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Gold nanoparticles and hepatitis B virus

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ABSTRACT

Hepatitis B virus (HBV) infection is one of the major health issues in the world presently with high tendency of leading to hepatocarcinoma, cirrhosis and liver cancer, especially if not properly managed. It has been estimated that there are about 2 billion people with a serological profile of HBV infection, and 360 million patients worldwide living with chronic HBV-associated liver disease, hence the need to find an efficient and precise diagnosis technique to drive a robust treatment for Hepatitis B virus cannot be over emphasized. The emergence of analytical device like biosensor which combines biological and physicochemical element to detect HBV in screened samples has been very helpful in providing a timely intervention to tame this virus. This review focuses on the current state of biosensor researches with respect to various in-depth application of gold nanoparticle for the detection of HBV.

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Introduction

Hepatitis B is a worldwide acknowledged public health issue with estimated 360 million infections, 600,000 deaths in addition to being a leading cause of high amount of infant and early childhood morbidity and mortality yearly [1]. According to a 2012 survey report, about 800 million people are living with the virus while in China alone, the carrying rate of the surface antigen is 9.7% [2]. This deadly disease is caused by a DNA virus called hepatitis B virus (HBV) which belongs to the Hepadnaviridae family. Humans are the only known natural hosts, where HBV primarily resides and replicates in the liver cells thereby causing either acute or chronic damages to the liver cells leading to deadly liver cirrhosis or hepatocellular carcinoma. The virus is also found in various amounts in circulating blood, saliva, breast milk, secretions of semen, and vagina [3,4]. HBV is transmitted sexually, mother to child at point of birth and via unprotected contacts with contaminated body fluids and objects such as needle stick. Fortunately, the burden of this lethal infection has been significantly reduced with the successful introduction of vaccines whose debut was recorded as far back as 1981 in the United States [5]. This breakthrough was further complemented with the coming of advanced testing, diagnostic and screening tools and devices. As such there is a pertinent need to devise a simple, fast but yet more efficient technique for the diagnosis of HBV [6]. In the past few decades, there has been an increasing number of the application of biosensors in detecting various pathogens including hepatitis virus [7]. According to its working principle, biosensors are highly

sensitive analytical devices made up of a biological element for detection or recognition of a specific analyte based on its inherent physicochemical characteristics and an electronic or optical transducer for signal transmission and measurement [8,9]. As reported by Haasnoot et al., majority of the biomedical research on biosensors have been focused on the immunological reaction or DNA hybridization techniques, and the bio-sensor were consistently efficient in giving results with a high degree of sensitivity [10]. In the light of the rapidly accumulating knowledge and applicability of nanotechnology, researchers have been exploring the unique optical and bio-conjugatory features of gold nanoparticles (AuNPs) in pathogen diagnosis and biomolecule detection devices [11]. Table 1 summarizes the available application of biosensor in detecting hepatitis sensor.

AuNPs have been instrumental in the detection of various pathogens apart from hepatitis virus (Table 2), however this review focuses mainly on the use of AuNP as a biosensor for detecting HBV gene and antigen [12]. It is important to state here that there have been some reports on other methods developed for the detection of HBV such as single strand conformation [13] and high performance liquid chromatography [14].

AuNPs as a biosensor and detector

In the past few years, AuNPs have been influential in the detection of DNA because of its unique optical and bio-conjugation features which early works were pioneered by

Mirkin et al paving way for several other studies such as use of spherical AuNPs targeted at detecting DNA [15–17]. Lately research attention has shifted to the elongated rod-like AuNPs called gold nanorods (AuNRs). AuNRs unlike other forms, gold particles possesses superior absorption and stronger light scattering properties thereby producing dual absorption peaks in the visible region and near the infrared regions which have been designated as the transverse and longitudinal surface plasmon bands, respectively [18]. AuNRs have been reported to be applicable in biosensor [19], gene delivery [20], and photothermal therapy [21,22]. Specifically Darbha et al attempted exploring the nonlinear optical features of AuNRs to screen HIV-1 viral DNA sequence [23,24] while Parab et al. worked on pathogen detection with the aid of AuNRs-based optical DNA biosensor [25]. Table 3 present a brief on some applications of AuNRs biosensors in target DNA detection on the basis of its unique optical features.

