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TOPIC HIGHLIGHT

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Biosensors for hepatitis B virus detection

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Abstract

A biosensor is an analytical device used for the detection of analytes, which combines a biological component with a physicochemical detector. Recently, an increasing number of biosensors have been used in clinical research, for example, the blood glucose biosensor. This review focuses on the current state of biosensor research with respect to efficient, specific and rapid detection of hepatitis B virus (HBV). The biosensors developed based on different techniques, including optical methods (*e.g.*, surface plasmon resonance), acoustic wave technologies (*e.g.*, quartz crystal microbalance), electrochemistry (amperometry, voltammetry and impedance) and novel nanotechnology, are also discussed.

Key words: Biosensor; Hepatitis B virus; Diagnosis; Detection; Quartz crystal microbalance

Core tip: This paper reviews the current state of biosensor research with respect to efficient, specific and rapid detection of hepatitis B virus. The biosensors developed based on different techniques, including optical methods (*e.g.*, surface plasmon resonance), acoustic wave technologies (*e.g.*, quartz crystal microbalance), electrochemistry (amperometry, voltammetry and impedance) and novel nanotechnology, are also discussed.

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INTRODUCTION

Hepatitis B virus (HBV) infection is one of the major health problems worldwide, and may lead to chronic hepatitis, cirrhosis and primary liver cancer, particularly if the patient's diet and health conditions are not well controlled[1-3]. It is estimated that there are 2 billion people with a serological profile of past or current HBV infection, and 360 million patients with chronic HBV-associated liver disease[4,5]. Patients are at an especially high risk when their serum viral load is > 10^5 copies/mL, according to a report by the REVEALHBV (risk evaluation of viral load elevation and associated liver disease/cancer-hepatitis B virus) group[6,7]. Hence, it is important to detect and monitor HBV at an early stage of infection. Hepatitis B is a familiar disease in China, and determination of virus concentration can provide the key foundation for diagnosis and treatment.

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[8]. Hepatitis B surface (HBs) antibody is the most commonly used immunoassay target

and appears even several months after the acute period of HBV infection[9]. In clinical application, PCR is traditionally the standard technique to identify HBV infection. Quantitative PCR has been used to estimate the initial amount of virus DNA by measuring the amount of amplified product. Real-time PCR is based on the evaluation of the threshold cycle (CT) when amplification of the PCR product is first detected[10]. PCR consists of 20-40 repeated cycles to achieve a detectable DNA concentration, so it requires efficient control of thermal cycles and thus has higher instrumentation costs. PCR is also known to have errors caused by its hybridization mechanism. Consequently, it is necessary to develop a detection technique that has the characteristics of cost-effectiveness, fast response, portable capability, and high sensitivity compared with traditional clinical diagnostic methods.

A biosensor is an analytical device used for the detection of analytes, which combines a biological component with a physicochemical detector[11]. Recently, an increasing number of biosensors have been used in clinical research, for example, the blood glucose biosensor[12,13]. Most of the clinical research on biosensors was based on immunological reaction or DNA hybridization, and the sensor always gave rapid results with a high sensitivity[14,15]. DNA biosensors based on nucleic acid hybridization are currently under intense investigation owing to their increasing importance in the diagnosis of disease, with low cost and low power requirements[16]. This type of biosensor can be prepared by immobilizing single-stranded DNA probes on different electrodes and physicochemical detectors are used to measure the hybridization events between the DNA probes and their cDNA fragments.

This review looks at the current state of biosensor research with respect to efficient, specific and rapid detection of HBV. The biosensors developed based on different techniques, including optical methods [*e.g.*, surface plasmon resonance (SPR)], acoustic wave technologies [*e.g.*, quartz crystal microbalance (QCM)], electrochemistry (amperometry, voltammetry and impedance) and novel nanotechnology, are also discussed.

BIOSENSORS BASED ON NANOTECHNOLOGY

Recently, with the development of nanotechnology, researchers are constantly expanding the applications of nanotechnology with unique properties to construct novel biosensors. For instance, quantum dots[17], carbon nanotubes[18], nanowires[19] and magnetic nanoparticles[20-22] have attracted much attention as signal transducers. Biosensors based on nanotechnology show high specificity and sensitivity after being labeled with DNA probe or antibody. The nanoparticle-based biosensor has high specificity, ease of operation, low cost, and the sensitivity needed for the rapid and reproducible detection of pathogenic microorganisms in clinical specimens. However, the covalent labeling with nanoparticles is time consuming and involves complicated synthetic procedures.

Gold nanorod material has high light absorption and a large scattering cross-section in the SPR wavelength regions. Therefore, Wang *et al*[23] designed a novel gold nanorod biosensor based on localized SPR (LSPR) to detect hepatitis B surface antigen (HBsAg). Investigation of the HBs-antibody-modified gold nanorod indicates that physical adsorption and successive blocking lead to a flexible shell enveloping the nanorod, which minimizes the nonspecific adsorption and allows the assays to work in buffer, serum and plasma. The experimental results indicate that a gold-nanorod-based LSPR biosensor is capable of measuring HBsAg concentration even at 0.01 IU/mL, which is about 40 times lower than the limit of detection of the ELISA method. The gold-nanorod-based biosensor can be utilized in quantitative analysis with a dose-dependent response ranging from 0.01 to 1 IU/mL.

