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Measurement of Hepatitis B Surface Antigen Concentrations Using a PEMS

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ABSTRACT

This paper describes a piezoelectric microcantilever PEMS mass sensor which detects presence of hepatitis B surface antigen by measuring concentration of HBsAg. This detects electrical signal and is label free. Through accurate mass micro balancing technique it measures mass of biomolecules attached. The probe is till cantilever area, the equivalent spring constant is high which minimizes the effect of changes in surface stress when biomolecules are attracted to it. The traditional dip and dry technique is employed for detection on sensor.

INTRODUCTION

Hepatitis B virus (HBV) infection causes various types of diseases like Hepatitis B, cirrhosis , hepatocellular carcinoma etc. millions of people are getting affected worldwide. to detect the hepatitis b . we need to detect HBsAg in very low concentration .Nowadays in hospitals , labs chemiluminescene immunoassay is used widely to detect HBsAg ,the detection limit is approximately 0.05 ng/ml. There are many advantages offered by PEMS and it is the most advanced technology for the detection of HBsAg. the principle on which target proteins work is that how the changes occurs in the resonant frequency of the PEMS , before and after a target protein is attached to it, through immunoreactions target proteins are captured on the probe area of the piezoelectric microcantilliver as a mass sensor. The change in the resonant frequency because of the target protein depends on following factors :-

(i)Change in surface stress o mass loading due to attached biomolecules .

(ii) The surface stress is inversely proportional to the effective stiffness of the PEMS (spring constant) in this paper we will discuss the experimental set up of PEMS and it's working .

PEMS consist of a part that measures the resonant frequency and another part of PEMS enables the probe area of the sensor to react with reagents. in the PEMS , impedance analyzer is used to measure the changing resonant frequency by detecting the phase angle , dielectric loss etc. here we will use "dip – and - dry " technique for detection [13].The detection of resonant frequency is done in air when the PEMS is dipped into reagents to either immobilize the antibody or bind the antigen. Here, we demonstrate the measurement of HBsAg concentration with a PEMS , where dip and dry technique is used and the PEMS has large effective stiffness and the probe area is confined to the end of the device . Also the masses of HBsAg were measured in different concentrations and with

reaction times. Also , a control test using a protein was performed.

ADVANTAGES

- PEMS is highly label free sensor.
- It is best suitable for the detection of HBsAg
- Small in size .
- Can be developed as a portable device .
- PEMS also allows multiplexed detection and electrical redout.

DISADVANTAGES

- Environmental condition like humidity, temperature must be controlled at a constant level because resonant frequency is affected by these .
- Quality factor should be large else accuracy of detection of resonant frequency will change.
- We know that detection of HBsAg with PEMS is carried in liquid where quality factor is reduced.

WORKING OF PEMS MASS SENSOR

The traditional dip and dry experimental setup for the sensor comprises of the part of sensor that measures resonant frequency and a part that enables probe area to react with the reagents, this method involved measurements of HBsAg in different concentrations and different reaction times. The impedance analyser employed to measure the resonant frequency. The sensor is dipped in reagents to either bind antigens or to immobilise the antibody and resonance frequency is sensed in air. Here we have used the method of measurement of HBsAg concentration with PEMS and developed it as mass function , the traditional way involved controlled testing using proteins.

Previously the sensors were designed to have sufficient sensitivity and reliability. The geometrical shape is depicted in figure1 [a]. The piezoelectric layer of the sensor is made up of lead zirconatetitanate (PZT), whose composition is Pb(Zr52Ti48)O3, and the Centerville structure is made up of gold. Gold is patterned at the end of Centerville area of probe. To understand working of PEMS a mechanical lumped model of the sensor is depicted in figure 1[b]. The lumped parameters can be found through modal analysis. The mass sensitivity of developed PEMS is much higher than that of commercial quarts crystal microbalance . The mass sensitivity for PEMS is found approximately 175 Hz/pg which is calculated through eigen value analysis before and after addition of mass to probe area. The mass sensitivity of commercial QCM with 5MHz crystal is approximately 75 Hz per micro gram. Since the surface stress effect on resonant frequency is less the developed PEMS can be used as mass sensor.

