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Original Article

Application of Gold Core-Shell Magnetic Nanoparticles Immunosensor for Detection of *Vibrio cholera*

Jamal Rashidiani[1], Khadijeh Eskandari[1], Reza Ranjbar[2*], Hamid Kooshki[1], Davoud Afshar[3], Farhad Safarpoor Dehkordi[4]

[1]Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
[2]Molecular Biology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran
[3]Department of Microbiology and Virology, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran
[4]Halal Research Center of IRI, FDA, Tehran, Iran

Corresponding Author: Reza Ranjbar, PhD, Professor, Molecular Biology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran. Tel: +98-2188039883, Email: ranjbar@bmsu.ac.ir

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Abstract

Introduction: For rapid and sensitive detection of *Vibrio cholerae* an accurate assay is needed and hence, in the present study, a gold coated magnetic nanoparticles (GMNPs) was investigated for the detection of *V. cholerae*

Materials and Methods: The GMNPs were synthesized and functionalized by 11-mercapto-undecanoic acid (MUA) as a linker for the immobilization of IgG against *V. cholera* OmpW antigen. In the next step, IgG was coupled with a carboxylic group of MUA using 1-ethyl 3-3 dimethyl aminopropyl carbodiimide hydrochloride (EDC)/N-Hydroxysuccinimide (NHS) and the IgG/GMNPs nanocomposite created and finally the bacterium was detected in a sandwich model enzyme-linked immuno sorbent assay (ELISA).

Results: The IgG/GMNPs nanocomposite which could detect *V. cholera* in a concentration range from 2.5×10^2 to 1.5×10^5 N/mL (number of *V. cholera* per milliliter) was detected. The correlation coefficient was 0.99 and the detection limit was 16 N/mL.

Conclusions: In this study, the procedure of antibody immobilization on magnetic nanoparticles was designed. By using the magnetic nanoparticles, the pre-concentration as a time-consuming step was removed and the sensitivity of *V. cholera* determination was increased. Also, this method can be an extension to detect another biological agent.

Keywords*: Vibrio cholera*, Gold Coated Magnetic Nanoparticles (GMNPs), Rapid Detection

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Introduction

Vibrio cholerae is a causative agent of Cholera, a disease represented with vomiting, severe watery diarrhea which results in severe dehydration and even death if left untreated. Several epidemics and pandemics of cholera have occurred and it seems to be a significant health problem worldwide.[1-4]

The traditional detection of *V. cholerae* is performed using selective media, biochemical tests, and microscopically examinations.[5] However, these methods are time-consuming and require viable organisms. Enrichment is one of the strategies, which is to increase the numbers of pathogens. However, these steps generally take time from 24 to 72 hours to complete and in the clinical cases, it will not be useful. Therefore, the application of a novel and rapid assay to detect organisms with even low numbers can be suitable for many objects. Several detection methods such as ELISA,[6,7] polymerase chain reaction (PCR), real-time PCR, multiplex PCR[8-14] and DNA hybridization probe have been introduced.[15]

Recently, micro-cantilever based biosensor with dynamic force microscopy has been introduced.[16] Magnetic nanoparticles due to their properties[17] has led to several applications such as: medical imaging,[18] drug delivery,[19,20] super-sensitive sensors and bio-detection.[21-23] Iron oxide magnetic nanoparticles have a paramagnetic response to external magnetic fields, which can be used for purification of biological samples.[24-27]

Usually, the magnetic nanoparticles are coated with either a non-metallic or a metallic layer that help its biocompatibility. The gold coated magnetic nanoparticles (GMNPs) are frequently used to immobilize biomolecules because of following advantages: (*i*) easy functionality of nanoparticle for immobilization of IgG, (*ii*) fast separation of targeted molecules from solutions and (*iii*) high surface-to-volume ratio for more loading of bio-molecules.

In the present study, an IgG/GMNPs complex for the detection of *V. cholerae* was applied and its sensitivity was evaluated by the ELISA method.

Materials and Methods

Apparatus and Procedure

In this study, a model (100 Bio-model, USA) of Cary spectrophotometer was used to UV-Vis spectroscopy of the samples. A model (Model Nexus 870, Thermo Nicolet Co. USA) of Fourier transform infrared spectrometer was used to record the spectrum of the Fourier Transform Infrared (FTIR). A model (LEO 440i, UK) of scanning electron microscope was used to obtain the scanning electron micrographs. For surface zeta potential measurement of the nanoparticles Malvern zeta sizer (Nano ZS model) was used.