ELECTROCHEMICAL DNA BIOSENSORS

Electrochemistry possesses attractive features over other existing measurement systems, therefore, electrochemical biosensors are expected to provide a simple, accurate and inexpensive platform for DNA analysis[24]. Electrochemical biosensors have attracted considerable attention in recent years, and have provided a sensitive, accurate, rapid and cost-effective platform for a variety of practical applications in the fields of medical diagnostics, clinical genetic analysis, forensic identification and environmental monitoring[25,26]. In clinical diagnosis, electrochemical transducers offer attractive advantages, for example, good sensitivity, high specificity, simplicity and low cost, for converting nucleic acid hybridization events into analytical signals[27,28]. The complex formed from the bioreceptor and biomolecular binding at the sensor surface results in a detectable change, converted into a quantitative amperometric, potentiometric or impedimetric signal. Electrochemical transducers are often used for detecting DNA hybridization events, due to their high sensitivity, small dimensions, low cost, and compatibility with microfabrication technology[29].

Voltammetric and amperometric biosensors

Zhang *et al*[30] have developed an electrochemical DNA biosensor for the detection of HBV DNA fragments. The biosensor relies on the covalent immobilization of the 21-mer single-stranded DNA (ssDNA) related to HBV genes on the modified glassy carbon electrode (GCE). Using $(CdL_2)^{2+}$ as a novel electroactive indicator, the hybridization between the probe and its complementary ssDNA was investigated by differential pulse voltammetry. Experiments with non-complementary oligonucleotides were carried out to assess the selectivity of the developed electrochemical DNA biosensor. The target HBV DNA could be quantified in the range from 1.01×10^{-8} to 1.62×10^{-6} mol/L with good linearity ($\gamma = 0.9962$). The detection limit was 7.19×10^{-9} mol/L.

In a study by Ding *et al*[31] a label-free electrochemical biosensor for the detection of oligonucleotides related to HBV sequences via the interactions of DNA with the redox-active complex 2,9-dimethyl-1,10-phenantroline cobalt [Co(dmp)(H_2O)(NO_3)$_2$] was described. The study was carried out by the hybridization of a 21-mer probe DNA modified on a GCE with target DNA, and [Co(dmp)(H_2O)(NO_3)$_2$], which was comparable in size to the small groove of native double-helix DNA, was used as an electrochemical indicator. Under the optimal conditions, the electrical signal had a linear relationship with the concentration of target DNA, ranging from 3.96×10^{-7} to 1.32×10^{-6} mol/L, and the detection limit was 1.94×10^{-8} mol/L (S/N = 3). The biosensor had good selectivity by detecting the three-base mismatch sequence.

Hashimoto *et al*[32] reported a microfabricated disposable-type electrochemical DNA biosensor, based on photolithography technique, for the detection of HBV genomic DNA. HBV DNA extracted from patients' sera was quantified in the range of 10^4-10^6 copies/mL, and the biosensor showed a good correlation with competitive PCR. The results suggested that the microfabricated disposable DNA biosensor can provide a specific and quantitative detection of HBV DNA in serum.

In a study by Meric *et al*[33], an electrochemical biosensor for voltammetric detection of DNA sequences related to HBV and TT virus (TTV) from PCR-amplified samples was described. The electrochemical DNA biosensor relied on the immobilization of the 21- or 24-mer single-stranded oligonucleotides (probe), which were related to the HBV and TTV sequences, and hybridization of these oligonucleotides with their complementary sequences (target) at a carbon paste electrode. The extent of hybridization between the probe and target sequences was determined using square wave voltammetry and methylene blue (MB) as a hybridization indicator. With its specific interaction with the guanine bases, the MB signals from the hybrid and the probe can easily be distinguished from each other. The point mutations, which include guanine bases in the DNA sequences, can rapidly be detected using this specific interaction of MB with unbound guanine bases. However, they did not give the detection range and limit for HBV detection.

Impedance biosensors

Electrochemical impedance spectroscopy is a sensitive technique, which monitors the electrical response of the studied system after application of a periodic small amplitude AC signal. System response provides information concerning the interface state (presence of adsorbed species) and can detect the occurrence of interfacial reactions[34,35].

Hassen *et al*[36] reported a new approach based on DNA hybridization for detecting HBV using an electrochemical impedance biosensor. DNA probes modified with biotin at the 5' position were immobilized on streptavidin-modified magnetic nanoparticles by biotin-streptavidin interaction. The hybridization reactions with specific cDNA target and non-complementary sequence were investigated by impedance spectroscopy. Results showed that the impedance biosensor allows detection of 50 pmol/L HBV DNA in a sample of 20 μL.

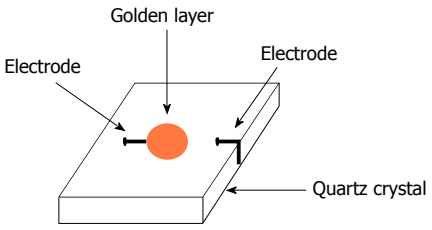

Figure 1 Schematic representation of constructed crystal for quartz crystal microbalance biosensor.

PIEZOELECTRIC BIOSENSORS

Piezoelectric biosensors are mass-sensitive and their resonant frequencies are directly dependent on the mass of the sensing layer, as well as the captured external biomolecules. Therefore, they have been developed for label-free monitoring of affinity interactions between molecules with real-time output, high sensitivity and good specificity[3,37,38]. Most of the reported piezoelectric biosensors utilize quartz crystal disks as transducers[39,40], and target DNA is normally the target analyte. However, these devices are subject to time-consuming procedures and inability for multi detection due to the physical nature of the quartz crystal.

