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ORIGINAL ARTICLE

Immunodiagnostic of *Vibrio cholerae O1* using localized surface plasmon resonance (LSPR) biosensor

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Abstract

V. cholerae O1 is a gram-negative bacilli that causes an acute gastrointestinal disease called cholera. *V. cholerae* can enter into the biofilm phase in a period of life; hence, it is challenging to recognize these bacteria. Accordingly, using localized surface plasmon resonance (LSPR) features of the nanoparticles, an accurate detection method based on the antigen-antibody reaction was used. Ordinarily, immobilization of plasmonic nanoparticles by monoclonal antibodies was performed and UV-visible spectroscopy, dynamic light scattering (DLS), and zeta potential (Zp) measurements verified the conjugation process. In the vicinity of several concentrations of *V. cholerae O1*, the consistency of the engineered nanobioprobe was then investigated using LSPR monitoring and colorimetric assay. Finally, the ELISA and PCR techniques contrasted the sensitivity of nanobiosensors. The results showed that by applying monoclonal antibodies as a sensor feature, the nanobioprobe showed high sensitivity to target bacterial analysis. Thus, the limit of detection in this immunoassay-based biosensor was calculated to be a sharp reduction in the absorption of 10 CFU/mL of *V. cholerae O1* with approximately 5 nm of redshift, while the shift of light refraction in the LSPR band was extended to approximately 18 nm by raising the antigen concentration to 10^4 CFU/mL. This LSPR biosensor can therefore be used for *V. cholerae O1* (Inaba strain) detection as a simple, sensitive, and reliable diagnostic tool. In conclusion, the built biosensor will facilitate and speed up *V. cholerae O1* (Inaba strain) classification by controlling the specific antigen to prevent the unintended spread of cholera disease.

Keywords *Vibrio cholerae O1* · Localized surface plasmon resonance · Immunoassay · Biosensor · Detection

Introduction

The protection of food and water is a big concern for the human population today. Infectious agents such as bacteria, viruses, and fungi may contaminate food in this region. Bacterial infections are prevalent in foodborne diseases, and *Vibrio cholerae*, which caused acute gastrointestinal disease called cholera, is one of the most significant bacteria (Zhang et al. 2019). This bacterium is a gram-negative bacterium belonging to the *Vibrionaceae* family (Tognotti 2011; Faruque et al. 1998 a, b). The pathogenicity process of this bacterium is due to the release of a potent poison from the group of enterocytes, which increases the activity of adenylate cyclase and ultimately leads to an uptick in electrolytes and severe infections (Ratna et al. 2015). In general, to managing immunological and indicative assays based on antibody agglutination, using from a relevant and specific antigen candidate is very important. Conventional biochemical and immunological methods for cholera toxin (CT) detection can only be concluded after the isolation of *V. cholerae O1*. The bacterial isolation and CT analysis is complex and usually take a few days, resulting in a considerable delay in the curing process and disease control (Bharati and Ganguly 2011; Faruque et al. 1998). In addition to toxins, one of the most specific markers of the *V. cholerae O1* is superficial lipopolysaccharide of antigens. The presence of LPS can perform an essential role in the immunologic response to the host immune system. Hence, the anti-serum against these superficial antigens has classified *V. cholerae* species into six groups (O1–O6). *V. cholerae O1* is divided into three serotypes based on

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specific surface antigens (A, B, and C), Inaba, Ogawa, and Hikojiam (Akbari et al. 2016; Huq et al. 2012).

There are several methods for diagnosis of *V. cholerae O1* such as culture-based methods, serological methods, and molecular methods. In this sense, the culture-based processes for this microorganism are time-consuming and may be dangerous due to bacteria's duplication time and the viable but nonculturable (VBNC) state of bacteria, respectively (Aulet et al. 2007). Recently, efforts have been made to develop more responsive methods, including a biosensor based on hybridoma (Zamani et al. 2016a, b). For the purpose of diagnosis, molecular detection assays such as polymerase chain reaction (PCR), multiplex PCR, were also commonly favored methods. But depending on individual instruments such as thermal cycler, DNA electrophoresis, and gel documentation, one of the most difficult problems in aforementioned methods is finding recognition (Mousavi et al. 2017; Zeinoddini et al. 2014). The existence of a new detection method based on color shifting is therefore attractive for a fast and accurate diagnosis of the microbial agents without using any individual devices. In the last decade, attempts have been made, including SPR-based biosensors, to establish more responsive methods (Sannomiya and Vo. 2011). On the other hand, in the field of microbial agent detection in water, air, and food, gold nanoparticles (GNPs) with unique optical features have been widely used.

Therefore, traditional chemical and physical approaches are substituted periodically by GNPs. The GNPs will convert the energy (light) of the incoming photons to the electron's collective oscillation. This approach will absorb light and spread it, which will work many times more than organic molecules, in the following. Many of the SPR nanobiosensors (Huang) have been focused on the dielectric constant in the atmosphere of the nanoparticle due to the effect on the absorption spectrum of the plasmonic GNP. Among the biosensors, the LSPR-based nanobiosensors are used as one of the most powerful devices in identification methods. The LSPR-based nanobiosensors, used as one of the most effective devices in identification methods, are among the biosensors. These simple methods involve preparation and skilled design to achieve high performance at the level of nanoparticle (Ahmed et al. 2014). In this work, using nanobioprobe-based gold nanoparticle and monoclonal antibody conjugates for immunodiagnosis of *V. cholerae O1*, we developed a new localized surface plasmon resonance (LSPR) biosensor.