Materials

$FeCl_2{\cdot}7H_2O$ (99.0%), $FeCl_3{\cdot}6H_2O$ (99.1%), NaOH, HCl (37%), tetramethylammonium hydroxide pentahydrate (TMAOH· $5H_2O$), 11-mercaptoundecanoic acid, 2-[N-morpholino] ethane sulfonic acid (MES) buffer, BM blue, N-Hydroxysuccinimide (NHS) and 1-ethyl 3-3 dimethyl aminopropyl carbodiimide hydrochloride (EDC) were purchased from Sigma–Aldrich, Germany. Trisodium citrate potassium, hydrogen tetrachlorocuprate (30% $HAuCl_4{\cdot}3H_2O$), sodium metaperiodate, ethylene glycol, dihydrogen phosphate (KH_2PO_4) and dipotassium hydrogen phosphate (K_2HPO_4) were also purchased from Merck, Germany. The solutions were prepared in deionized double distilled water (OES-SDLL-20L, USA) and all experiments were carried out at room temperature. Polyclonal antibody of *V. cholera* recombinant outer membrane protein (ompW) was obtained from Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

Preparation of Gold Coated Magnetic Nanoparticles

Co-precipitation of Fe (III) and Fe (II) ions under alkaline condition was applied to synthesize the magnetic nanoparticles. Briefly, $FeCl_2{\cdot}7H_2O$ (5.4 g) and $FeCl_3{\cdot}6H_2O$ (2.0 g) (Sigma, Germany) were dissolved in HCl (10 mM, 33 mL) and then, 167 mL of aqueous NaOH (1.5 M) was also added into the solution, dropwise. After precipitation of iron nanoparticles, they were washed 3-5 times with distilled water to remove excess NaOH and then, heated at 60°C to dry it up.

In the next step, iron nanoparticles (100 mg) were suspended in 5 mL tetramethylammonium hydroxide pentahydrate (TMAOH) solution (0.1M, pH 12) and then, dispersed in citric acid (95 mL, 5 mM) and stirred vigorously for 2 hours. About 0.2 M NH_2OH-HCl and 1% $HAuCl_4{\cdot}3H_2O$ were alternatively added into the nanoparticle solution and were stirred strongly. Finally, $NH_2OH{\cdot}HCl$ (0.2 M) and $HAuCl_4$ (1%) were also added into solution dropwise and were stirred until the solution became purple in color.[28,29]

Functionalization of Gold Coated Magnetic Nanoparticles

The GMNPs were functionalized in three sequential steps: at the first step, 150 µL of 1% suspend GMNPs was added into 1.5 mL of 2 mM 11-mercapto-undecanoic acid (MUA) and was gently stirred for 1 hour. Then, the carboxylic-functionalized GMNPs was collected by a magnet and washed three times with deionized water to remove additional 11-MUA.

Carboxylated GMNPs were suspended into MES buffer (0.5 M, pH 6.1) to 1 mL final volume. About 600 µL (50 mg/mL) NHS (must be prepared freshly) was also added into solution and following vigorous stirring, transferred into a microtube containing 400 µL (10 mg/mL) EDC and finally, stirred at room temperature for 45 minutes. The modified nanoparticles were washed with 0.2 M MES buffer and resolved again into 1.8 mL of 0.5 M MES buffer (pH 7.5).

In the third step, 200 µL pure rat anti-OmpW IgG (1 mg/mL)[30] was added into the composite nanoparticles solution and slightly stirred at room temperature for 20 minutes. The IgG/GMNPs magnetic nanocomposites were washed and resolved in 1.8 mL of 0.5 M MES buffer and finally stored at 4°C in dark bottle (pH 7.4).[28,29]

Capturing of Vibrio cholera by IgG/GMNPs Nanocomposite

The IgG/GMNPs nanocomposites were first treated with BSA (containing 1% BSA and 0.2% glycine) while shaking at 220 rpm for 1 hour and washed three times with PBST buffer (PBS containing 0.1% Tween-20 and 0.1 mM EDTA). The IgG/GMNPs magnetic nanocomposites were then dissolved in the 50 mM MES buffer and stored at 40°C until use.

About 100 µL of IgG/GMNPs nanocomposite was treated with several concentrations of *V. cholera* (containing 2.5×10^2 - 1.5×10^4 cells/mL) in PBS (Total reaction volume =50 mL) and incubated at room temperature for 20 minutes, while shaking. *V. cholera*/IgG/GMNPs nanocomposite was washed three-time by PBS and dispersed in 1 mL PBS.

