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組裝式半導體生醫感測裝置之設計與應用

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摘要

與血中的膽固醇量過高相關的腦血管疾病、糖尿病和高血壓性疾病穩居國人十大死因之中。雖然定期的健康檢查是預防這些慢性疾病最佳的方式，但目前一般醫療檢驗儀器單價高導致不普及；赴醫院檢查難以即時得知檢驗結果，長期下來花費也不少。故繼續研發新型即時、簡便、可攜式的檢驗器材，是預防慢性疾病的另一個選擇。本研究利用市售的光二極體(photodiode, Hamamatsu S2387-1010R)與發光二極體(light emitting diode, LED)組成一光感測器，搭配酵素法偵測膽固醇濃度。酵素法是以膽固醇氧化酵素(Cholesterol oxidase)催化膽固醇的氧化反應，反應產生的H_2O_2 再與phenol 與4-aminoantipyrine 作用，經由過氧化酵素(Horseradish ,HRP)催化可生成紅色的產物quinoneimine。藉由紀錄光子被光二極體吸收而後轉變的電流，可分析不同的光度變化對應的膽固醇濃度。同時我們以具有公認性的可見光/紫外光光譜儀（Hitachi UV-3300）進行平行測試，確保組裝式的感應平台可行性。實驗結果顯示：組裝式感測平台所測得的膽固醇氧化酵素K_m 值為31.3 ± 2.0 μM，與可見光/紫外光光譜儀所測得之K_m 值19.7 ± 2.6 μM 相近。血清檢體做測試的結果也顯示：利用組裝式感測平台所測得的膽固醇量與全自動生化分析儀(Hitachi 747)有高度相關。故未來希望能將更多此組裝式感測平台應用在更多檢驗項目下，並朝微小化整體感測平台的目標前進。

Abstract

Cardiovascular diseases, diabetes and high blood pressure are among the top ten death causes in Taiwan. . High cholesterol level in blood is one of the main causes to these diseases. As for chronic diseases, it is important to urge people to have a regular health examination. However, high cost and inconvenience of health examination make people away from doing regular health examination. Therefore, to develop a portable, easy-to-use and accurate diagnosis system will be an alternative option to solve this problem. We use commercially available photodiodes (S2387-1010R, Hamamatsu) and LEDs (light emitting diode) to assemble a photo sensor, and detect cholesterol concentration by enzymatic method. Cholesterol oxidase catalyzes cholesterol oxidation and produce H_2O_2. The product H_2O_2 reacts with 4-aminoantipyrine and phenol to generate red quinoneimine product. By measuring the external current of the photodiode, we can calculate the concentration of cholesterol. In the meanwhile, the same enzymatic reaction is tested on traditional UV-vis spectrometer (Hitachi UV-3300) to make sure the accuracy of our assembled sensing device. The result shows that the K_m value of cholesterol oxidase obtained form assembled sensing device is 31.3 ± 2.0μM which approaches the K_m value 19.7 ± 2.6μM acquired from UV-3300. And results for serum cholesterol obtained using assembled sensing device did not significantly differ from those obtained using a chemistry analyzer (Roche Hitachi 747). In conclusion, our assembled semiconductor sensing device could work. We'll try to apply more diagnostic items to our device, and miniaturize this device in the near future.

Introduction

Market of in vitro diagnostics (IVD)

In the year 2000, the global market for biotech industry totaled approximately $ 6.2 billion in U.S. dollars. By 2005 the global market for biotech industry is expected to be worth $ 8.9 billion in U.S. dollars, pharmaceutical market will take up 64% of the global market for biotech industry, in vitro diagnostic market will take up 24% of the global market for biotech industry and others take up 12% of the global market for biotech industry. [1] Although there is such huge market value in biotech industry, the investors always think the biotech industry as a high risk, high cost and most importantly long term industry. With so many drawbacks, biotech industry in Taiwan attracts fewer investors to fund. However, comparing with pharmaceutical market, in vitro diagnostics must be the best choice as a start-up biotech industry because it only needs shorter-term investigation and investment and have good performance after combining with other technologies such as microelectromechanical system (MEMS), computer science, communication engineering and so on. Furthermore, according to the estimation of Decision Resources, the use of diagnostics in hospitals and diagnostic center takes approximately 75%, the use of over-the-counter diagnostics in pharmacy and family occupies 8.8%, and use in clinic and point-of-care center takes 7.8% while blood bank and others occupy around 4% respectively. In recent years the over-the-counter diagnostics market is increasing remarkably. The reason is that over-the-counter diagnostics, such as fertility test strip, pregnant test strip, glucose strip, urine strip, are handy, parity and have quick respond time. However, we should still notice that the use of diagnostics in hospitals and diagnostic center still takes major part, and it is because an average family can't afford a delicate but expensive, large-sized diagnostic machine. Hence we take advantage of low-cost, small size semiconductor elements to make a usable assembled diagnostic biosensor.