Materials and methods

Strain and chemicals materials

The clinically isolated samples of *V. cholerae O1* (Inaba strain) and *Shigella flexneri* have been provided from the Iranian Health Reference Laboratory (Bu-Ali Hospital). The hydrogen tetrachloroaurate ($HAuCL_4$), trisodium citrate ($Na_3C_6H_5O_7$), the phosphate buffer saline solution (PBS), and bovine serum albumin (BSA) were procured from Sigma, USA. Moreover, the monoclonal antibody was prepared by creative diagnostic (Cat. No. DMAB9687). The O-phenylenediamine (OPD) tablet was obtained from Merck.

Synthesis of stable Au nanoparticles

Turkovitch's technique (Dong et al. 2020) is the most common procedure for GNP synthesis. The GNPs were provided by reduction of Au^{3+} ($HAuCl_4$) to Au^0. Accordingly, the reduction reaction between trisodium citrate ($Na_3C_6H_5O_7$) and tetrachloroauric acid ($HAuCl_4 .3H_2O$) was managed in 10 mL of deionized water. Stirring continued for 5 min after adding the sodium citrate solution until the solution turned bright red-colored. Ultimately, this solution was adjusted to pH = 9 and stored at 25 °C for further application. The nanoparticles were characterized applying dynamic light scattering (DLS), zeta potential (Zp), and transmission electron microscopy (TEM) to determine the electrical surface charge, size, and size distributions, respectively. On the other hand, UV-visible spectroscopy within 400–700 nm wavelength ranges was utilized to characterize the configure of globular gold nanoparticles (Lee et al. 2018; Masereel et al. 2011 a,b).

Surface modification of GNPs by antibody

Against particular antigens of Inaba strains, the monoclonal antibody was added. It should be noted that all the working containers were thoroughly washed and cleaned with the solution (HNO_3-HCl) to use the nanoparticles at all stages of the experiment. Deionized water was also used to make all the solutions that were required. In addition, this approach is a qualitative assay to assess the capacity of nanoparticles to bind to an antibody. The positive charge is also abundant in the antibody due to the positive charge of the amino acid and its N-terminal fraction which establishes an electrostatic bond with the negative charge of the nanoparticle. According to this, at first, K_2CO_3 was added to the gold colloidal solution (D525 = 1.0) until pH = 9 was reached. Then, the different concentration of antibody stock was added to 125 μL of the solution with pH = 9. After 5 min, 125 μL of 1 M NaCl was added. Then, the color shift was observed 10 min later, and the minimum amount of antibody for stabilizing colloidal gold particles was determined (Lee et al. 2018).

Confirmation of designed nanobioprobe

First, the nanobioprobe mixture was incubated for 60 min at 20–22 °C, and then, BSA was applied with a final concentration of 10%. By centrifugation at 12,000*g* for 20 min, the

immobilized colloidal gold particles with antibodies were isolated from unbound antibodies. The final precipitate (pellet) in a buffer containing 1% BSA (Pecora 2000; Lee et al. 2018 a, b) was substituted after the removal of the supernatant. In the second step, the conjugation process was characterized in terms of size, surface charge, and an array quality of optical absorption through the LSPR monitoring utilizing dynamic light scattering (DLS), zeta potential, and (UV/Vis) spectroscopy techniques, respectively (Pecora 2000; Lee et al. 2018 a, b).

Immunoassay for biological activities

The functional structure of the conjugated products was also assessed, using the indirect ELISA method. At first, 100 μL of different concentrations of *V. cholerae O1* as antigen (10–10^2–10^3–10^4 CFU/mL) were coated onto the floor of the ELISA plate and were incubated in 4 °C overnight. It should be noted that one well in each series was considered as a negative control, and the Shigella flexneri bacterium was used as the target antigen. Also, the well has been held without any antigen and covered with a normal buffer and was washed for 4 times. Next, 200 μL of blocking solution (% 0.3 BSA in PBST buffer) was added to each well and were incubated for 2 h at 37 °C. After washed 3 times by washing buffer, 100 μL of normal antibody (appropriately diluted in 0.5 mM PBS buffer) and conjugated antibody were combined to each well in two series and held for 1 h at 37 °C. After that, were washed 2 times in a washing buffer. Moreover, 100 μL enzyme-conjugated secondary antibodies (appropriately diluted in PBST buffer) were joined to each well and incubated for 1 h at 37 °C and 4 times in washing buffer. Ultimately, 100 μL of the fit substrate/chromogenic solution to each well was attached and kept at room temperature (in the dark) for 30 min or until the color change was achieved. Finally, 100 μL of stop solution was added and the absorbance at the appropriate wavelength (450–630 nm) was immediately observed (Lequin 2005; Kaur et al. 2002 a,b).