QCM biosensors

QCM is based on the property of an AT cut quartz crystal that oscillates when an alternating voltage is applied. The quartz surface always has a thin gold (or other) metal layer deposited onto it, which doubles as a conductor for the AC current flowing and also provides an attachment surface for a bioreceptor to be immobilized (Figure 1). This deposition can be monitored by the frequency decrease caused by the additional mass on the metal layer that is related to the mass change via the Sauerbrey equation[41]. Recently, increasing attention has been paid to the development of QCM biosensors because of their many merits such as compact size, high sensitivity and good specificity, low cost, label-free detection, and rapid response[3,42]. Moreover, advances in biosensors make multiple-analyte diagnosis possible, which may eliminate expensive and time-consuming detection procedures in clinical diagnosis.

Xu *et al*[43] have developed a diaphragm-based QCM immunoassay chip to detect simultaneously HBV and α-fetoprotein (AFP) antibodies. The total assay time was < 2 h. The frequency shift-based calibration curves showed a detection limit of 0.1 ng/mL and a dynamic detection range of 0.1-10 000 ng/mL for both anti-HBsAg and anti-AFP. The small frequency variations of the reference sensors during the whole detection processes show that the washing processes and nonspecific

biomaterials absorption have negligible effects on the sensor resonant frequency. The results demonstrate that the piezoelectric immunoassay chip has potential applications for rapid, specific, sensitive, and multiple detection of HBV.

Yao *et al*[39] have used peptide nucleic acid (PNA) probes instead of DNA probes to construct PNA-QCM biosensors for real-time monitoring of the hybridization assay of HBV. The PNA probe can combine target sequences more effectively and specifically than DNA probes. The PNA probe is designed and immobilized on the surface of the biosensor to substitute the conventional DNA probe for direct detection of HBV genomic DNA without previous amplification by PCR. The hybridization assay is completed within 50 min. The detection limit is 8.6 pg/L and the clinical specificity is 94.44% compared with real time-PCR. The PNA probe can distinguish sequences that differ in only one base. Detection sensitivity can be improved and detection time can be reduced by adding RecA-protein-coated complementary ssDNA, which complements HBV gene regions.

Rolling circle amplification (RCA) is an isothermal amplification technique for small circular DNA templates, with the unique property of product localization[44]. Yao *et al*[45] have described the application of RCA-based QCM biosensors for direct detection of HBV genomic DNA from clinical samples. The covalent bonding between the capture probes and the gold electrode surface guarantees that the linkage between the capture probes and the amplified RCA products is maintained during the assay. Making use of the high amplification efficiency of Phi29 DNA polymerase and the remarkable precision of *Escherichia coli* DNA ligase in differentiating mismatched bases at the ligation site, we can detect as few as 10^4 copies/mL HBV DNA. The experimental results show that RCA has significantly enhanced sensitivity for the target strand compared to the single-base mismatch strand. With 60 min RCA duration, the proposed detection shows over 10 times amplification of the original signals.

In a study by Zhou *et al*[40] a highly sensitive piezoelectric DNA biosensor has been developed based on the sensitive mass-transducing function of the QCM and the specificity of the nucleic acid hybridization reaction. An HBV nucleic acid probe was immobilized onto the gold electrodes of a 9-MHz AT-cut piezoelectric quartz crystal by the polyethyleneimine adhesion, glutaraldehyde cross-linking or physical adsorption method. The frequency shifts of hybridization have a better linear relationship with the amount of HBV DNA, when the amount is 0.02-0.14 μg/mL.

Microcantilever biosensors

Microcantilevers have emerged as viable biosensors, demonstrating prominent performance[46,47]. Recently, the structures and materials of microcantilevers have been improved due to advances in the micro-/nano-electromechanical system, and the applications of microcantilevers have been significantly expanded due to their utilization in the nano-/biotechnology field. Microcantilever biosensors have several advantages over the aforementioned methods, such as rapid detection, simple equipment operation, low cost, and label-free detection within complex biological serum. Thus, microcantilever biosensors have been used successfully for DNA hybridization, immunoassays and particle detection including viruses, bacteria and cells[48,49].

Huang *et al*[50] have developed a DNA-sensing device based on microcantilevers. The experimental results revealed that the DNA detection system-on-a-chip (SoC) has good selectivity between 1-bp-mismatched DNA and all-matched DNA. Utilizing the N^+ polysilicon 2 embedded layer as a piezoresistor, the stress induced by the microcantilever-beam deflection can be measured. The developed SoC was the first fully integrated monolithic microcantilever biosensor system, which includes sensors, interface circuits, digital processor, and wireless communication on a single chip for HBV DNA detection. The experimental results showed that the HBV DNA SoC can successfully distinguish the signal of all-mismatched, 1-bp-mismatched, and all-matched sequences of 19-mer HBV-conserved DNA sequences. At the same time, the concentration detection range has been identified from 1 pmol to 10 nmol. That was the first time that the HBV DNA detection capability has been demonstrated by a fully integrated wireless SoC. The microcantilever SoC offers the characteristics of real-time and label-free detection with high sensitivity and selectivity.