Biosensors

Biosensor is defined as a compact analytical device incorporating a biological or biologically-derived sensing element either integrated within or intimately associated with a physicochemical transducer. The usual aim of a biosensor is to produce either discrete or continuous digital electronic signals which are proportional to a single analyte or a related group of analytes. At present several different types of transducers are used in combinations with bio-specific elements, including electrochemical, thermal, acoustic and optical transducers. As a whole, a biosensor basically has 6 characteristics: (1) specificity: only to detect one target. (2) sensitivity: small amount of sample required. (3) reproducibility: results of a test or measure are identical or closely similar each time it is conducted. (4) simplicity: handy operation. (5) inexpensiveness: an afforded price. (6) stability: mechanically rugged, offering high reliability. The current trends of a good biosensor are miniaturization, portability, low cost of mass production and easy of use. Although there are many kinds of biosensors, however, the semiconductor sensor is differentiated from others by its small size and its manufacturing techniques.

Semiconductor sensors could be fabricated by standard batch processing; hundreds of thousands of identical semiconductor sensors can be produced in one run, thus

substantially improving their performance-to-cost ratio.

Spectrophotometry

Several methods have been proposed for determination of the concentration of regents, the foremost using UV-vis spectrophotometry, HPLC, chemiluminescence and electrochemical methods. Nevertheless, spectrophotometry which determines absorption spectra of compounds, performs kinetic assays or determines the concentration of organic or inorganic analytes in solution is generally used in clinical tests. The absorption of a molecule at a particular wavelength can be related to the concentration of that molecule in solution as described by Beer's law:

$A = \varepsilon \; c \; l$

where A is the absorbance of the sample at some wavelength , c is the concentration of sample in molarity units, l is the path length of sample the light beam traverses (in centimeters), and l is an intrinsic constant of the molecule, known as the extinction coefficient, or molar absorptivity. However, a common error associated with absorption measurements is deviation from Beer's law. The form of Beer's law only suggests that the absorption of a sample will increase linearly with the concentration of the molecule being analyzed.

Indeed, optical instruments, such as UV-vis absorption spectrophotometer, fluorescence meters, and luminescence meters are commonly used to measure biochemical reactions. Moreover, photomultiplier tube (PMT) is the most common light sensor used in these spectrophotometers. PMT is an effective and sensitive light sensor. Nevertheless, the needs for high power (about 500 to 1000 V), the size and the price of PMT limit its application in a variety fields such as personalized diagnosis kits. Contrary to PMT, some semiconductor transducers as we mentioned above could be very useful as light sensor for a portable health care instrument. So, in this report, we tried to construct an assembled semiconductor element base setup for the quantification of biological reaction.

Complementary Metal-Oxide Semiconductor (CMOS) photodiode was used as sensors to monitor the change of chemiluminescence or absorption of light in enzymatic reactions that were specifically designed for each target molecule. However, there is a main drawback, the manufacture and testing of the IC design for biochemical testing has uncertain waiting period. Fortunately, there are electronic devices available in the market to be an alternative choice. To integrate semiconductor elements sold in the market can save time waiting for self-design semiconductor element manufacturing. And then we can depend on what results we discover to design the more suitable semiconductor elements for biochemical reactions. Thus, we use LED (light emitting diode) as light source and photodiode as light detector plus biological reaction to develop a portable, easy-to-use and accurate diagnostic setup.

LED

Light emitting diodes (LEDs) are the ultimate light source in the light technology. The LED technology has flourished for the past few decades. High efficiency, reliability, rugged construction, low power consumption, and durability are among the key factors for rapid development of solid-state lighting based on high-brightness visible LEDs. Conventional light sources, such as filament light bulbs and fluorescent lamps depends

on either incandescence or discharge in gases. These two processes are accompanied by large energy losses, which are attributed to the high temperatures and large Stokes shift characteristics. On the other hand, semiconductors allow an efficient way of light generation. LEDs made of semiconductor materials have the potential of converting electricity to light with near unity efficiency. Presently, most of the LEDs are made of III-V compound semiconductor materials with a small number fabricated by II-VI and group IV semiconductor materials. Conventional LEDs includes GaAsP (yellow to red) and GaP (green to red) devices. A new development is directed to various materials used for high brightness (HB) LEDs based on AlGaAs (red), AlInGaP (yellow-green to red) and InGaN (blue, green and white) devices. The development of LEDs is dependent on epitaxial growth advances in compound semiconductor technologies, mainly molecular beam epitaxy (MBE) and metal-organic vapour phase epitaxy (MOVPE). The LEDs are fabricated from semiconductor materials. The basic LED consists of a p-n junction. Under forward bias condition, electrons are injected into p-type region, and holes are injected into the n-type region. Recombination of these minority carriers with the majority carriers at the p-n junction leads to light generation. The wavelength and color of the light is determined by the difference in the energy levels of the electrons and holes.