Function and sensitivity of nanobiosensor

The sensitivity of the designed nanobioprobe (conjugated GNP to antibody) was investigated in the presence of various concentrations of *V. cholerae* O1 strain, Inaba serotypes. Therefore, serial concentrations (10–10^4 CFU/mL) of this bacterium were provided in the nanobioprobe. Ultimately, using a UV-visible spectrophotometer in the visible light range (wavelength 400–700 nm), the vials were evaluated (Nagatsuka et al. 2013). Similar to the peculiar optical properties of GNPs, the solution's color is also affected by the gold atoms' accumulation and dispersion. For this reason, in the presence of different *V. cholerae O1* concentrations, the vials practiced in the previous process were analyzed as ocular, and their color variations were also reported at different times (Iarossi et al. 2018). The wavelength shift ($\Delta\lambda_{max}$) of metal nanoparticles is expressed by the following equation:

$$\Delta\lambda_{max} = m\,(n_{ads} - n_{medium})\,[1 - \exp\,(-2d/l_d)]$$

m is the sensitivity factor, n_{ads} and n_{medium} are the refractive indices of the adsorbate and medium surrounding the nanoparticle, d is the effective thickness of the adsorbate layer, and l_d is electromagnetic field decay length, respectively. Red shift happens when the refractive index in the presence of the antibody is greater than the refractive index of the surrounding area. The distance from the surface allows the l_d to increase by binding the antibody to GNP. As large molecules such as biomolecules are positioned on the surface, they allow more volume to be occupied and lead to d increase and allow the LSPR wavelength shift to increase. Also, both tests were replicated three times and conducted.

Results

Property and characterization of gold nanoparticles

Another critical feature of the characterization of gold nanoparticles is spectrophotometry. In this way, the absorption peak changes to a longer wavelength by increasing the particle size, and the width of the absorption spectra is related to the distribution pattern of size. A specific peak of surface plasmon resonance (SPR) in the visible light range between 400 and 700 nm was therefore indicated for the sensitized gold nanospheres. According to Fig. 1, the diagram has a strong absorption peak at 530 nm, indicating the formation of a spherical gold nanoparticle (Fig. 1(A, B)). The TEM picture showed that, due to the negatively charged layer of citrate ions that redirect atoms from each other, the gold colloid solution is in a suspension condition. In addition, the TEM picture showed

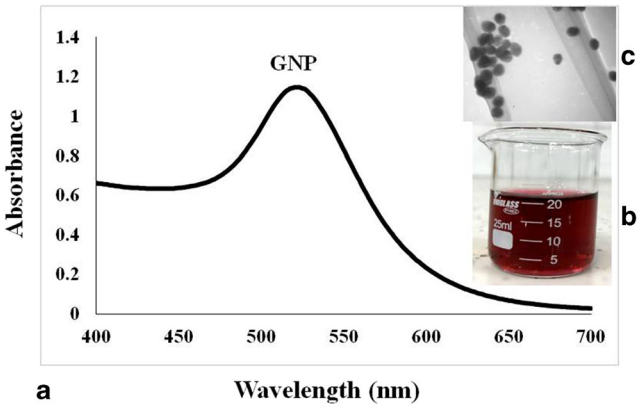

Fig. 1 (A) UV-visible absorbance spectra of plasmonic GNPs based on surface plasmonic resonance (SPR). (B) The image is related to the gold colloidal solution. (C) Microstructural studies of GNPs by visual observation of transmission electron microscopy (TEM)

that the maximum shape of the gold nanospheres was globular and around 43 nm in size (Fig. 1(C)).

On the other hand, a physical method used to determine the distribution, size, and also the surface load of the particles contained in the suspensions based on their brownie movement is the dynamic light scattering (DLS) study. DLS therefore measures the intensity of the light diffused by the suspension nanoparticles as a function of time and calculates the hydrodynamic diameter of the particles. In addition, a zeta potential analysis (Zp) was used to validate the negative charge of citrate-stabilized AuNPs, suggesting the influence of pH on the surface charge due to the distribution of the Zp values. The size of the nanoparticles is approximately 43 nm, as shown in Fig. 2a, and their zeta potential is approximately − 33 (Fig. 2b), with the antibody binding to the nanoparticle, thus increasing the zeta potential, or, in other words, reducing the negative charge. Noteworthy, the designed nanobioprobe solution should be kept in 4 °C for 1 month.

Property and characterization of nanobioprobe

- Firstly, the visual analysis was designed to test the ability of gold nanoparticles to bind to the antibodies. Na^{1+} ion is bound to GNPs by attaching NaCl and prevents repellent, inducing GNP aggregation and changing the color of the nanoparticles from red to blue. If nanoparticles are bonded to the molecule, and their environment occupied, after loading NaCl salt into the medium, Na^{1+} cannot bond to the nanoparticles, and consequently, the aggregation process not happened. According to Fig. 3(B), the concentration of about 110 μg/mL of the monoclonal antibody was considered as a proper concentration for carrying out the conjugation process. In addition, the range of nanoparticle spectra revealed that plasmonic nanoparticles reduced the absorption rate after conjugation with antibodies, and variations in the refractive index around the nanobioprobe reported light dispersion with a numerical value in monoclonal antibody attachments at about 5 nm wavelength (Fig. 3(A)). The results of DLS and zeta potential measurements showed that the characteristics of gold nanoparticles after conjugation were changed. Hence, the size and surface charge of functionalized GNP by monoclonal antibodies were estimated at 63 nm and − 18, respectively (Fig. 4).

