MULTIFUNCTIONAL BIOCHIP FOR MEDICAL DIAGNOSTICS AND PATHOGEN DETECTION

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ABSTRACT

An important factor in medical diagnostics is rapid, selective, and sensitive detection of biochemical substances (proteins, metabolites, nucleic acids), biological species or living systems (bacteria, virus or related components) at trace levels in biological samples (e.g., tissues, blood and other bodily fluids). This manuscript provides an overview of the operating principle and application of an integrated multi-functional biochip based on integrated circuit complementary metal oxide semiconductor (CMOS) sensor array for use in medical diagnostics and pathogen detection. The usefulness and potential of the biochip as a rapid diagnostic tool at the physician's office and as a practical, inexpensive screening tool for pathogens under field conditions are discussed.

1. INTRODUCTION

Infectious diseases are responsible for almost half of mortality in developing countries. These deaths occur primarily among the poorest people because they do not have access to the diagnostics tools, drugs and commodities necessary for rapid detection, prevention or cure. Approximately half of infectious disease mortality can be attributed to just three diseases – human immunodefficiency virus (HIV), tuberculosis (TB) and malaria. According to the world health organization, these three diseases cause over 300 million illnesses and more than 5 million deaths each year. Rapid, simple, cost-effective medical devices for screening multiple medical diseases and infectious pathogens are essential for early diagnosis and improved treatments of many illnesses. Biosensors and biochips are diagnostic devices that employ the powerful molecular recognition capability of bioreceptors such as antibodies, DNA, enzymes and cellular components of living systems. Bioreceptors can generally be classified into five different major categories: (1) antibody/antigen, (2) enzymes, (3) nucleic acids/DNA, (4) cellular structures/cells, and (5) biomimetic. The operating principle of a biosensor involves detection of this molecular recognition and transforming it into another type of signal using a transducer that may produce either an optical signal (i.e., optical biosensors) or an electrochemical signal (i.e., electrochemical biosensors). A biosensor that involves the use of a microchip system in an array format for detection is referred to as a biochip.

In general either nucleic acid or antibody probes are often used as biological probes for biochips [1-10]. Biochips using nucleic acid probes are often called gene chips, and biochips with antibody probes are often called protein chips. We have developed an integrated biochip that combines nucleic acid probes, antibody probes, and a detection system into a self-contained microdevice [1-7]. This presentation describes a unique biochip system that uses multiple bioreceptors with different functionalities on the same biochip, allowing simultaneous detection of several types of biotargets on a single platform. This device is referred to as the multi-functional biochip (MFB).

2. THE MULTI-FUNCTIONAL BIOCHIP

The MFB is an integrated multi-array biochip, which is designed by combining integrated circuit elements, an electro-optics excitation/detection system, and bioreceptor probes into a self-contained and integrated microdevice [3,4]. Fig. 1 depicts a schematic diagram of the MFB device.

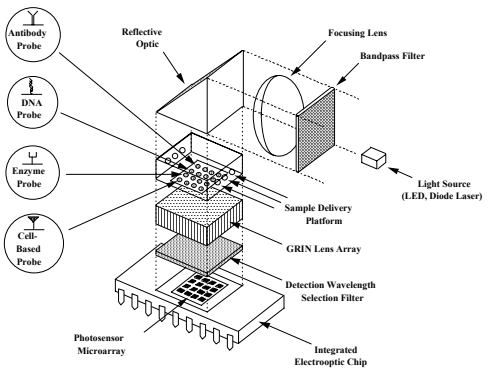

Figure 1. Schematic Diagram of the Multi-Functional Biochip (MFB) optical system.

The MFB system includes the following elements: 1) an excitation light source, 2) multiple bioprobes having different types of bioreceptors, 3) a sampling platform, 4) sensing elements, and 5) a signal amplification and treatment system. The development of the multichannel sampling platform involves immobilization of bioprobes

on multiarray (4 x 4 or 10 x 10 channels for current biochips) substrates, which can be performed on a transducer detection surface to ensure optimal contact and maximum detection. When immobilized onto a substrate, the bioprobes are stabilized and, therefore, can be reused repetitively.

Labeled and unlabeled DNA probes were prepared in our laboratory when needed, or were purchased from a commercial source (Oligos Etc., Wilsonville, Oregon). We synthesized desired strands of oligonucleotides and labeled them with fluorescent labels (e.g., fluorescein and Cy5 dyes) (further details are described later in the Methods Section). Several methods have been investigated to bind bioprobes to different supports. The method commonly used for binding bioprobes to glass involves silanization of the glass surface followed by activation with carbodiimide or glutaraldehyde. Immobilization of the bioreceptor probes onto a substrate or membrane and subsequently attaching the membrane to the transducer detection surface is another approach that can be used.