Photodiode

Photodiodes are semiconductor devices responsive to high energy particles and photons. There are many types of photodiodes, however, the planar diffusion type silicon photodiodes which yield low level dark current are suitable for biomedical sensor. Planar diffused silicon photodiodes are simply P-N junction diodes. A P-N junction can be formed by diffusing either a P-type impurity (anode), such as Boron, into a N-type bulk silicon wafer, or a N-type impurity, such as Phosphororous, into a P-type bulk silicon wafer. However, the p-n junction of photodiode is unusual because the top p layer is very thin. The thickness of this layer is determined by wavelength of radiation to be detected. Near the p-n junction the silicon becomes depleted of electrical charges. This is known as the "depletion region". The depth of the depletion can be varied by applying a reverse bias voltage across the junction, and the depletion region is important to photodiode performance since most of the sensitivity to radiation originates there. Photodiodes operate by absorption of photons or charged particles and generate a flow of current in an external circuit, proportional to the incident power. Below is a diagram of a planar diffused silicon photodiode under reverse bias voltage. The responsivity (R_λ, A/W) of a silicon photodiode is a measure of the sensitivity to light, or a measure of the effectiveness of the conversion of the light power into electrical current. It is defined as the ratio of the photocurrent I_p (A/cm^2) to the incident light power P (W/cm^2) at a given wavelength:

$$R_\lambda = I_p/P$$

However, responsivity increase slightly with applied reverse bias due to improved charged collection efficiency in the photodiode, and increase in temperature which decreases the band gap also cause responsivity variations. By measuring the
responsivity of a photodiode, we can know the spectral responsivity of a specific photodiode. When a photodiode is exposed to light, the current-voltage characteristic of a photodiode is:

$$I_{Total} = I_{SAT}\left(e^{qV_A/k_BT} - 1\right) - I_P$$

where the I_{SAT} is the reverse saturation current, q is the electron charge, V_A is the applied bias voltage, k_B =1.38 x 10^{-23} J/K, is Boltzmann Constant, T is the absolute temperature and I_P is the photocurrent. A photodiode's capability to convert light energy to electrical energy, expressed as percentage, is its Quantum Efficiency (Q.E.). It is defined as the fraction of the incident photos that contribute to photocurrent. It is related to responsivity by:

Q.E. = $R_{\lambda\ Observed}$ / $R_{\lambda\ Ideal}$

= R_λ hc/λq = 1240 R_λ/λ

where h= 6.63 x 10^{-34} J/s, is the Planck constant, c=3 x 10^8 m/s, is the speed of light, q= 1.6 x 10^{-19} C, is the electron charge, R_λ is the responsivity in A/W and λ is the wavelength in nm. Operating under ideal conditions of reflectance, crystal structure and internal resistance, a high quality silicon photodiode of optimum design should be capable of approaching a Q.E. of 80%.

Cholesterol

Cholesterol is an unsaturated steroid alcohol of high molecular weight, consisting of a perhydrocyclopentanthroline ring and a side chain of eight carbon atoms. In its esterified form, it contains one fatty acid molecule. Cholesterol is found almost exclusively in animals. Virtually all cells and body fluids contain some cholesterol.

Cholesterol is used for the manufacture and repair of cell membranes, for synthesis of bile acids and vitamin D, and is the precursor of five major classes of steroid hormones: progestins, glucocorticoids, mineralcocorticoids, androgens, and estrogens. There are different kinds of lipoproteins in the body but the two kinds which indicate the risk of heart attack are "Low Density Lipoproteins" or LDL and "High Density Lipoprotein" or HDL. Low Density Lipoprotein is the major cholesterol carrier in the blood. It is also known as the "bad" cholesterol. HDL cholesterol also known as the "good" cholesterol carries the cholesterol from other parts of the body back to the liver. The liver removes cholesterol from the body.

According to the survey of department of health, cardiovascular disease, diabetes and high blood pressure are among the leading 10 death causes in Taiwan in 2004. The assertion that when cholesterol concentration in blood is higher than normal, it will lead to an increased chance of developing either atherosclerosis or coronary heart disease (CHD) is long established.There are many cholesterol-measured methods but the enzymatic method is now more popular than other methods. And the determination of total cholesterol in human serum is very important to diagnose any abnormality in hypertension, arteriosclerosis or in lipid metabolism. Richmond (1973) was the first person who described the preparation of an oxidase from *Nocardia* and its application to the enzymatic assay of cholesterol in saponified serum extracts. The product of this enzymatic assay could be detected by direct UV measurement at 240nm. This work was extended by Allain et al. (1974) to include the enzymatic hydrolysis of cholesterol esters and the coupled reaction with 4-aminoantipyrine and phenol. The reaction followed by the three-enzyme assay and indicator.