The sensitivity and selectivity of nanobioprobe

The specificity and sensitivity of monoclonal antibodies (before and after conjugation) were contrasted by the ELISA assay in order to control the binding of antibodies to the nanoparticles and their proper functioning (Fig. 5). A parallel and close relationship between the sensitivity ratio between non-conjugated antibodies and the nanobioprobe was shown in the results of this experiment. Via rising the antigen concentration, the interactive process (antibody-antigen) was increased, which has been indicated an advance in the sensitivity of the nanobioprobe in the proximity of target analytes. In addition to the specific function of the antibody, this test also confirms the intact structure of the protein. In the following, no significant adsorption was observed in other wells, such as negative controls (*Shigella flexneri*) and zero concentration. In addition to the specificity of the nanobioprobe, and the correct handling, this assay was explained a complete blocking of the vacant capacity around the nanoparticles. In the comparison of the series ELISA stripe, a small decrease in the antibody susceptibility was observed in the nanobioprobe attachments. In this process, it should be noted that noncovalent contact was occurred based on the sharing of electrostatic bands in

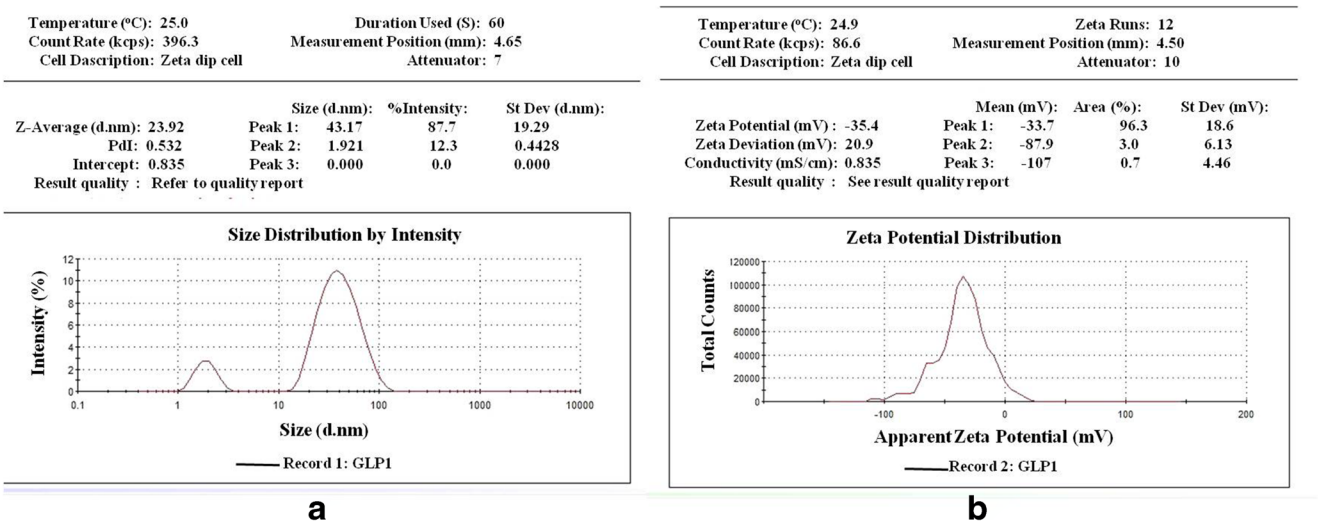

Fig. 2 Particle size (**a**) and surface charge (**b**) analysis of GNPs using dynamic light scattering (DLS) and zeta potential measurements

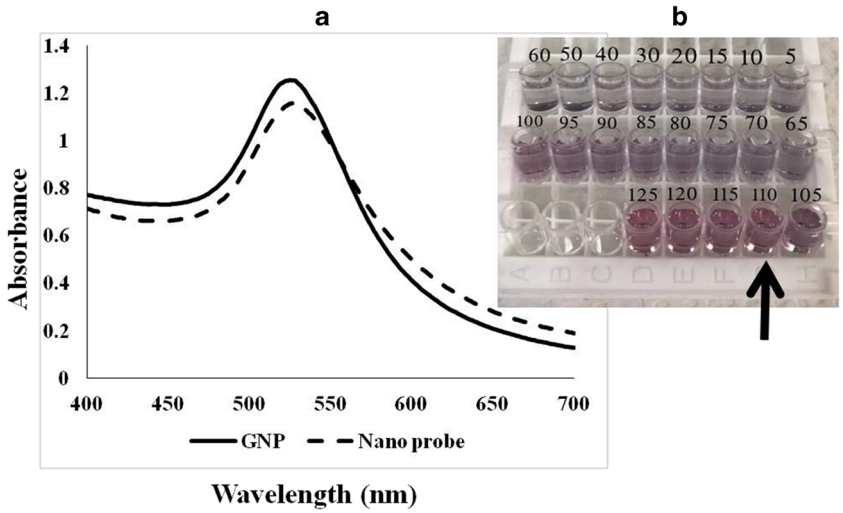

Fig. 3 (A) UV-visible absorbance spectra of the functionalized GNPs using the monoclonal antibody. (B) The minimum concentration of the monoclonal antibody for surface modification of nanoparticles in biosensor substrate preparation (range of antibody concentration: 5–125 μg/mL)

laboratory conditions. So it should not be considered a 100% response, and accordingly, this sensitivity reduction was ignored. In general, the findings of the investigations suggested that no negative association between the structure and the consistency of functional sensitivity was produced by the labeling of antibodies with gold nanoparticles. Finally, after half an hour, the LSPR-based biosensor immunodiagnosis was authorized. LSPR monitoring results showed that using gold nanoparticle-labeled antibody to recognize *V. cholerae O1* could adjust the frequency of LSPR in the vicinity of this bacterium's different concentrations. According to Fig. 6, the limit of detection (LOD) was estimated at 10 CFU/mL for this LSPR-based nanobiosensor. Meanwhile, a higher concentration (up to10^4 CFU/mL) of antigen was employed; the LSPR band was extended (Table 1).