Cha *et al*[51] have reported HBV DNA detection using a silica nanoparticle-enhanced dynamic microcantilever biosensor. A 243-mer nucleotide of HBV DNA precore/core region was used as the target DNA. For detection purposes, the capture probe on the microcantilever surface and the detection probe conjugated with silica nanoparticles were designed specifically for the target DNA. HBV target DNAs of 23.1 fmol/L to 2.31 nmol/L, which are obtained from PCR products, are detected using a silica nanoparticle-enhanced microcantilever. The HBV target DNA of 243-mer is detected down to the picomolar level without nanoparticle enhancement and femtomolar level using a nanoparticle-based signal amplification process. There is an almost 2-3 orders of magnitude increase in sensitivity, and the microcantilever is a highly sensitive and reliable diagnostic tool for DNA detection.

Surface acoustic wave biosensors

Surface acoustic wave (SAW) sensors, which are a type of piezoelectric sensor, were first introduced by White and Voltmer, and are potential biochemical sensing platforms with excellent sensitivity, speed and reliability. Since Wohltjen and Dessy's application, SAW sensors based on Rayleigh surface waves have been used as highly sensitive sensors[52].

Lee *et al*[53] have demonstrated an application of low-wave mode SAW immunosensors to detect HBs antibody in aqueous conditions. The resonance frequency shift has been monitored to detect specific binding of HBs antibody to the immobilized HBsAg. The sensor shows bind-

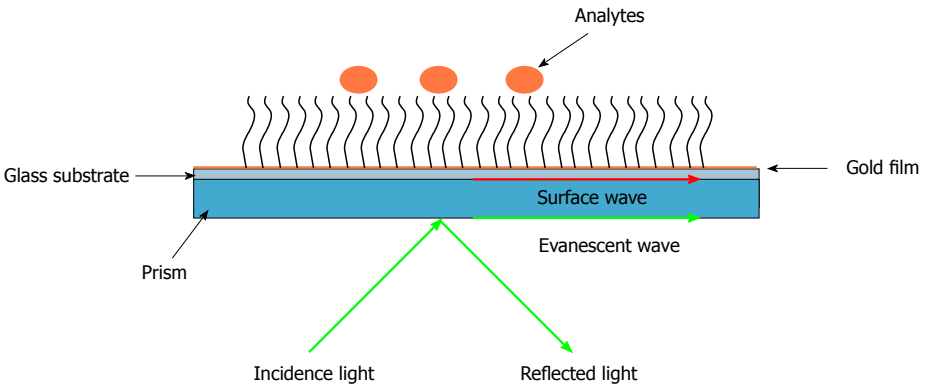

Figure 2 Schematic representation of surface plasmon resonance biosensor. Incidence light illuminates thin gold film, causing electrons to be excited and form a surface plasmon wave.

ing specificity to HBs antibody and a linear relationship between the frequency shift and antibody concentration with a sensitivity of 0.74 Hz/(pg/μL) and a detection limit < 10 pg/μL. The SAW immunosensor can successfully detect HBs antibody in whole blood samples without any pretreatment.

SPR BIOSENSORS

The SPR method is based on total internal reflection in a glass prism onto which a thin metal film (usually gold) is deposited. When total internal reflection of the incident light occurs, an evanescent wave is set up that exerts a field outside the prism. This can only occur when the film is thin enough, about 50 nm, as the delocalized electrons within the metal cause a surface plasmon to be established at the metal-ambient interface. This causes a wave to propagate along the surface and therefore reduces the intensity of light reflected[41]. The advantages of SPR biosensors are the direct detection, without any additional labeling, and the possibility of online monitoring of affinity interactions[54]. The SPR method is popular because it allows label-free, high-sensitivity, real-time analysis and flexible system design. For example, the design of various orderly nanostructures has been shown to provide strong local electrical field enhancement, allowing a higher sensitivity and a wider dynamic range[37,55]. SPR sensor device design is trending toward miniaturization, low cost and user friendliness[56,57] (Figure 2).

To develop a high-sensitivity, low-cost screening system for HBV detection, Chuang *et al*[58] demonstrated the detection of the LAMP reaction by the SPR analytical method without immobilizing probes. The HBV template mixed in 10 μL LAMP solution could be detected using the SPR-LAMP system within 17 min, even at the detection-limited concentration of 2 fg/mL. They also analyzed the correlation coefficients between the initial concentrations of HBV DNA templates and the system response (ΔRU) at different amplification times to establish an optimal endpoint of 25 min ($R^2 = 0.98$).

Chung *et al*[9] have described the use of SPR to detect captured human HBV antibodies using an HBsAg in conjunction with a secondary antibody. The detection limit was found to be 10 nmol HBV antibody. The detection limit of the SPR biosensor for the medical diagnosis was similar to the commercial ELISA kit.

TRANSLATIONAL PERSPECTIVES

In recent years, biosensors have been shown to provide complementary and additional information contributed by the well-established bioanalytical techniques. Particular advantages of biosensors are as follows: the possibility of minimalizing the setup, in principle down to the molecular scale; the use of well-established microsystem technologies during manufacture (of at least certain sensor components); integration of signal preprocessing steps on a chip; and building arrays for more complex analysis. Detecting viruses and viral components using biosensors is a relatively new concept and still in its infancy. Traditional viral detection methods are time consuming and expensive, and consequently, demand for accurate viral biosensors by rapid detection systems is increasing. In most cases, only limited quantities of HBV are available from clinical samples. In most biosensor studies, both the sensitivity and specificity of the HBV detection were unaffected by serum samples (Table 1). However, it is necessary to point out that more detailed work is required before the clinical application of biosensors.