Application of AuNP for hepatitis C virus (HCV RNA) sensing

A number of research works have established that AuNPs based devices can be used to detect HCV. For instance, Griffin et al conceived and developed a procedure known as size and distance dependent nanoparticle surface energy transfer technique for the purpose of HCV RNA sensing and detection [26]. In brief, the underlining principle of the technique shows that RNA probe is branded with

fluorophores and absorbed onto the surface of the AuNPs such that once the probe binds to the targeted RNA, the dsRNA complex is released into the solution and a fluorescence emission is reestablished. It is important to note that the resultant solution undergo a color change from red to blue due to aggregation of AuNPs (Figure 1). The fluorescence intensity is directly proportional to the concentration of the target RNA in the solution hence could be utilized in determining the quantity of HCV RNA [26]. In a related development, Glynou et al fabricated a dry-reagent strip biosensor for target DNA detection using AuNPs as visual probe. The results obtained compares favorably with no marked differences between it and COBAS AMPLICORTM, a proven and an efficient kit. Further to it merit, the test is guileless with a reported turnaround time of ~10 min [27].

Application of AuNP in detecting HB surface antigen

There have been quite few research studies that demonstrated the application of AuNPs in detecting HBV [28]. AuNR have been used to develop biosensor for detecting hepatitis B surface antigen (HBsAg) in biological sample or specimen such as buffer, blood serum and plasma [29]. HBsAg is considered the most crucial biomarker for the lab-based diagnosis of hepatitis B virus. The presence of HBsAg in the blood or serum sample signifies a chronic and acute level of hepatitis B virus infection [30,31]. The operating principle involves labeling the AuNR surface with a monoclonal hepatitis B surface antibody (HBsAb) to detect HBsAg and the technique was validated via ELISA [32]. Integrating the antibody with concentrated AuNRs through nonspecific physisorption lead to the synthesis of the antibody-AuNR complex (Figure 2) called the biosensor, which can characterize target proteins.

Application of AuNR as a fluorescent biosensor in detecting HBV gene

In another study conducted by Xiaocui et al., demonstrated utilization of AuNRs-based fluorescent biosensor for the detection of HBV sequences in which emission spectrum of fluorescein (FAM)-ssDNA was designated as the probe DNA.

Table 1. Application of various biosensor techniques in detecting HBV.

Detecting target	Biosensor features	Detection limit	Ref.
HBsAg	Nanorods biosensor	0.01 IU/mL	[29]
HBsAb	QCM	0.1 pg/ μ L	[40]
	SAW	10 pg/ μ L	[41]
	SPR	10 nmol/L	[42]
HBV DNA	Electrochemical biosensor	7.19×10^{-9} mol/L	[43]
		2.5×10^{-6} mol/L	[44]
	QCM	8.6×10^{-12} g/L	[45]
	Microcantilever biosensor	10^{-12} mol/L	[46]
		2.31×10^{-14} mol/L	[47]
	SPR	2×10^{-12} g/L	[48]

HBsAb, hepatitis B surface antibody; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; QCM, quartz crystal microbalance; SAW, surface acoustic wave; SPR, surface plasmon resonance.

This table adapted from [39] with copyright permission.

Table 2. Showing several applications of gold nanoparticles as biosensors to detecting other pathogens.

Method	Types of nanoparticles	Targets
Colorimetric/scanometric	Gold	SNP (rs2131877) in human DNA [50] Mutations in EGFR gene in genomic DNA [51] SNPs in β -thalassemia gene in genomic DNA—mediated by PCR [52] Hepatitis C virus RNA [53] BCR-ABL fusion transcript in clinical samples [54] Plasmodium falciparum heat shock protein in infected blood cultures [55]
Fluorescence-based	Gold	Human and mouse IgG antibody in human and mouse serum [56] Interleukin-8 (IL-8) cancer biomarker in human serum [57] Interleukin-6 (IL-6) cancer biomarker in calf serum [58] Tumor necrosis factor α (TNF- α) in human serum [59]
Electric/Electrochemical	Gold	Anti-hepatitis B virus antibodies and human IgG in human serum [60] Nicotinic acid adenine dinucleotide phosphate (NAADP) [61] in cell extracts Multiple pathogen DNA in clinical specimens [62] Lymph nodes in mouse [63] Brain vessels in mouse [64] HER2 cancer biomarker in breast adenocarcinoma cells [65]
SERS (surface-enhanced Raman scattering)	Gold	
NIR (near infrared region)	Gold	

Table 3. Showing various applications of gold nanorods as DNA biosensors.