Generally, using monoclonal antibody-conjugated GNP to the detection of *V. cholerae O1* could improve the limit of detection (LOD) by LSPR-based nanobiosensor to 10 CFU/mL beside the redshift of 5 nm. In the higher concentration (10^4 CFU/mL) of antigen, the LSPR band was increased and had manifested a change of wavelength about 18 nm (Fig. 6a). The colorimetry applying the nanobiosensor was identified after 1 h with a slight color change from red to purple. This slight discoloration was clearly confirmed after 6 h. According to the above results, a designed nanobioprobe using the monoclonal antibody could be estimated ultrasensitive for immunodiagnostics of *V. cholerae O1*.

Discussion

V. cholerae is a very infectious bacterium that can pollute water, so it is critical to achieve a precise and highly sensitive detection of *V. cholerae O1* from environmental samples. The

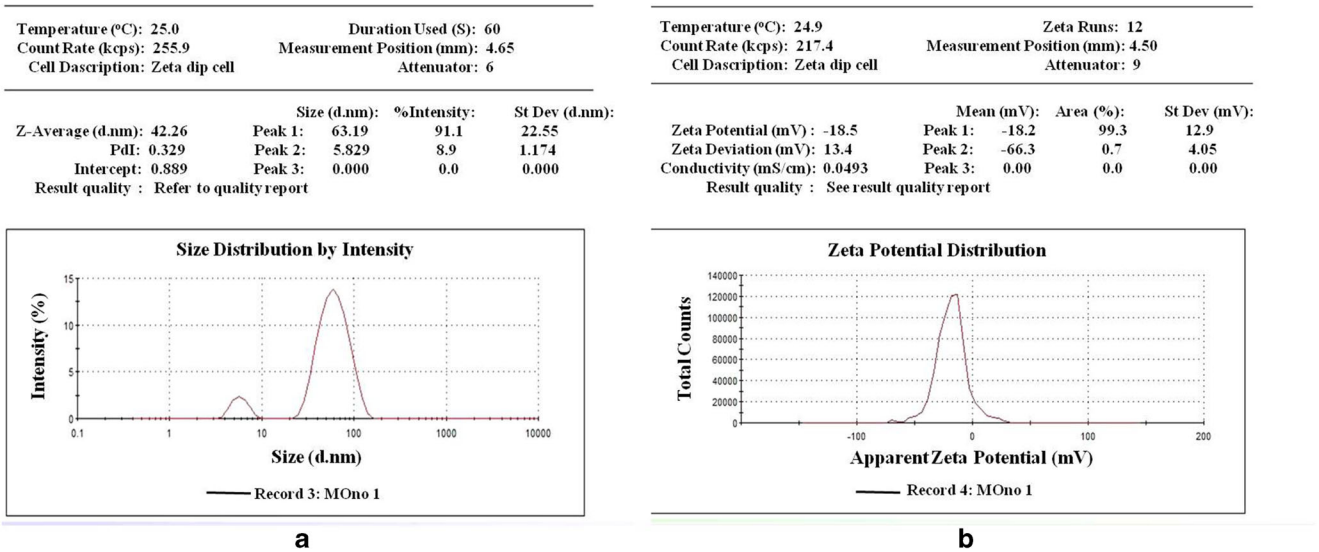

Fig. 4 Particle size (**a**) and surface charge (**b**) analysis of functionalized GNP with monoclonal antibody using dynamic light scattering (DLS) and zeta potential

Table 1 The LSPR band of functionalized GNPs in the presence of different concentrations from antigen

Spectra of nanobioprobe/antigen (CFU/mL)	Wavelength (nm)
GNP	523/0
Nanoprobe (GNP + antibody)	527/5
10	532/5
10^2	537/7
10^3	538/9
10^4	545/0

viable but nonculturable (VBNC) is a unique stability strategy of these bacteria in the environment that is regulated by the expression of relevant genes and the production of an external matrix exopolysaccharide around the bacteria and will be able to preserve the bacteria in response to environmental degradation effects. Moreover, the VBNC bacteria cannot be cultured on customary microbiological media; other environmental specimens may also interfere with the diagnostic analysis process, which has challenged the identification of this bacterium by current methods (Fakruddin et al. 2013).

Following recent advancements in nanotechnology and the use of gold nanoparticles (GNPs), biosensors have been used to enhance diagnostic efficiency (Zeinoddini et al. 2018). One of the most influential and useful features of the GNPs are the special color and optical properties due to their plasmonic and customizable effects (Huang and El-Sayed. 2010). There are many methods which reported for molecular detection of this infectious element like PCR-based methods such as conventional PCR, multiplex PCR, and real-time PCR, or other molecular methods like loop-mediated amplification method (LAMP) (Monazah et al. 2017; Zeinoddini et al. 2017). Molecular approaches detect a selected region in the genomic area, and for this case, the CtxA, CtxB, Zot, and OmpW genes were chosen and reported (Mousavi et al. 2017; Zeinoddini et al. 2014). Moreover, in modern techniques such as dot blotting assay, although the combination of the two sets of methods provides a powerful system for tracking, isolation, and characterization of pathogens at about 10 CFU/mL, there have needed to trained personnel and advanced equipment for extractive processes and amplification of the targeted genes (Zeinoddini et al. 2015).