About 100 µL from different concentrations of *V. cholerae*/IgG/GMNPs were added into each wells and after 30 minutes, 100 µL of anti-*V. cholerae* polyclonal antibody (1:8000 dilution in PBS) added into microplate wells (96-well, U-bottom, Falcon) and incubated for 1 hour at room temperature, while shaking. After incubation, horseradish peroxidase (HRP) -conjugated anti-antibody was added into micro-wells and incubated 30 min. Microplate was washed three times with PBS (pH 7.4) and 100 µL of 3,3',5,5'-tetramethylbenzidine as substrate added into each well. After incubation in dark room (until the color of negative control well has changed), the reaction was stopped with 50 µL of 1N H_2SO_4 and their absorbance were measured at 405 nm.

Results

Micrograph of Gold Coated Magnetic Nanoparticles

Scanning the electron microscope micrograph of $Au@Fe_3o_4$ core shells showed that they were spherical in shape with sizes less than 100 nm (Figure 1). Response to external magnetic field and separation in Becher just in 10 seconds revealed that the GMNPs nanoparticles were functional and had a high magnetic power (Figure 2).

Infrared Spectroscopy

The immobilization of IgG and nanoparticles was studied by Fourier transform infrared spectrometer (FT-IR). The UV-Vis FTIR spectra of magnetic nanoparticles (MNPs) and GMNPs have been presented in Figure 3, which represents the FTIR spectra of MUA/GMNPs (upper) and IgG/MUA/GMNPs (down). As shown in FTIR spectra, the C—H, C═O, C—O and C—OH bands appeared which confirm the MUA

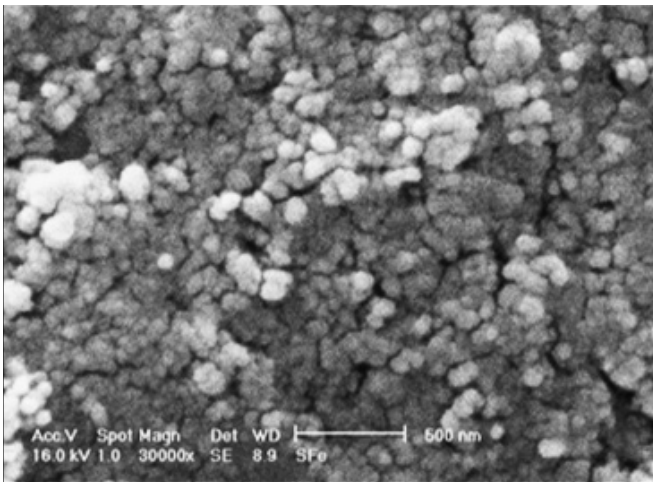

Figure 1. SEM Micrograph of Synthesized Gold-Coated Magnetic Nanoparticles.

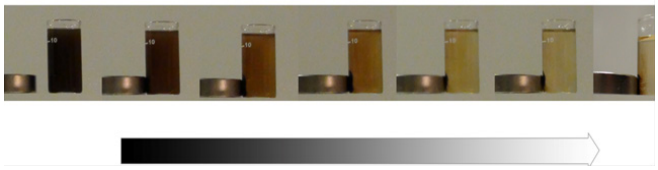

Figure 2. The Effect of External Magnetic Field on MGNP.

immobilization on GMNPs (upper). Also, the C—N and N—H bands were completely visible which established the IgG immobilization on MUA/GMNPs (down).

Detection of Vibrio cholera Via IgG/GMNPs Magnetic Nanocomposite

Vibrio cholera was detected at the concentration ranging from 2.5×10^2 to 1.5×10^5 N/mL (number of *V. cholera* per milliliter) with a correlation coefficient of 0.9926 and a detection limit of 16 N/mL. An association between the increase of the absorption intensity (at 405 nm) and the *V. cholera* number by the sandwich model of IgG/GMNPs are shown in Figure 4 which were calculated with I-Formula.

I-formula: $3.3\delta/S \longrightarrow 3.3\times0.9926/0.0004$

Specificity of the Immunosensor

In the current study, the selectivity of the proposed immunosensor was evaluated in the presence of four different gram negative and gram-positive bacteria (*Escherichia coli, Klebsiella pneumoniae, Shigella sonnei, and Staphylococcus aureus*). They had any response to IgG/GMNPs nanocomposite, showing the selectivity of the system to *V. cholera*.