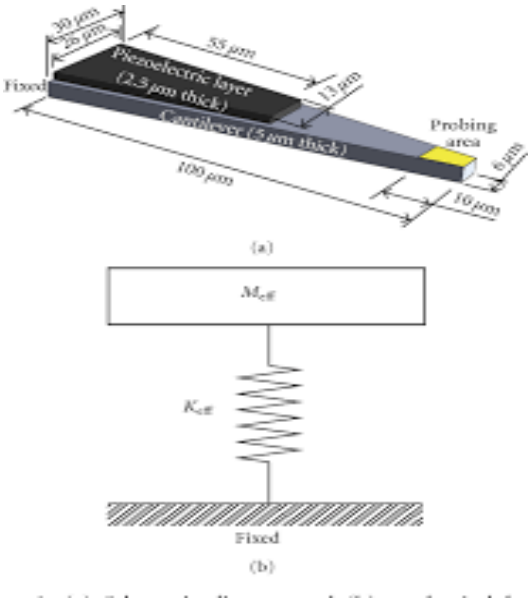

Figure1

EXPERIMENT

Figure 3 depicts the experimental setup for detection HBsAg using PEMS. The probe area of the sensor is dipped into the biochemical solution (<10microL), dipping depth of the sensor is controlled using a CCD camera. The resonant frequency of the sensor is monitored using a computer based measuring system i.e PXI system. Mechanical resonant frequency is the peak point of conductance spectra. The frequency at the peak point id calculated using a graphical programming language LABview. A thermohygrostatwas used to maintain constant temperature and humidity during the experiment.[9]

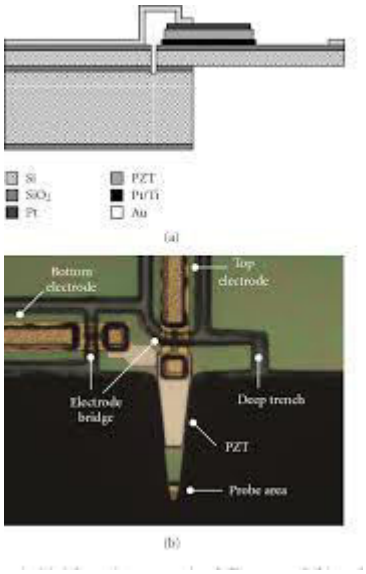

Figure 3

DETECTION PROCESS- The detection process pf the sensor is illustrated in figure 4. Initially the sensor is exposed to cleaning process which requires ultraviolet (UV) light (254nm, UV5D Short-Wave Lamp, Spectronics) and wet cleaning is also applied, initial step should be carried out for thirty minutes. [7] Second step includes reaction of gold present on the probe with thiolated protein A/G. which was required for binding the antibodies. In the third stage the sensor was passed through BSA to ensure nonspecific binding. In the fourth step anti HBsAg is immobilised to reaction site of the sensor. In the fifth step a test is conducted using PBS and AFP for comparison with the detection results. Finally while controlling the reaction time HBsAg was detected at a specific concentration. After every step PBS rinsing and DI water rinsing were carried out, also measurement of resonant frequency is carried out after every step after drying with nitrogen gas. The different between resonant frequency of HBsAg before and after and mass sensitivity of PEMS is used to determine the mass of detected HBsAg. Immobilized anti HBsAg, concentration and reaction time is found experimentally during the process. To compensate the effect of evaporation on concentration the biochemical droplets are replaced several times.[8]

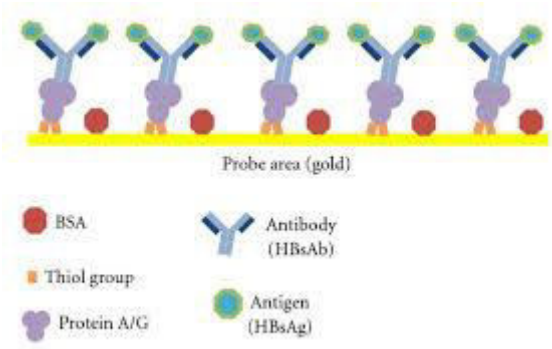

Figure 4

FUTURE SCOPE:

There are various types of biosensors developed based on various different techniques such as optical methods (e.g. surface Plasmon resonance),acoustic wave technologies(e.g. quartz crystal microbalance),electrochemistry(amperometery, voltammetry and impedance),novel nanotechnology and still counting. Currently the most commonly used clinical diagnostic methods for HBV detection are immunoassay and polymerase chain reaction (PCR). Immunoassays are based on serological methods that

target viral antigens or antibodies, which can present a good selectivity and achieve 100% accuracy. However, the immunoassay method does not provide quantitative results and the detection is limited by serological response[1].

Piezoelectric biosensors are mass sensitive and their resonant frequencies are directly proportional to the mass of sensing layer, as well as the captured external biomolecules.[2]They have been developed for label free monitoring of affinity interactions between with real time output,good specificity and high sensitivity. Most of the piezoelectric biosensors developed till the date uses quartz disks as transducers and target DNA is generally the target analyte. But these devices are subjected to various time consuming processes and inability to multi detection due to the physical nature of quartz crystal. So, further research work can be carried out which would be give us a new method which is less time consuming and is able for multi detection.