The development of isothermal amplification technology, such as strand displacement amplification and RCA, makes the amplification of nucleic acid sequences without PCR possible, which provides an opportunity for biosensor miniaturization. RCA is an isothermal amplification technique that is adaptable to the on-chip signal amplification format. Unlike other amplification procedures, RCA produces a single amplified product that remains linked to the DNA primer. Circularization of the padlock probes in the RCA reaction is specific in that 1-bp mismatch can be specifically discriminated[59-62]. As a result of its simplicity, specificity and high sensitivity, RCA has attracted considerable attention in biosensor detection. Aptamers are a new class of molecules with great potential to rival monoclonal antibodies in thera-

Table 1 Biosensor techniques discussed in this review

Detection target	Characteristics of biosensor	Detection limit	Ref.
HBsAg	Nanorods biosensor	0.01 IU/mL	[23]
HBsAb	QCM	0.1 pg/μL	[43]
	SAW	10 pg/μL	[53]
	SPR	10 nmol/L	[9]
HBV DNA	Electrochemical biosensor	7.19×10^{-9} mol /L	[30]
		1.94×10^{-8} mol /L	[31]
		1.77×10^{-14} mol /L	[32]
		2.5×10^{-6} mol /L	[36]
	QCM	8.6×10^{-12} g/L	[39]
		1.77×10^{-14} mol/L	[45]
		2×10^{-8} g/L	[40]
	Microcantilever biosensor	10^{-12} mol/L	[50]
		2.31×10^{-14} mol/L	[51]
	SPR	2×10^{-12} g/L	[58]

HBsAg: Hepatitis B surface antigen; HBsAb: Hepatitis B surface antibody; HBV: Hepatitis B virus; QCM: Quartz crystal microbalance; SAW: Surface acoustic wave; SPR: Surface plasmon resonance.

peutic, diagnostic and analytical, as well as basic research applications. In comparison to antibodies as capture molecules in biosensors, aptamers have several advantages. Aptamers are produced *in vitro*, and their binding affinity, specificity, and stability can easily be manipulated and improved by rational design. At the same time, aptamers can be modified with functional groups to allow covalent or direct immobilization on biochips, resulting in highly ordered receptor layers that can improve detection sensitivity significantly[63,64]. As stable RNA or DNA oligonucleotides, aptamers can be denatured and refolded many times without loss of activity[65]. The characteristics and low cost of synthesis make aptamers particularly suitable for biosensor detection.

Since their development in the 1990s, SPR and QCM have been the most researched techniques to date. However, the novel biosensors will be more attractive with the progress of isothermal amplification technology and aptamer techniques. We hope that a portable, label-free biosensor for the sensitive and specific HBV detection will be available in the near future.

REFERENCES

1 **Beasley RP**. Hepatitis B virus. The major etiology of hepatocellular carcinoma. *Cancer* 1988; **61**: 1942-1956 [PMID: 2834034 DOI: 10.1002/1097-0142]

2 **Lok AS**, McMahon BJ. Chronic hepatitis B. *Hepatology* 2001; **34**: 1225-1241 [PMID: 11732013 DOI: 10.1053/jhep.2001.29401]

3 **March C**, Manclús JJ, Jiménez Y, Arnau A, Montoya A. A piezoelectric immunosensor for the determination of pesticide residues and metabolites in fruit juices. *Talanta* 2009; **78**: 827-833 [PMID: 19269436 DOI: 10.1016/j.talanta.2008.12.058]

4 Hepatitis B vaccines. *Wkly Epidemiol Rec* 2009; **84**: 405-419 [PMID: 19817017]

5 **Shepard CW**, Simard EP, Finelli L, Fiore AE, Bell BP. Hepatitis B virus infection: epidemiology and vaccination. *Epidemiol Rev* 2006; **28**: 112-125 [PMID: 16754644 DOI: 10.1093/epirev/mxj009]

6 **Iloeje UH**, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology* 2006; **130**: 678-686 [PMID: 16530509 DOI: 10.1053/j.gastro.2005.11.016]

7 **Lee CZ**, Huang GT, Yang PM, Sheu JC, Lai MY, Chen DS. Correlation of HBV DNA levels in serum and liver of chronic hepatitis B patients with cirrhosis. *Liver* 2002; **22**: 130-135 [PMID: 12028407 DOI: 10.1034/j.1600-0676.2002.01525.x]

8 **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. *Biosens Bioelectron* 2013; **42**: 261-266 [PMID: 23208096 DOI: 10.1016/j.bios.2012.10.078]

9 **Chung JW**, Kim SD, Bernhardt R, Pyun JC. Application of SPR biosensor for medical diagnostics of human hepatitis B virus (hHBV). *Sens Actuators B Chem* 2005; **111-112**: 416-422 [DOI: 10.1016/j.snb.2005.03.055]

10 **Paraskevis D**, Haida C, Tassopoulos N, Raptopoulou M, Tsantoulas D, Papachristou H, Sypsa V, Hatzakis A. Development and assessment of a novel real-time PCR assay for quantitation of HBV DNA. *J Virol Methods* 2002; **103**: 201-212 [PMID: 12008014 DOI: 10.1016/S0166-0934(02)00033-2]

11 **Ziegler C**, Göpel W. Biosensor development. *Curr Opin Chem Biol* 1998; **2**: 585-591 [PMID: 9818183 DOI: 10.1016/S1367-5931(98)80087-2]

12 **Quershi A**, Gurbuz Y, Kang WP, Davidson JL. A novel interdigitated capacitor based biosensor for detection of cardiovascular risk marker. *Biosens Bioelectron* 2009; **25**: 877-882 [PMID: 19775885 DOI: 10.1016/j.bios.2009.08.043]