Discussion

In the present study, we developed a sandwich model of IgG/GMNPs nanocomposite to detect *V. cholera* with a correlation coefficient of 0.998 and detection limit of 16 N/mL (number of bacterium per milliliter). The specificity of the nanocomposite in the presence of gram negative and gram positive bacteria showed that the assay is reliable. Molecular methods may have a low specificity due to the presence of sequences similarity among bacteria, which lead to false positive results. Instead, using specific antibodies is commonly used in nanocomposites and in our opinion; it will result in a high specificity which has also been confirmed previously.[31] One of the reasons may be the capture antibody used in this research, which was against OmpW, a conserved and surface-exposed outer membrane protein which is specific to all *V. cholerae* strains.[32,33]

In addition to high specificity, the presented procedure needs only 40 min to detect *V. cholerae*, which is comparable with molecular and new advanced methods which usually requires more than 1 h. Molecular assays have several steps such as DNA or RNA extraction, target amplification and product electrophoresis steps that are time consuming.[34]

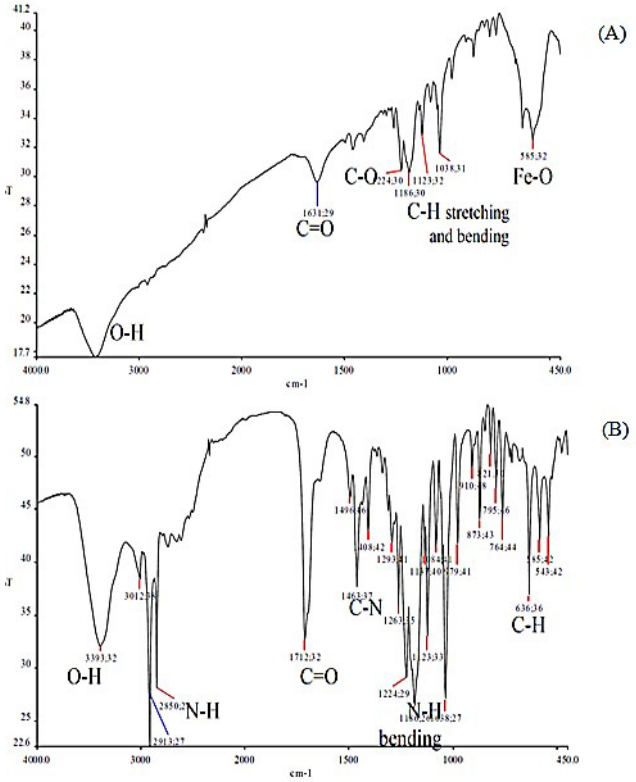

Figure 3. FTIR Spectra of MUA/GM (A) and IgG/MUA/GMNPs (B).

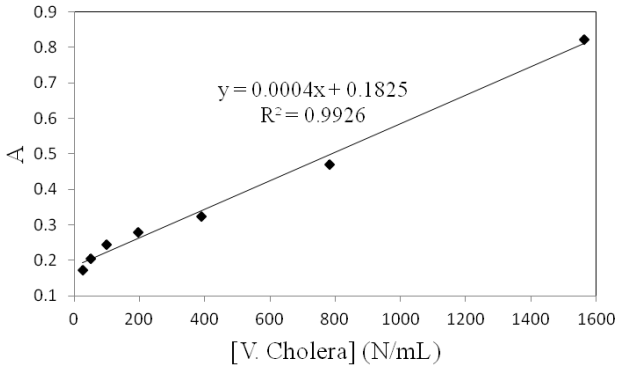

Figure 4. Calibration Curve for *V. cholera* Determination

Although the IgG/GMNPs nanocomposite detection based is similar to the ELISA method, but many steps which take a long time in conventional ELISA, was excluded from the procedure.

The method presented here showed also a high sensitivity with a detection limit of 16 N/mL, which is more sensitive than other directional methods which are frequently used in clinical or research laboratories. Laczka et al developed a specific sandwich-model assay to detect 12 *Vibrio* species based on avidin-biotin binding strategy, which had the detection limits between 7×10^3 to 3×10^4 cells/mL.[35]

The different values in the detection limit of the assay may result from the types of antibody, which is used as a capture antibody. In the study by Martínez-Govea et al, it was revealed that the application of antibody against Omp antigens in the ELISA assay leads to a high sensitivity in comparison with the ELISA assay, in which the antibody against whole-bacterial cell has been used.[36]

Conclusions

Using the IgG/GMNPs nanocomposite for the detection of *V. cholerae* is simple, reliable and faster than other methods such as Polymerase Chain Reaction (PCR) and it may be effective and affordable to replace it with other molecular assays.

Authors' Contributions

All authors contributed equally to this research.

Conflict of Interest Disclosures

The authors declare that they no conflicts of interest.

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