Following improved identification methods, offered a sensor-based for the outer membrane protein detection of *V. cholerae*, were attempting to apply a diagnostic system by plasmonic nanoparticles with high sensitivity and specificity. Although they were successful in the direct diagnosis of the *V. cholerae* at about 43 CFU/mL, this method has some limitations, so that in this biosensor, the antibody was immobilized on gold nanorods by chemical modification (Taheri et al. 2016). In an improved diagnostic method, the self-assembly of gold nanoparticles (AuNPs) was used to give the AuNP monolayers a controlled diameter of 20 nm to detect the pathogenic bacteria responsible for food poisoning. In pure culture, this highly sensitive, quantitative, and fast (within 30–35 min) chip provided for *Salmonella* identification via an upper detection limit of 10^4 CFU/mL. Moreover, the applicability of the developed plan requires to be established in various food matrices; it should be reduced by the detection processes before its commercialization (Oh et al.2017). In addition, the sensing of gold nanotubes (GNRs) with an average length of 50 nm and a diameter of 14 nm (Maximilien Cotta and his colleagues) was investigated in order to classify such proteins and to demonstrate their ability as plasmon nanobiosensors. However, they could be able to demonstrate the sensitivity of such an LSPR sensor around 10^9 M/nm on a concentration range from 10^{-8} to 10^{-10} M (Cottat et al. 2013).

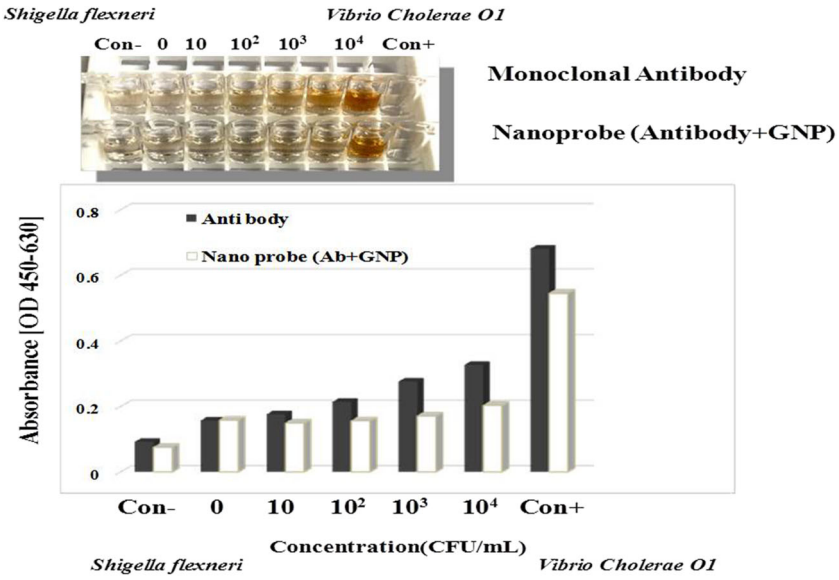

Fig. 5 Indirect ELISA for measuring the function of a labeled monoclonal antibody with GNP

