuit technology, integration of the suitable sensing layer with the detection circuitry has become easier than ever. Until recently the integration for the support of fluidic transport with such kind of biosensing application drew very little attention even though many of the surface functionalization and detection steps rely on the handling of fluids. Microfluidic technology, in this case, can not only provide convenience in the fluidic transport, it can also ensure precise and high stringent washing – one of the most important part of target molecule selection, precise control of solutions – important for low volumetric detection, shrinking the device size and automation.

To acquire the signal during the sensing process, a good design of the circuit is a necessity. Usually a good design is a factor of various requirements dependent on specific biosensing system. The sensitivity of signal transduction are often governed by the careful selection of amplifier in the design of the acquisition circuit. In biosensing system with very low electrolyte impedances, the noise voltage – the variation of potential on the working electrode can be a big concern. On the other hand a small DC quiescent current can cause a significantly high potential drop in high impedance media causing error in measured signal. Therefore, the good design mostly depend on the right selection of the amplifiers and proper trade-offs in their properties. The designed circuit has been fabricated with the mm scale surface mountable small outline integrated circuit (SOIC) having lower resistance and inductance at the connectivity and soldered on the printed circuit board (PCB) ensuring thermal and electrical benefits.

A compact device has been developed for the purpose of label free on-chip electrical detection of biomolecules such as DNA and miRNA for future clinical and biological applications. It provides flexibility in fluid handling through the integration of microfluidic device and low cost and simplicity in detection through fully electrical measurement. The device has been developed with first creating the recognition layer on top of substrate and then patterning small multiple sensing areas on the same layer through conventional photolithographic technique. The sensing areas has next been covered with microfluidic channels made of PDMS. The channels have been designed using AutoCAD’14 software and transformed into plastic mold using Roland Milling Machine. The PDMS layer was then developed on top of the mold and replaced on sensing area after curing. The development of the

total system was completed with a miniature analog circuit used for detection and attached to the bottom of the substrate. The total system that is smaller than a palm has been developed in such a way that it can be used as a standalone device and offer future point of care solution.

The developed device has been used for the electrical detection of Nucleic Acid with surface modified gold electrodes. There are number of reasons behind the selection of gold electrodes for modification with immobile capture molecules in order to observe target molecule sensing. The fabrication of thin film of gold on top of substrate such glass is quite straight forward and offer easier way to pattern multiple sensing areas through conventional lithographic and available chemical etchant. But most importantly, It does not react with most of the chemicals making it very convenient to handle and manipulate in biological experiment. It also binds with sulfur (-S) through covalent bond known as thiolated-gold bond. Therefore, capture probe molecule can be modified with thiol to immobile onto the surface and make a self-assembled monolayer that remains stable over long period of time. The observation of molecules has been done in two steps. At first, DNA-DNA interactions has been observed on gold surface modified with DNA molecule. The observation has been done through both continuous and non-continuous real-time acquisition of surface potential. In the continuous measurement technique, the surface has been continuously observed during the time of surface modification (ex. capture molecule immobilization, target molecule capturing etc.) as opposed to conventional methods where surface is observed only after a modification step is complete. We then used the device for the detection of DNA-miRNA interaction. miRNA which are short, single stranded, non-protein coding RNAs are expected to act as an excellent biomarker since their expression correlates to specific diseases. But due to their shorter length, detection of such kind of molecular interaction brings new challenges. We tried to overcome the challenges through careful modification of the capture probe DNA molecules and tried to take advantage of the stacking effect that occurs in between neighbor nucleic acid bases. With such kind of modification we were able to achieve high selectivity in detection of target miRNA (miR-16) from very closely matched non-target sequences (miR21 & let7a). We also were able to show a relationship between percent of sequence matching and observed signal through this study.

The surface-modified single crystal gallium oxide (β-Ga_2O_3) has been studied and examined as a new recognition surface for the biosensing application. Electrically conductive oxide also known as transparent conductive oxides (TCO) have shown some promising and technologically important properties such as visible light transparency, chemical stability and good electrical conductivity. Among them most unique property is the coexistence of optical transparency in the visible light region and controllability of conduction, something metal electrodes cannot offer. In this regard, β-Ga_2O_3, a transparent conducting oxide, has advantages over other conventional TCOs owing to its unique important properties

such as large band gap (4.9 eV), deep UV-transparency, and good electrical conductivity in bulk form in addition to the common TCO properties. We have chosen single crystal β-Ga_2O_3 for its unique properties over other TCO's and investigated its potential as electrode material for biomolecular detection.The surface modification has been carried out through salinization process at first and then thiol modified capture probe molecule were attached there through a linker known as EMCS. The observation of the surface modifications through salinization, capture molecules, and subsequent detection of target molecules has been carried out through observation of the current. The biomolecules on the surface and their electrostatic interactions with salt ions heavily influences the ionic barrier formed on the surface at the equilibrium condition and the free movement of ions in the bulk electrolyte. The effect is reflected to the observed current caused by the change in resistivity when constant potential is applied to the solutions. We investigated DNA – DNA interactions by exploring such influence on the surface and was able to separate target DNA molecule from others. A miniature liquid junction diode array has further been fabricated on the β-Ga_2O_3 surface and the device has been used to sequence highly similar miRNA sequences of let-7 family. let-7a molecule has been separated from single base mismatch let-7f and let-7c molecules through relative resistivity shift with a reasonable margin through the measurement of diode current.

With the number of cancer patients increasing every year, a point of care biosensing tool capable of detecting cancer biomarker has become a crying need for the generation. While several DNA sensing have been established over the past few years, detection of miRNA that act as a strong cancer biomarker and can directly be associated with specific diseases through their expression remains a challenge to date. Therefore, a fully electronic, highly efficient, lab-on-chip type device for biomarker detection would certainly be a blessing for clinical applications and can make cancer detection easier and low cost. We have developed a novel micro-electro-fluidic device and the ability of this biosensor has been justified through the detection of molecular interactions. Different recognition layers such as gold and single crystal gallium oxide has been studied to identify their potential in target selection and sensitivity for future use in point of care devices. The sequence based selection of very short but closely related miRNA detection demonstrated here hold promise for using it for future cancer biomarker detection. Further work can also been planned to explore one of its (β-Ga_2O_3) very important and unique property - deep UV transparency. The double bond of nucleic acid bases like thymine and cytosine absorbs UV light and the UV modified base forms direct covalent bond (*Goodsell,D.S, The Oncologist* ***6****, 298-299, 2001*) with neighbor bases. Investigation of such kind of strong molecular interaction would be convenient through gallium oxide because of its deep UV transparency. Therefore, gallium oxide holds more promise for future on-chip biosensing.