Microcantilevers have emerged as viable biosensors, demonstrating prominent[3].Recently the studies have improved the structures and materials of microcantilevers due to advances in micro/nano electromechanical system leading to expansion in applications in the field of nano-biotechnology. Microcantilever biosensors have several features such as rapid detection, low cost, simple equipment operation, and label free detection within complex biological serum.[4]

Cha[5] ethave reported HBV DNA detection using a silica nanoparticle enhanced dynamic microcantilevel biosensor. In which a 243-mer nucleotide of HBV DNA precore/core region was used as target DNA. For detection purposes, the capture probe on the microcantilever surface and detection probe conjugated with silica nanoparticles were designed specially to target DNA. HBA target DNAs of 23.1 fmol/L to 2.31nmol/L ,which is obtained from PCR products , are detected using a silica nanoparticle-enhanced microcantilever .The detection of HBV target DNA of 243-mer down to picomolar level without nanoparticle senhancement and femtomolar lever is carried out using a nanoparticle based process. This gives a highly sensitive and reliable diagnosis of DNA.

The piezoelectric microcantilevers so developed are label free which detects the sensing signal electrically, designed to measure the mass of biomolecules attached to it using accurate mass microbalancing technique. Further researchers are working on to improve efficiency and quality factor which would lead to higher accuracy.

RESULTS AND CONCLUSION

The resonant frequency of piezoelectric micro cantilever decreased as the biomolecules gets attached to it. The resonant frequency decreases at each step in the process of immobilization of the anti-HBsAg on the probe area as can be seen in the conductance spectra in figure 5. It takes even less than 20 sec to obtain this conductance spectrum with the quality factor of PEMS being approximately 200.

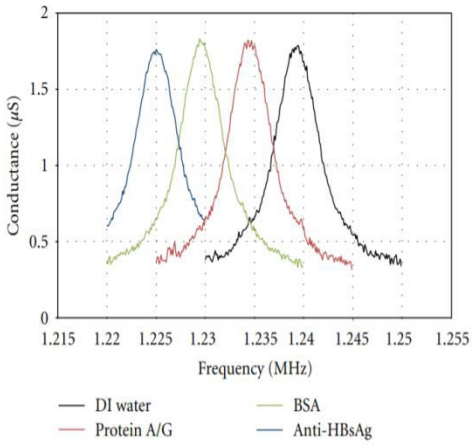

Fig 5:Conductance spectra of PEMS in the process of immobilizing anti-HBsAg on probe area.

The factors on which the mass of detected HBsAg concentration depends are (i) concentration of target solution ,(ii)reaction time,(iii)density of the immobilized anti-HBsAg,(iv) the area it occupies on the probe. So, we can conclude the amount of mass of HBsAg depends only on the concentration of HBsAg in the target solution which can be measured by measuring the mass of detected HBsAg which is in range of 0.1-100 ng/mL which can be seen in the following figure 6 . It also shows the result of measured mass that was obtained for control tests which can be done using PBS (related to the minimum detectable mass) and AFP(related to the binding selectivity of the detection test of HBsAg).

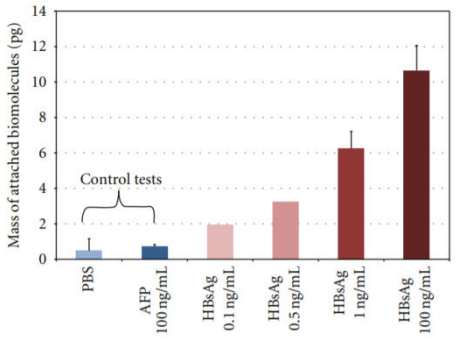

Fig6:Mass of detected HBsAg in several concentration

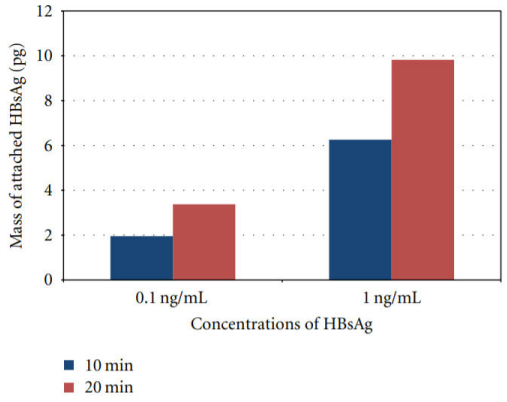

Fig7:Time dependence of immunoreactions for different concentrations of HBsAg

The above figure 7 shows the dependence of immunoreactions on time for different concentrations of HBsAg. So, from this graph we can conclude that at the concentration of 0.1ng/mL there is about 75% increase in mass of attached HBsAg and at 1ng/mL there is about 55% increase in mass, as the reaction time is increased twofold. The binding speed of HBsAg is zero at the saturation point so the binding speed decreases as the reaction time increases. The increasing ratios of mass of detected HBsAg have lower values at high concentration of HBsAg, as the binding speed increases to reach the saturation point quickly.