In the blood circulation, two thirds of the cholesterol is esterified, and one third is in free from, so we should use cholesterol esterase (EC 3.1.1.13) to catalyze cholesterol ester to free cholesterol first. And then cholesterol oxidase (EC 1.1.3.6) catalyzes the oxidation and isomerization of 3β-hydroxy steroids with the concomitant formation of

H_2O_2. Hydrogen peroxide oxidoreductase (EC 1.11.1.7), also known as peroxidase, catalyzes the oxidation of aqueous aromatic compound by hydrogen peroxide. Cholesterol is usually measured in milligrams (mg) of cholesterol per deciliter (dl) of blood. The range of total cholesterol level recommended by National Cholesterol peroxidase Educational Program (NCEP) is: Desirable, 200mg/dL or less; Borderline-high, 200 to 239mg/dL and High, 240mg/dL. It is said: prevention is better than treating disease; therefore attending for a health examination regularly helps us discover diseases early, and hence receive timely treatment. However, high cost and inconvenience of health examination cause people not to make the periodic health examination. For this reason, as we have mentioned several times before, the development of an accurate, portable, relatively inexpensive and easy-to-use biosensor will become the most important issue in the healthcare industry. Hence we use LED (light emitting diode) as light source and photodiode as light detector plus biological reaction to develop the diagnostic setup which will meet all the demands.

Results and Discussion

This study used all commercially available semiconductor elements to integrate a low-cost photosensors. Although the assembled semiconductor device has lower sensitivity than UV-3300, but our result has illustrated that the assembled setup is still a simple but useful bioanalytical tool. The potential application of a light sensitive photodiode in biochemical diagnosis can be shown in Table 4. Photo sensor has advantages of remote sensing, low cost, miniaturization, multiple modes such as absorbance, reflectance, fluorescence and extensive electromagnetic range. Thus, photo sensors are conventional instruments in diagnostics field. Some published papers indicate Complementary Metal-Oxide Semiconductor (CMOS) photodiode are photo sensors to monitor the change of chemiluminescence or absorption of light in enzymatic reactions that were specifically designed for each target molecule. However, there is a main drawback, the manufacture and testing of the IC design for biochemical testing has uncertain waiting period. For this reason, we change experimental strategy: to integrate semiconductor elements sold in the market first to save time waiting for self-design semiconductor element manufacturing and then according to what results we find to modify IC design to produce more suitable semiconductor sensor. Fortunately, our assembled devices could work in diagnostics field. Nevertheless, there is still room for improvement of our assembled sensing device, for example, when the light of a LED is unstable, we can add a spectroscope to detect the light from the LED and to correct and calibrate the real amount of light from the LED. Besides, we also could add an easy temperature-controlled circuit to make sure our experiment is under the same temperature. Or we could change a more light-sensitive photodiode such as PIN photodiode to improve the sensitivity in measurement. After refinement of the assembled sensing device, our next step is to apply to more biomedical reactions and then integrate this circuit and elements into IC manufacture to make a real diagnostic chip. Finally, by combining a novel A/D converter, miniaturization will be the ultimate goal.

In conclusion, optimization studies for several of the ingredients are: 0.82 mM 4-aminoantipyrine, 14 mM phenol, pH 7.0 and 0.05 M phosphate buffer. Enzyme kinetic

analysis of cholesterol oxidase observed with our assembled semiconductor sensing device indicates the K_m value of cholesterol oxidase is 31.3 ± 2.0 μ M which approaches the K_m = 19.7 ± 2.6 μ M obtained with traditional UV-vis spectrophotometer (UV-3300). And the cholesterol detection limit of assembled semiconductor device is 0.02 mM while the cholesterol detection limit of UV-vis spectrophotometer is 0.01 mM. However, the comparison of measured serum cholesterol levels in 9 individuals determined with assembled setup and Hitachi 747 tell us: the regression line for serum cholesterol is y = 0.9711x + 1.8143 and the correlation coefficient is r_2 = 0.9972. It means that the result for serum cholesterol obtained using the assembled device does not significantly differ from those obtained using Roche Hitachi 747, traditional chemistry analyzer used in hospital. Although the assembled semiconductor setup is less sensitivity than UV-3300, but our result illustrated that commercially available electronic device could establish a simple and useful bioanalytical tool.