The analytical methods of DNA detection	Linear range/nmol/L	LODa/nmol/L	Real sample	References
Gold-nanorod-based sensing of sequence specific HIV-1 virus DNA by using hyper-Rayleigh scattering spectroscopy	0.1–50	0.10		
Monitoring human telomere DNA hybridization and G-quadruplex formation using gold nanorods	13.4–67	4.47		
One-step label-free optical genosensing system for sequence-specific DNA related to the human immunodeficiency virus based on the measurements of light scattering signals of gold nanorods	0.17–11.67	0.080	HIV-1 LTR real sample	
A gold nanorod-based optical DNA biosensor for the diagnosis of pathogens	0.25–75	0.083	Human urine sample	
A gold nanorods-based fluorescent biosensor for the detection of hepatitis B virus DNA based on fluorescence resonance energy transfer	0.045–6	0.015	Human urine sample	

This table adapted from [66] with copyright permission.

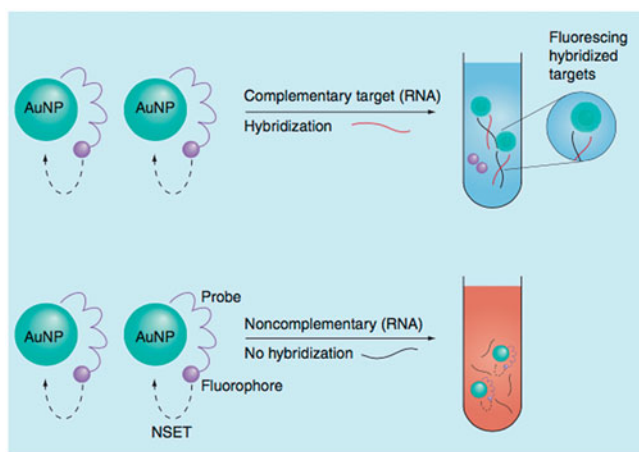


Figure 1. Gold nanoparticle-based assay to detect hepatitis C virus RNA. In hepatitis C virus (HCV)-positive specimens, the fluorophore-labeled probe hybridizes to the target HCV RNA and fluorescence is detected. In addition, the color of the solution will change from red to blue, owing to the aggregation of AuNPs (a qualitative colorimetric signal indicating the presence of HCV RNA). AuNP, gold nanoparticle; NSET, nanoparticle surface-energy transfer. This image adapted from [26] with copyright permission.

Increase in the concentration of DNA probe in the AuNRs suspension is proportional to a decrease in the fluorescence intensity of FAM as a result of the Fluorescence resonance energy transfer (FRET) which spans from FAM to AuNRs. Also when the complementary DNA (cDNA) is introduced into the system, a further decrease in the fluorescence intensity of FAM is observed due to increase in both electrostatic interaction and FRET effectiveness. Ideally, the regression of the fluorescence intensity of FAM correlated linearly to the concentration of the cDNA with values ranging from 0.045 to 6.0 nmol L⁻¹, and the expression of HBV gene via polymerase chain reaction (PCR) was successfully carried out [11,33].

Application of AuNR and PCR in detecting HBV gene

According to a study conducted by Xiaocui et al. which successfully synthesized AuNRs in aqueous solution in accordance to the seed-mediated method as reported by Murphy et al. [34,35]. The procedure begins with preparation of the seed solution done by mixing cetyltrimethylammonium bromide

(CTAB) and HAuCl₄ with ice-cold NaBH₄ [36] rat. After 2 h, the obtained seed solution is then used for the synthesis of AuNRs. CTAB was carefully mixed with AgNO₃ and HAuCl₄, while 140 μL of ascorbic acid was added to the resultant solution. AuNRs growth is initiated with the addition of 24 μL of the seed solution to the growth solution and kept at 30 °C for a day. Finally, the excess CTAB is removed from the solution via centrifugation and AuNRs produced are re-dispersed in distilled water and characterization and morphology of the synthesized AuNRs were carried out using transmission electron microscopy (TEM; Figure 3). Furthermore, Xiaocui et al. reported a sharp reduction in the fluorescence intensity of FAM for the positive sample, whereas the negative sample displayed a slight decrease in FAM. However, PCR of the blank solution gave a signal proportional in intensity to that of the probe signal. The significant changes observed in the fluorescence intensity of FAM demonstrates that AuNRs based fluorescent DNA biosensor could efficiently detect the PCR product of HBV gene and potentially be applied in the detection of the HBV gene.