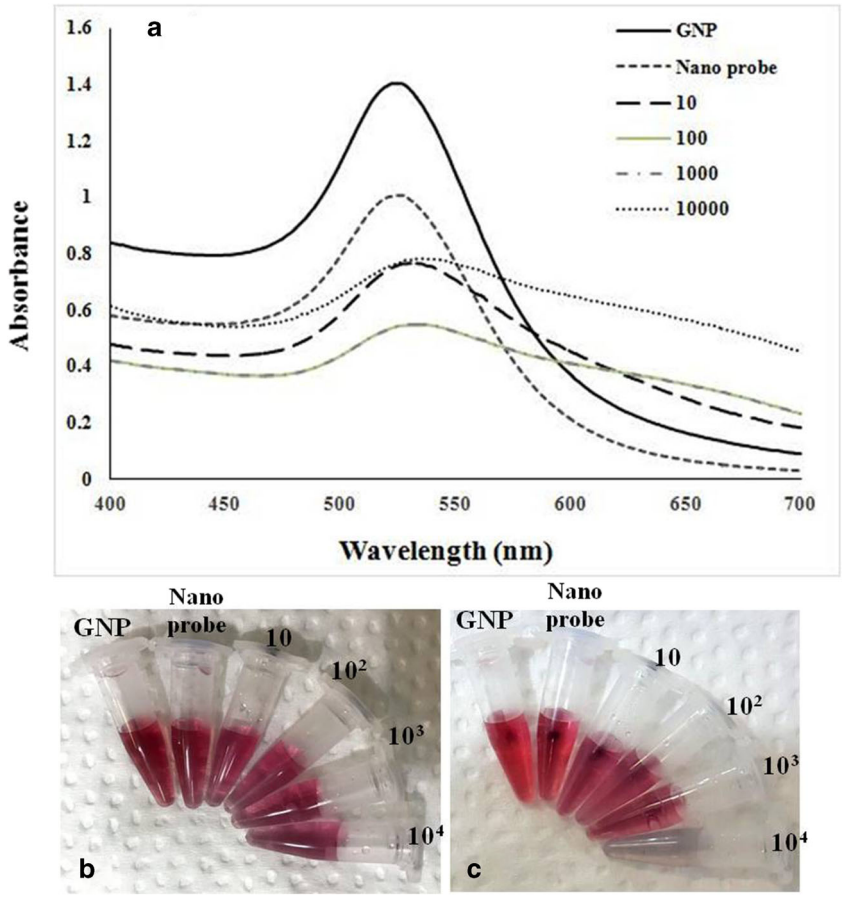

Fig. 6 **a** UV-visible absorbance spectra of plasmonic nanobioprobe (conjugated monoclonal antibody) based on surface plasmon resonance(LSPR). Tuning the gold nanoparticle colorimetric assay for the rapid detection of *V. cholerae O1* (CFU/mL) after **b** 1 h and **c** 6 h

In a related and complicated scheme, the LSPR chip with immobilized aptamer ligand was improved for the exposure of three distinct bacterial species by observing changes in peak extinction rate. Capacity of this sensor optimized by different concentrations of the probe, incubation time for aptamer immobilization, and incubation time for cell binding. The sensor revealed a detection limit of 30 CFU per assay in all instances. As a result, the sensor system could recognize different target bacterial species in multiple modes with high specificity on a single chip (Yoo et al. 2015). Consequently, the expense of materials and machinery, simple methods of synthesis, and lack of surface chemical manipulation (electrostatic band) have been taken into account in the pursuit of these objectives. Finally, a detection limit of about 10 CFU/mL through a redshift of about 5 nm was shown by the built LSPR biosensor. The displacement of the 18 nm wavelength in the presence of 10^4 CFU/mL was observed.

Conclusions

Results have shown that adding the monoclonal antibody to the colorimetric assay makes it easy to identify the infectious agent of cholera disease. Generally, antigen monitoring using an immunogold-based biosensor was considered to be a system of high specificity, precision, speed, and could be a valuable tool for the prevention and release of disease from toxic clinical strains of nontoxic *V. cholerae*. The immunoassay-based LSPR biosensors are therefore a cost-effective plan to replace cultivation methods, existing methods of traditional PCR, and ELISA in the isolation of these VBNC bacteria from clinical specimens.

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Compliance with ethical standards

Conflict of interests The authors declare that they have no conflict of interest.

References

Ahmed A, Rushworth JV, Hirst NA, Millner PA (2014) Biosensors for whole-cell bacterial detection. Clin Microbiol Rev 27(3):631–646. https://doi.org/10.1128/CMR.00120-13

Akbari MR, Ahmadi A, Salimian J (2016) Evaluation of antibodies produced against lipopolysaccharides of *Vibrio cholerae* for rapid

detection of cholera. Pathobiology Research 18(4):23–31 http://journals.modares.ac.ir/article-30-6377-en.html

Aulet O, Silva C, Fraga SG, Pichel M, Cangemi R, Gaudioso C et al (2007) Detection of viable and viable nonculturable *Vibrio cholerae O1* through cultures and immunofluorescence in the Tucumán rivers, Argentina. *Revista da sociedade brasileira de Medicina Tropical* 40(4):385–390. https://doi.org/10.1590/S0037-86822007000400002

Bharati K, Ganguly NK (2011) Cholera toxin: a paradigm of a multifunctional protein. Indian J Med Res 133(2):179

Cottat M, Thioune N, Gabudean AM, Lidgi-Guigui N, Focsan M, Astilean S, de la Chapelle ML (2013) Localized surface plasmon resonance (LSPR) biosensor for the protein detection. Plasmonics 8(2):699–704. https://doi.org/10.1007/s11468-012-9460-3

Dong J, Carpinone PL, Pyrgiotakis G, Demokritou P, & Moudgil B M (2020) Synthesis of precision gold nanoparticles using Turkevich method. *KONA Powder and Particle Journal*, 2020011. https://doi.org/10.14356/kona.2020011

Fakruddin M, Mannan KSB, Andrews S (2013) Viable but nonculturable bacteria: food safety and public health perspective. ISRN microbiology 2013:1–6. https://doi.org/10.1155/2013/703813

Faruque SM, Albert MJ, Mekalanos JJ (1998) Epidemiology, genetics, and ecology of toxigenic*Vibrio cholerae*. Microbiol Mol Biol Rev 62(4):1301–1314

Huang X, El-Sayed MA (2010) Gold nanoparticles: optical properties and implementations in cancer diagnosis and photothermal therapy. J Adv Res 1(1):13–28. https://doi.org/10.2217/17435889.2.5.681

Huq A, Haley BJ, Taviani E, Chen A, Hasan NA, Colwell RR (2012) Detection, isolation and identification of *Vibrio cholera* from the environment. Curr Protoc Microbiol 6:6A.5. https://doi.org/10.1002/9780471729259.mc06a05s26

Iarossi M, Schiattarella C, Rea I, De Stefano L, Fittipaldi R, Vecchione A et al (2018) Colorimetric immunosensor by aggregation of photochemically functionalized gold nanoparticles. ACS omega 3(4):3805–3812. https://doi.org/10.1021/acsomega.8b00265