13 **Qureshi A**, Niazi JH, Kallempudi S, Gurbuz Y. Label-free capacitive biosensor for sensitive detection of multiple biomarkers using gold interdigitated capacitor arrays. *Biosens Bioelectron* 2010; **25**: 2318-2323 [PMID: 20381333 DOI: 10.1016/j.bios.2010.03.018]

14 **Haasnoot W**, Gerçek H, Cazemier G, Nielen MW. Biosensor immunoassay for flumequine in broiler serum and muscle. *Anal Chim Acta* 2007; **586**: 312-318 [PMID: 17386729 DOI: 10.1016/j.aca.2006.10.003]

15 **Kalogianni DP**, Koraki T, Christopoulos TK, Ioannou PC. Nanoparticle-based DNA biosensor for visual detection of genetically modified organisms. *Biosens Bioelectron* 2006; **21**: 1069-1076 [PMID: 15935636 DOI: 10.1016/j.bios.2005.04.016]

16 **Drummond TG**, Hill MG, Barton JK. Electrochemical DNA sensors. *Nat Biotechnol* 2003; **21**: 1192-1199 [PMID: 14520405 DOI: 10.1038/nbt873]

17 **Shingyoji M**, Gerion D, Pinkel D, Gray JW, Chen F. Quantum dots-based reverse phase protein microarray. *Talanta* 2005; **67**: 472-478 [PMID: 18970191 DOI: 10.1016/j.talanta.2005.06.064]

18 **Ghosh S**, Sood AK, Kumar N. Carbon nanotube flow sensors. *Science* 2003; **299**: 1042-1044 [PMID: 12532025 DOI: 10.1126/science.1079080]

19 **Zheng G**, Patolsky F, Cui Y, Wang WU, Lieber CM. Multiplexed electrical detection of cancer markers with nanowire sensor arrays. *Nat Biotechnol* 2005; **23**: 1294-1301 [PMID: 16170313 DOI: 10.1038/nbt1138]

20 **Baselt DR**, Lee GU, Natesan M, Metzger SW, Sheehan PE, Colton RJ. A biosensor based on magnetoresistance technology. *Biosens Bioelectron* 1998; **13**: 731-739 [PMID: 9828367 DOI: 10.1016/S0956-5663(98)00037-2]

21 **Li G**, Sun S, Wilson RJ, White RL, Pourmand N, Wang SX. Spin valve sensors for ultrasensitive detection of superparamagnetic nanoparticles for biological applications. *Sens Actuators A Phys* 2006; **126**: 98-106 [PMID: 18414592 DOI: 10.1016/j.sna.2005.10.001]

22 **Millen RL**, Kawaguchi T, Granger MC, Porter MD, Tondra M. Giant magnetoresistive sensors and superparamagnetic nanoparticles: a chip-scale detection strategy for immunosorbent assays. *Anal Chem* 2005; **77**: 6581-6587 [PMID: 16223243 DOI: 10.1021/ac0509049]

23 **Wang X**, Li Y, Wang H, Fu Q, Peng J, Wang Y, Du J, Zhou Y, Zhan L. Gold nanorod-based localized surface plasmon resonance biosensor for sensitive detection of hepatitis B virus in