Integrated Circuit Development for the Biochip

The integrated electro-optic microchip system developed for this work involved integrated electrooptic sensing photodetectors for the biosensor microchips. Such an integrated microchip system with on-board integrated circuit (IC) electronics is not currently available commercially. Therefore, we have designed IC electrooptic systems for the microchip detection elements at Oak Ridge National Laboratory. Highly integrated biosensors are made possible partly through the capability of fabricating multiple optical sensing elements and microelectronics on a single integrated circuit [11, 12]. Such an integrated microchip system is not currently available commercially.

To develop a biochip system with optimized performance, we have developed and evaluated several biochip IC systems based on photodiode circuitry, one system having 16 channels (4 x 4 array), and another having 64 channels (8 x 8 array) having four types of electronic circuits on a single platform. The biochips include a large-area, n-well integrated amplifier-photodiode array that has been designed as a single, custom IC, fabricated for the biochip. This IC device is coupled to the multiarray sampling platform and is designed for monitoring very low light levels. The individual photodiodes have 900-µm square sizes and are arrayed on a 1-mm spacing grid. The photodiodes and the accompanying electronic circuitry were fabricated using a standard n-well CMOS process. The use of this type of standard process allows the production of photodiodes and phototransistors as well as other numerous types of analog and digital circuitry in a single IC chip. This feature is the main advantage of the CMOS technology in comparison to other detector technologies such as charge-coupled devices or charge-injection devices. The photodiodes themselves are produced using the n-well structure that is generally used to make resistors or as the body material for transistors. Since the anode of the diode is the p-type substrate material, which is common to every circuit on the IC chip, only the cathode is available for monitoring the photocurrent and the photodiode is constrained to operate with a reverse bias.

We designed an analog multiplexer that allows any of the elements in the array to be connected to an amplifier. In the final device, each photodiode could be supplied with its own amplifier. The multiplexer is made from 16 cells for the 4 x 4 array device. Each cell has a CMOS switch controlled by the output of the address decoder cell. The switch is open when connecting the addressed diode to an amplifier. This arrangement allows connecting a 4 x 4 (or 10x10) array of light sources (different fluorescent probes, for example) to the photodiode array and reading out the signal levels sequentially. With some modification, a parallel reading system can be designed. Using a single photodiode detector would require mechanical motion to scan the source array. The additional switches and amplifier serve to correctly bias and capture the charge generated by the other photodiodes. The additional amplifier and switches allow the IC to be used as a single, large area (nearly 4 mm square) photodetector.

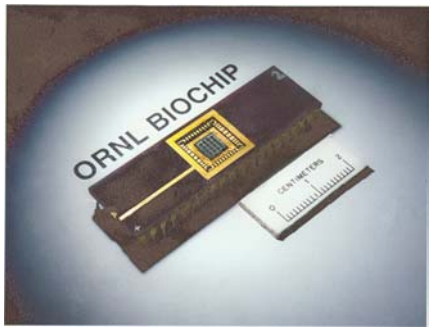

Figure 2. Photograph of the 4x4 integrated circuit microchip.

Fig. 2 shows a photograph of the 4x4 IC microchip. With the CMOS technology, highly integrated biosensors are made possible partly through the capability of fabricating multiple optical sensing elements and microelectronics on a single IC. A two-dimensional array of optical detector-amplifiers was integrated on a single IC chip. To evaluate

and select an improved IC system for the biochip, we have also designed and fabricated a chip with an 8x8 CMOS sensor array. This microchip contains 4 quadrants, each having a different electronic design, which was evaluated for optimal performance.

3. APLICATIONS IN MEDICAL DIAGNOSTICS AND PATHOGEN DETECTION

3.1. Medical Diagnostics

In general, high-density microarray technology having relatively expensive and bulky detection systems is useful for laboratory applications, but they are not appropriate for clinical use at the physician's office. On the other hand, integrated biochip systems having a medium-density array (10 –100 probes) and a miniaturized detection microchip are most appropriate for medical diagnostics at the point-of-care, i.e., the physician' s office. Recently, an integrated biochip device with a 16-photodiode array was developed and evaluated for the detection of HIV1 gene fragments. This system is an illustration of the usefulness of the DNA biochip for detection of a specific HIV gene sequence. Actual detection of the HIV viruses will require simultaneous detection of multiple gene sequence regions of the viruses. It has been observed that progression of the AIDS disease causes an increase in the genotype diversity in HIV viruses. It has been reported that the HIV viruses appear to defeat the immune system by producing and accumulating these gene mutations as the disease progresses. In this study, a specific DNA sequence fragment was used as the model system for a feasibility demonstration.