We have to maintain constant reaction time to measure the concentration of HBsAg but we need to adjust the reaction time as the detection of region of the concentration .i.e. shorter time is needed for lower concentration and vice versa.

The flow injection analysis (FIA) is widely used as it allows tracking the kinetics of immunoreactions and for multiplexed detection or automated system. But FIA needs more complicated systems compared to “dip-and-dry” technique. Moreover PEMS has lower quality factor and lower resonant frequency in liquid due to viscous damping effect and added mass affect of liquid thus FIA would lower the sensitivity and reliability of the PEMS and so “dip-and-dry” approach can be called best approach to measure the concentration 0f HBsAg with the development of PEMS.

In this paper we have discussed how the concentrations of HBsAg which is the surface antigen for hepatitis B can be measured in range of 0.1-100ng/mL using Piezoelectric Micro cantilever as a Mass Sensor. The concentration of HBsAg can be measured by measuring the mass of detected HBsAg when the reaction time for the target solution, the anti HBsAg density ,and its area on probe are constant. And so from this paper we conclude that PEMS can be utilized to serve the purpose of measuring the concentration of HBsAg

and can also be used for sensitive diagnostic testing of HBV infection.

REFERENCES

[1]Huang H, Jin L, Yang X, Song Q, Zou B, Jiang S, Sun L, Zhou G." An internal amplification control for quantitative nucleic acid analysis using nanoparticle-based dipstick biosensors." BiosensBioelectron. 2013;42:261–266. [PubMed] [Google Scholar]].

[2]World J Gastroenterol. 2014 Sep 21; 20(35): 12485–12492.Published online 2014 Sep PMCID: PMC4168081 PMID: 25253948Biosensors for hepatitis B virus detectionChun-Yan Yao and Wei-Ling Fu

[3]Fritz J. Cantilever biosensors. Analyst 2008; 133: 855-863 [PMID: 18575634 DOI: 10.1039/b718174d]

[4]Waggoner PS, Craighead HG. "Micro- and nano-mechanical sensors for environmental, chemical, and biological detection." Lab Chip 2007; 7: 1238-1255 [PMID: 17896006 DOI: 10.1039/b707401h]

[5] Cha BH, Lee SM, Park JC, Hwang KS, Kim SK, Lee YS, Ju BK, Kim TS." Detection of Hepatitis B Virus (HBV) DNA at femtomolar concentrations using a silica nanoparticle-enhanced microcantilever sensor". BiosensBioelectron. 2009;25:130–135. [PubMed] [Google Scholar]

[6]. S. Q. Liu, Z. Z. Zheng and X. Y. Li, Anal. Bioanal. Chem., 405 (2013) 63.

[7] J. John Livingston, M. Hemalatha, Charging an Electronic Gadget using Piezoelectricity, Indian Journal of Science and Technology, Vol 7(7), 945948, July 2014

[8]. A. Shelke, A. Habib, U. Amjad, M. Pluta, T. Kundu, U. Pietsch and W. Grill, in T. Kundu (Editor), Health Monitoring of Structural and Biological Systems 2011, Spie-Int Soc Optical Engineering, Bellingham, 2011.

[9] Yamuna. M. B1, K. Shanmukha Sundar, Design of Piezoelectric Energy Harvesting and Storage Devices, International Journal of Advanced Research in Electrical, Electronics and Instrumentation Engineering,Vol. 3, Issue 8, August 2014

[10] J. Hees, N. Heidrich, W. Pletschen, R. E. Sah, M. Wolfer, O. A. Williams, V. Lebedev, C. E. Nebel and O. Ambacher, Nanotechnology, 24 (2013) 0957.

[11] H. Wang and A. A. Wereszczak, IEEE Trans UltrasonFerroelectr Freq Control, 55 (2008) 2559.

[12] T. K. Sinha, S. K. Ghosh, R. Maiti, S. Jana, B. Adhikari, D. Mandal and S. K. Ray, ACS Appl. Mater. Interfaces, 8 (2016) 14986.

[13] Measurement of Hepatitis B Surface Antigen Concentrations Using a Piezoelectric Microcantilever as a Mass Sensor Sangkyu Lee,1 Jongyun Cho,1 Yeolho Lee,2 Sangmin Jeon,3 Hyung Joon Cha,3 and Wonkyu Moon1