Application of AuNP as immuno-sensor in detecting HB surface antigen

Qiu et al. reported another study which fabricated a label-free HBsAg immunosensor achieved through the combined features of the biocompatibility and redox electrochemistry of PAA-Fc and the excellent adsorption affinity of AuNPs to HBsAb [37]. This technique's operation is established on the principle of specificity of antigen–antibody interface coupled with electro-chemical transduction for analytical purpose. The immune-sensor as designed by Qiu et al. is illustrated in Figure 4 below in a step-by-step preparation.

In an attempt to further elucidate the immunoreaction between the immobilized HBsAb and HBsAg in the blood sample using the fabricated immune-sensor, Jian-Ding et al. used the differential pulse voltammetry (DPV) approach. The recorded quantitative measurement was determined from 0.2 to 0.68 V with pulse amplitude of 50 mV. DPV peak currents were found to decrease with an increase in the value of HBsAg concentration (Figure 5) thus were interpreted as the amperometric response which decreases with increase in HBsAg concentration [38].

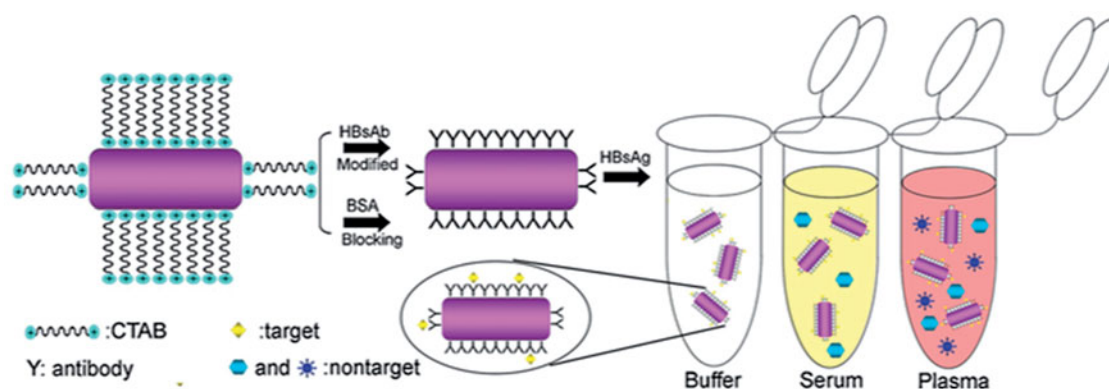


Figure 2. Showing pictorial representation of the synthesis of antibody-functionalized AuNRs and the detection mechanism for the biosensor immunoassay in capturing targets in different matrixes. This image adapted from [29] with copyright permission. AuNRs, gold nano-rods.

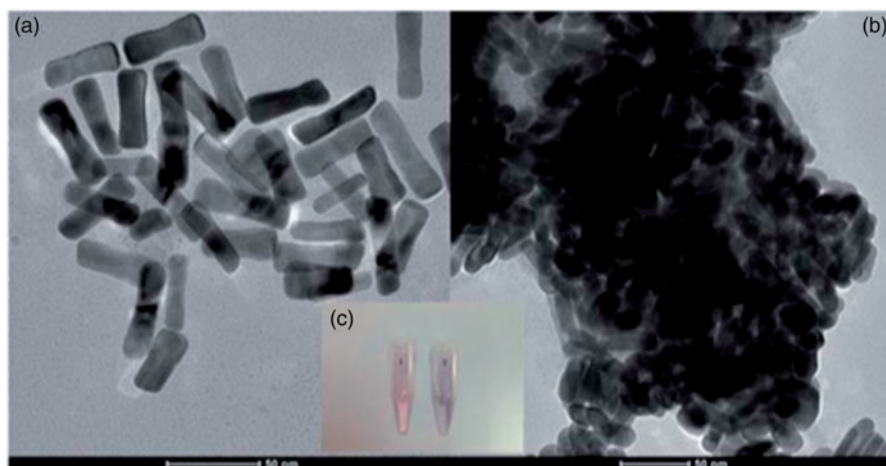


Figure 3. Showing transmission electron microscopy (TEM) images of the synthesized gold nano-rods (AuNRs) and TEM images of FAM-ssDNA-CTAB-AuNRs conjugates before (a) and after hybridization with cDNA (b) at 37 °C. The color of spectrum of fluorescein (FAM)-ssDNA-cetyltrimethylammonium bromide (CTAB)-AuNRs conjugates changes from red to light purple (c). Red denotes the color of ternary complexes and light purple denotes the color after hybridization with target DNA. This image adapted from [11] with copyright permission.