In general biosensors and biochips employ only one type of bioreceptor as probes, i.e., either nucleic acid or antibody probes. Biochips with DNA probes are often called gene chips, and biochips with antibody probes are often called protein chips. An integrated DNA biochip that uses multiple bioreceptors with different functionalities on the same biochip, allowing simultaneous detection of several types of biotargets on a single platform has been developed. This device is referred to as the multi-functional biochip (MFB). The unique feature of the MFB device is the capability to perform different types of bioassay on a single platform using DNA and antibody probes simultaneously *E. coli and B. anthracis* using antibody and DNA probes, respectively. Hybridization of a nucleic acid probe to DNA biotargets (e.g., gene sequences, bacteria, viral DNA) offers a very high degree of accuracy for identifying DNA sequences complementary to that of the probe. In addition to DNA probes, the MFB uses also another type of bioreceptor, i.e., antibody probes that take advantage of the specificity of the immunological recognition. The results of this study using antibody against *E coli* and DNA probes for *B. anthracis* demonstrate the feasibility of the multi-functional biochip for the detection of multiple biotargets of different functionality (DNA, proteins, etc.) using a single biochip platform.

Mycobacterium tuberculosis bacterium or the *p53* cancer protein have also been detected on the same biochip (Fig. 3). The two first rows of the biochip were used for the detection of TB gene segment using DNA probes, while last row used to the detection of the p53 protein using antibody probes. The third row was the blank.

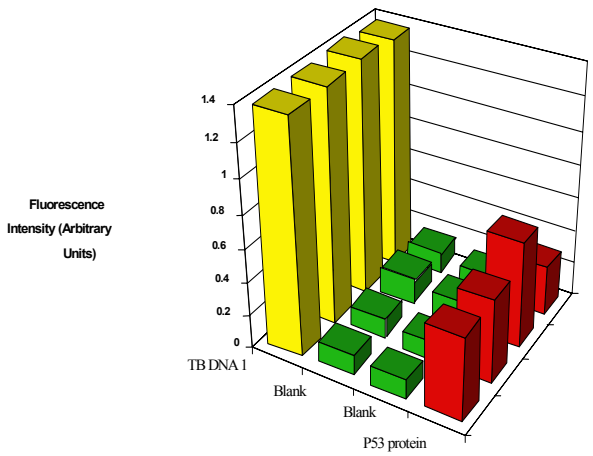

Figure 3. Detection of the *Mycobacterium tuberculosis* gene fragment and the *p53* cancer protein using the multifunctional biochip system.

3.2. Pathogen Detection

Cost-effective biochips can provide inexpensive tools to detect pathogens at field locations located far from sophisticated laboratories since they do not require skilled workers. Another important application of biochip devices involves the detection of biological pathogens (e.g., bacteria and viruses) present in the environment, at occupational sites such as small clinics and offices, or in public places. To achieve the required level of sensitivity and specificity in detection, it is often necessary to use a device that is capable of identifying and differentiating a large number of biochemical constituents in complex environmental samples. DNA biochip technologies could offer a unique combination of performance capabilities and analytical features of merit not available in any other current bioanalytical system. Biochip devices that combine automated sample collection systems and multichannel sensing capability will allow simultaneous detection of

multiple pathogens present in complex environmental samples. In this application the biochip technology could provide an important tool to warn of exposure to pathogenic agents for use in human health protection and disease prevention.

4. SUMMARY

Infectious diseases are moving across borders, becoming a global treat to our lifestyle and well-being. Over half of TB cases in some wealthy countries are among foreign-born populations. With increasing travel, increasing cases of malaria were reported among travelers. Infectious diseases are becoming a matter of national security for many developing countries. Sustainable development is feasible if countries can tame the infectious diseases that disempower their people. If these diseases remain unchecked, they could damage the social fabric, diminish agricultural ouput, affect industrial production, undermine political, social and economic stability, and ultimately contribute to regional and global insecurity.

For the above reasons, there is an urgent need to develop rapid, simple, cost-effective medical devices for screening multiple medical diseases simultaneously and to monitor infectious pathogens for early medical diagnosis. Such a system will also be useful in physician offices or for personal use at home. Other important applications involve monitoring pathogens for diseases by relatively unskilled personnel in the field far remote from clinical laboratories. The MFB, which is a truly integrated biochip system that comprises probes, samplers, detector as well as amplifier, and logic circuitry on board, could enable a rapid and inexpensive test for multiple diseases and for a wide variety of applications. With its multi-functional capability, the MFB technology is a system that allows simultaneous detection of multiple biotargets simultaneously. Such a device could provide information on both gene mutation (with DNA probes) and gene expression (with antibody probes against proteins) simultaneously. It is expected that advances in miniaturization and mass production technology will significantly reduce the cost of fabrication of biochip systems for widespread use worldwide to address the urging need for improved health care at a reduced cost.

5. ACKNOWLEDGEMENTS

The author acknowledges the contribution of G.D. Griffin, A.L. Wintenberg, M.N. Ericson, D.L. Stokes, J. Mobley, D. Stratis, B. M. Cullum, J.M. Song, M.D. Askari, and R. Maples. This work was sponsored by the U.S. Department of Energy, Chemical and Biological National Security Program, and the Office of Environmental and Biological Research, under contract DE-AC05-00OR22725 with UT-Battelle, LLC.

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