buffer, blood serum and plasma. *Biosens Bioelectron* 2010; **26**: 404-410 [PMID: 20729056 DOI: 10.1016/j.bios.2010.07.121]
24 **Pänke O**, Kirbs A, Lisdat F. Voltammetric detection of single base-pair mismatches and quantification of label-free target ssDNA using a competitive binding assay. *Biosens Bioelectron* 2007; **22**: 2656-2662 [PMID: 17141493 DOI: 10.1016/j.bios.2006.10.03]
25 **Li F**, Feng Y, Dong P, Tang B. Gold nanoparticles modified electrode via a mercapto-diazoaminobenzene monolayer and its development in DNA electrochemical biosensor. *Biosens Bioelectron* 2010; **25**: 2084-2088 [PMID: 20207131 DOI: 10.1016/j.bios.2010.02.004]
26 **Luo X**, Lee TM, Hsing IM. Immobilization-free sequence-specific electrochemical detection of DNA using ferrocene-labeled peptide nucleic acid. *Anal Chem* 2008; **80**: 7341-7346 [PMID: 18771276 DOI: 10.1021/ac8010236]
27 **Kostecka P**, Havran L, Pivonkova H, Fojta M. Voltammetry of osmium-modified DNA at a mercury film electrode: application in detecting DNA hybridization. *Bioelectrochemistry* 2004; **63**: 245-248 [PMID: 15110280 DOI: 10.1016/j.bioelechem.2003.11.005]
28 **Palecek E**. Past, present and future of nucleic acids electrochemistry. *Talanta* 2002; **56**: 809-819 [PMID: 18968559 DOI: 10.1016/S0039-9140(01)00649-X]
29 **Xue D**, Elliott CM, Gong P, Grainger DW, Bignozzi CA, Caramori S. Indirect electrochemical sensing of DNA hybridization based on the catalytic oxidation of cobalt (II). *J Am Chem Soc* 2007; **129**: 1854-1855 [PMID: 17260990 DOI: 10.1021/ja067339r]
30 **Zhang S**, Tan Q, Li F, Zhang X. Hybridization biosensor using diaquabis[N-(2-pyridinylmethyl)benzamide-κ2N,O]-cadmium(II) dinitrate as a new electroactive indicator for detection of human hepatitis B virus DNA. *Sens Actuators B Chem* 2007; **124**: 290-296 [DOI: 10.1016/j.snb.2006.12.040]
31 **Ding C**, Zhao F, Zhang M, Zhang S. Hybridization biosensor using 2,9-dimethyl-1,10-phenantroline cobalt as electrochemical indicator for detection of hepatitis B virus DNA. *Bioelectrochemistry* 2008; **72**: 28-33 [PMID: 18096445 DOI: 10.1016/j.bioelechem.2007.11.001]
32 **Hashimoto K**, Ito K, Ishimori Y. Microfabricated disposable DNA sensor for detection of hepatitis B virus DNA. *Sens Actuators B Chem* 1998; **46**: 220-225 [DOI: 10.1016/S0925-4005(98)00121-X]
33 **Meric B**, Kerman K, Ozkan D, Kara P, Erensoy S, Akarca US, Mascini M, Ozsoz M. Electrochemical DNA biosensor for the detection of TT and Hepatitis B virus from PCR amplified real samples by using methylene blue. *Talanta* 2002; **56**: 837-846 [PMID: 18968562 DOI: 10.1016/S0039-9140(01)00650-6]
34 **Janek RP**, Fawcett WR, Ulman A. Impedance spectroscopy of self-assembled monolayers on Au (III): evidence for complex double-layer structure in aqueous NaClO4 at the potentia of zero charge. *J Phys Chem B* 1997; **101**: 8550-8558 [DOI: 10.1021/jp971698e]
35 **Janek RP**, Fawcett WR, Ulman A. Impedance spectroscopy of self-assembled monolayers on Au (III): sodium ferrocyanide charge transfer at modified electrode. *Langmuir* 1998; **14**: 3011-3018 [DOI: 10.1021/la970980]
36 **Hassen WM**, Chaix C, Abdelghani A, Bessueille F. An impedimetric DNA sensor based on functionalized magnetic nanoparticles for HIV and HBV detection. *Sens Actuators B Chem* 2008; **134**: 755-760 [DOI: 10.1016/j.snb.2008.06.020]
37 **Chang CC**, Chiu NF, Lin DS, Chu-Su Y, Liang YH, Lin CW. High-sensitivity detection of carbohydrate antigen 15-3 using a gold/zinc oxide thin film surface plasmon resonance-based biosensor. *Anal Chem* 2010; **82**: 1207-1212 [PMID: 20102177 DOI: 10.1021/ac901797j]
38 **Poitras C**, Tufenkji N. A QCM-D-based biosensor for E. coli O157: H7 highlighting the relevance of the dissipation slope as a transduction signal. *Biosens Bioelectron* 2009; **24**: 2137-2142 [PMID: 19118996 DOI: 10.1016/j.bios.2008.11.016]
39 **Yao C**, Zhu T, Tang J, Wu R, Chen Q, Chen M, Zhang B, Huang J, Fu W. Hybridization assay of hepatitis B virus by QCM peptide nucleic acid biosensor. *Biosens Bioelectron* 2008; **23**: 879-885 [PMID: 17951045 DOI: 10.1016/j.bios.2007.09.003]
40 **Zhou X**, Liu L, Hu M, Wang L, Hu J. Detection of hepatitis B virus by piezoelectric biosensor. *J Pharm Biomed Anal* 2002; 27: 341-345 [PMID: 11682242 DOI: 10.1016/j.aca.2010.09.038]
41 **Caygill RL**, Blair GE, Millner PA. A review on viral biosensors to detect human pathogens. *Anal Chim Acta* 2010; **681**: 8-15 [PMID: 21035597 DOI: 10.1016/j.aca.2010.09.038]
42 **Shen G**, Wang H, Tan S, Li J, Shen G, Yu R. Detection of anti-sperm antibody in human serum using a piezoelectric immunosensor based on mixed self-assembled monolayers. *Anal Chim Acta* 2005; **540**: 279-284 [DOI: 10.1016/j.aca.2005.03.035]
43 **Xu T**, Miao J, Wang Z, Yu L, Li C. Micro-piezoelectric immunoassay chip for simultaneous detection of Hepatitis B virus and α-fetoprotein. *Sens Actuators B Chem* 2011; **151**: 370-376 [DOI: 10.1016/j.snb.2010.08.013]
44 **de la Torre TZ**, Mezger A, Herthnek D, Johansson C, Svedlindh P, Nilsson M, Strømme M. Detection of rolling circle amplified DNA molecules using probe-tagged magnetic nanobeads in a portable AC susceptometer. *Biosens Bioelectron* 2011; **29**: 195-199 [PMID: 21907556 DOI: 10.1016/j.bios.2011.08.019]
45 **Yao C**, Qi Y, Zhao Y, Xiang Y, Chen Q, Fu W. Aptamer-based piezoelectric quartz crystal microbalance biosensor array for the quantification of IgE. *Biosens Bioelectron* 2009; **24**: 2499-2503 [PMID: 19188059 DOI: 10.1016/j.bios.2008.12.036]
46 **Fritz J**. Cantilever biosensors. *Analyst* 2008; **133**: 855-863 [PMID: 18575634 DOI: 10.1039/b718174d]
47 **Waggoner PS**, Craighead HG. Micro- and nanomechanical sensors for environmental, chemical, and biological detection. *Lab Chip* 2007; **7**: 1238-1255 [PMID: 17896006 DOI: 10.1039/b707401h]
48 **Wu G**, Ji H, Hansen K, Thundat T, Datar R, Cote R, Hagan MF, Chakraborty AK, Majumdar A. Origin of nanomechanical cantilever motion generated from biomolecular interactions. *Proc Natl Acad Sci USA* 2001; **98**: 1560-1564 [PMID: 11171990 DOI: 10.1073/pnas.98.4.1560]
49 **Wu G**, Datar RH, Hansen KM, Thundat T, Cote RJ, Majumdar A. Bioassay of prostate-specific antigen (PSA) using microcantilevers. *Nat Biotechnol* 2001; **19**: 856-860 [PMID: 11533645 DOI: 10.1038/nbt0901-856]
50 **Huang CW**, Hsueh HT, Huang YJ, Liao HH, Tsai HH, Juang YZ, Lin TH, Lu SS, Lin CT. A fully integrated wireless CMOS microcantilever lab chip for detection of DNA from Hepatitis B virus (HBV). *Sens Actuators B Chem* 2013; **181**: 867-873 [DOI: 10.1016/j.snb.2013.02.061]
51 **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. *Biosens Bioelectron* 2009; **25**: 130-135 [PMID: 19616931 DOI: 10.1016/j.bios.2009.06.015]
52 **Mah C**, Thurbide KB. Acoustic methods of detection in gas chromatography. *J Sep Sci* 2006; **29**: 1922-1930 [PMID: 16970192 DOI: 10.1002/jssc.200500424]
53 **Lee HJ**, Namkoong K, Cho EC, Ko C, Park JC, Lee SS. Surface acoustic wave immunosensor for real-time detection of hepatitis B surface antibodies in whole blood samples. *Biosens Bioelectron* 2009; **24**: 3120-3125 [PMID: 19423329 DOI: 10.1016/j.bios.2009.04.009]
54 **Kim SA**, Das S, Lee H, Kim J, Song YM, Kim IS, Byun KM, Hwang SJ, Kim SJ. Preliminary approach of real-time monitoring in vitro matrix mineralization based on surface plasmon resonance detection. *Biotechnol Bioeng* 2011; **108**: 1473-1478 [PMID: 21192003 DOI: 10.1002/bit.23049]
55 **Lin CY**, Fang YY, Lin CW, Tunney JJ, Ho KC. Fabrication of NOx gas sensors using In2O3–ZnO composite films. *Sens Actuators B Chem* 2010; **146**: 28-34 [DOI: 10.1016/