Kaur R, Dikshit KL, Raje M (2002) Optimization of immunogold labeling TEM: an ELISA-based method for evaluation of blocking agents for quantitative detection of antigen. Journal of Histochemistry & Cytochemistry 50(6):863–873. https://doi.org/10.1177/002215540205000612

Lee JH, Cho HY, Choi HK, Lee JY, Choi JW (2018) Application of gold nanoparticle to plasmonic biosensors. Int J Mol Sci 19(7):2021. https://doi.org/10.3390/ijms19072021

Lequin RM (2005) Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA). Clin Chem 51(12):2415–2418. https://doi.org/10.1373/clinchem.2005.051532

Masereel B, Dinguizli M, Bouzin C, Moniotte N, Feron O, Gallez B, Vander Borght T, Michiels C, Lucas S (2011) Antibody immobilization on gold nanoparticles coated layer-by-layer with polyelectrolytes. J Nanopart Res 13(4):1573–1580. https://doi.org/10.1007/s11051-010-9908-3

Monazah A, Zeinoddini M, Saeeidinia AR (2017) Evaluation of a rapid detection for Coxsackievirus B3 using one-step reverse transcription loop-mediated isothermal amplification (RT-LAMP). J Virol Methods 246:27–33. https://doi.org/10.1016/j.jviromet.2017.04.006

Mousavi SM, Zeinoddini M, Azizi A, Saeedinia A, Monazah A (2017) Molecular detection of zonula occludens toxin (zot) genes in *Vibrio Cholerae O1* using PCR. Research in Molecular Medicine 5(3):37–40 http://rmm.mazums.ac.ir/article-1-248-en.html

Nagatsuka T, Uzawa H, Sato K, Kondo S, Izumi M, Yokoyama K et al (2013) Localized surface plasmon resonance detection of biological toxins using cell surface oligosaccharides on glyco chips. ACS Appl Mater Interfaces 5(10):4173–4180. https://doi.org/10.1021/am4002937

Oh SY, Heo NS, Shukla S, Cho HJ, Vilian AE, Kim J et al (2017) Development of gold nanoparticle-aptamer-based LSPR sensing chips for the rapid detection of Salmonella typhimurium in pork meat. Sci Rep 7(1):1–10. https://doi.org/10.1038/s41598-017-10188-2

Pecora R (2000) Dynamic light scattering measurement of nanometer particles in liquids. J Nanopart Res 2(2):123–131. https://doi.org/10.1023/A:1010067107182

Ratna PVJ, Sandjarara T, Rajkumar S (2015) Genotyping of *Vibrio cholera* strains based on the cholera toxin and virulence associated genes. Indian Journal of Applied Research 5:197–198

Taheri RA, Rezayan AH, Rahimi F, Mohammadnejad J, Kamali M (2016) Development of an immunosensor using oriented immobilized anti-OmpW for sensitive detection of *Vibrio cholerae* by surface plasmon resonance. Biosens Bioelectron 86:484–488. https://doi.org/10.1016/j.bios.2016.07.006

Tognotti E (2011) The dawn of medical microbiology: germ hunters and the discovery of the cause of cholera. J Med Microbiol 60(4):555–558. https://doi.org/10.1099/jmm.0.025700-0

Sannomiya T, Vo J (2011) Single plasmonic nanoparticles for biosensing. Trend Biotechnol 29:343–351. https://doi.org/10.1016/j.tibtech.2011.03.003

Yoo SM, Kim DK, Lee SY (2015) Aptamer-functionalized localized surface plasmon resonance sensor for the multiplexed detection of different bacterial species. Talanta 132:112–117. https://doi.org/10.1016/j.talanta.2014.09.003

Zamani P, Sajedi RH, Hosseinkhani S, Zeinoddini M, Bakhshi B (2016a) A luminescent hybridoma-based biosensor for rapid detection of *V. cholerae* upon induction of calcium signaling pathway. Biosens Bioelectron 79:213–219. https://doi.org/10.1016/j.bios.2015.12.018

Zamani P, Sajedi RH, Hosseinkhani S, Zeinoddini M (2016b) Hybridoma as a specific, sensitive, and ready to use sensing element: a rapid fluorescence assay for detection of *Vibrio cholerae O1*. Anal Bioanal Chem 408(23):6443–6451. https://doi.org/10.1007/s00216-016-9762-y

Zeinoddini M, Azizi A, Bayat S, Tavasoli Z (2018) Localized surface plasmon resonance (LSPR) detection of diphtheria toxoid using gold nanoparticle-monoclonal antibody conjugates. Plasmonics 13(2):583–590. https://doi.org/10.1007/s11468-017-0548-7

Zeinoddini M, Monazah A, Saeedinia AR (2017) Comparison between RT-PCR, NASBA and RT-LAMP methods for detection of coxsackievirus B3. Biom J 3(2):100–106

Zeinoddini M, Saeedinia AR, Sadeghi V (2014) Rapid detection of *Vibrio cholerae* using hexaplex PCR assay. Journal of Police Medicine 3:77–84 http://teb.police.ir/teb/article-1-216-en.html

Zeinoddini M, Saeedinia AR, Sadeghi V, Shamsara M, Hajia M, Rahbar M (2015) Simple and accurate detection of Vibrio cholera using triplex dot blotting assay. Biom J 1(1):52–57

Zhang Z, Zhou J, Du X (2019) Electrochemical biosensors for detection of foodborne pathogens. Micromachines. 10(4):222. https://doi.org/10.3390/mi10040222