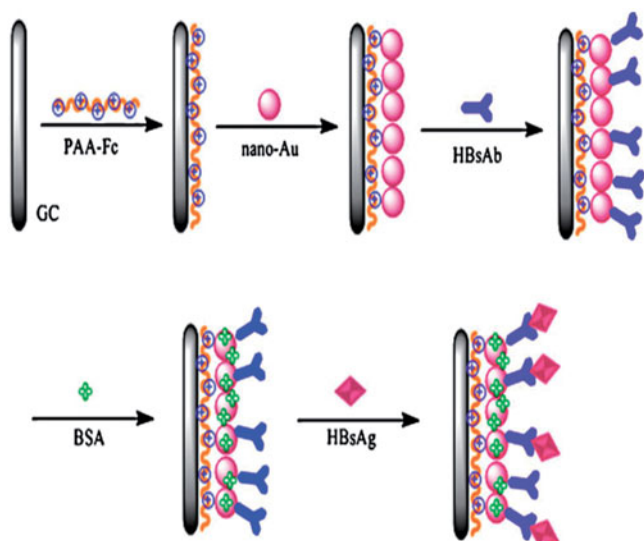


Figure 4. Showing step-by-step preparation of the immune sensor using AuNP. This image adapted from [37] with copyright permission. AuNP, gold nanoparticle.

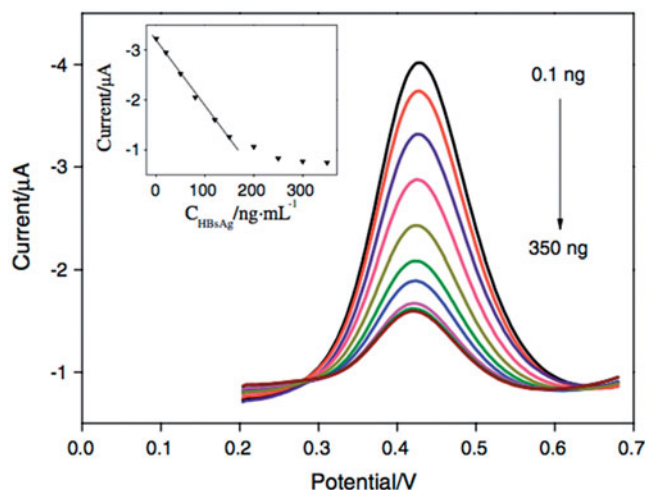


Figure 5. Showing DPV curves of the immunosensor after incubating with various concentrations of HBsAg in 0.1 M pH 5.5 acetate buffer solution. This image adapted from [37] with copyright permission. DPV, differential pulse voltammetry; HBsAg, hepatitis B virus surface antigen.

The fabricated electro-chemical immunosensor was shown as been capable of determining HBsAg in human serum (six samples) and the results obtained were compared to those

gotten via ELISA technique. The relative deviations between the two techniques were in the range of -5.76% to $+6.12\%$, which suggested that the results of two techniques were

almost the same. Conclusively, the new invented AuNP based immunosensor might be a promising tool for determining the HBsAg in human serum clinically.

Conclusion

AuNRs-based fluorescent biosensor has been demonstrated to detect hepatitis B virus DNA. The fabricated biosensor was efficient in detecting complementary DNA sequences and most importantly it successfully detected PCR product of the HBV gene.

This newly developed bio-sensing strategy based on combination of PCR (for specific target gene amplification before detection) with FRET technique could successfully detect HBV or HCV gene from human sample, plasma sample in a concentration range of 0.045–6.0 nmol L⁻¹. In addition electrochemical DNA biosensor based on AuNRs on a gold-electrode surface has also been demonstrated for detecting HBV, furthermore AuNP based immunosensor was used successfully to detect HBsAg in human serum. However more clinical research is still needed to increase the concentration range and detective and sensitivity of all these AuNP based sensors.

Disclosure statement

The authors deny any conflict of interest in any terms or by any means during the study.

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