j.snb.2010.02.040]
56 **Feltis BN**, Sexton BA, Glenn FL, Best MJ, Wilkins M, Davis TJ. A hand-held surface plasmon resonance biosensor for the detection of ricin and other biological agents. *Biosens Bioelectron* 2008; **23**: 1131-1136 [PMID: 18155516 DOI: 10.1016/j.bios.2007.11.005]
57 **Chinowsky TM**, Grow MS, Johnston KS, Nelson K, Edwards T, Fu E, Yager P. Compact, high performance surface plasmon resonance imaging system. *Biosens Bioelectron* 2007; **22**: 2208-2215 [PMID: 17150350 DOI: 10.1016/j.bios.2006.10.030]
58 **Chuang TL**, Wei SC, Lee SY, Lin CW. A polycarbonate based surface plasmon resonance sensing cartridge for high sensitivity HBV loop-mediated isothermal amplification. *Biosens Bioelectron* 2012; **32**: 89-95 [PMID: 22209071 DOI: 10.1016/j.bios.2011.11.037]
59 **Cheng Y**, Zhang X, Li Z, Jiao X, Wang Y, Zhang Y. Highly sensitive determination of microRNA using target-primed and branched rolling-circle amplification. *Angew Chem Int Ed Engl* 2009; **48**: 3268-3272 [PMID: 19219883 DOI: 10.1002/anie.200805665]
60 **Su Q**, Xing D, Zhou X. Magnetic beads based rolling circle amplification-electrochemiluminescence assay for highly sensitive detection of point mutation. *Biosens Bioelectron* 2010; **25**: 1615-1621 [PMID: 20034781 DOI: 10.1016/j.bios.2009.11.025]
61 **Nallur G**, Luo C, Fang L, Cooley S, Dave V, Lambert J, Kukanskis K, Kingsmore S, Lasken R, Schweitzer B. Signal amplification by rolling circle amplification on DNA microarrays. *Nucleic Acids Res* 2001; **29**: E118 [PMID: 11726701]
62 **Jarvius J**, Melin J, Göransson J, Stenberg J, Fredriksson S, Gonzalez-Rey C, Bertilsson S, Nilsson M. Digital quantification using amplified single-molecule detection. *Nat Methods* 2006; **3**: 725-727 [PMID: 16929318 DOI: 10.1038/NMETH916]
63 **Liss M**, Petersen B, Wolf H, Prohaska E. An aptamer-based quartz crystal protein biosensor. *Anal Chem* 2002; **74**: 4488-4495 [PMID: 12236360 DOI: 10.1021/ac011294p]
64 **Ellington AD**, Szostak JW. Selection in vitro of single-stranded DNA molecules that fold into specific ligand-binding structures. *Nature* 1992; **355**: 850-852 [PMID: 1538766 DOI: 10.1038/355850a0]
65 **Jenison RD**, Gill SC, Pardi A, Polisky B. High-resolution molecular discrimination by RNA. *Science* 1994; **263**: 1425-1429 [PMID: 7510417 DOI: 10.1126/science.7510417]

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