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Precise, direct, and rapid detection of *Shigella Spa* gene by a novel unmodified AuNPs-based optical genosensing system

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ABSTRACT

Early detection of infectious bacteria is a necessity for combating infectious diseases. Due to low infectious dose of *Shigella*, rapid and sensitive detection is needed. Compared to the presented genes, *Spa* gene can be introduced as a novel sequence for all species of *Shigella* detection. Herein, the possibility of *Spa* genes for detection of four species of *Shigella* was investigated for the first time by AuNPs-based optical genosensing system. In this method, AuNP–DNA probes were hybridized with *Spa* gene sequence. When the complementary target is present, it prevents the aggregation of the complex under acid environment and the solution remains red whereas in the absence of the specific sequence, it turns to purple. Therefore, visual detection is possible with bare eye. The comparison of this Optical DNA biosensor and PCR-based method showed that the proposed method is simple, cost-effective, rapid operation, with high or comparable detection limit of (LOD and LOQ: 8.14 and 26.6 ng mLl^{-1}, respectively), without need of any expensive techniques, and equipments compared to the conventional methods. In conclusion, the described method may develop into a platform that could be utilized for detection of various bacterial species with high accuracy and prompt screening of samples.

1. Introduction

Shigellae as a common prevalent foodborne pathogen, represents a major burden of disease in developing countries (Kotloff et al., 2013; Priyanka et al., 2016; Zhao et al., 2014). The estimated 165 million cases of shigellosis that occur annually result in the deaths of ~1.1 million people, mostly in children under 5 years (Kotloff et al., 2017; Sahl et al., 2015). Numerous studies confirm that *Shigella* spp. is an endemic pathogen in the world particularly in developing countries and industrialized countries such as Norway, Denmark, Australia, Portugal and the United States, causing severe diarrheal disease that requires hospitalization in young children (Jiménez et al., 2010).Shigella, intracellular Gram-negative pathogen, non-spore forming and rod shaped bacterium, causes significant diarrheal disease and mortality in humans (Kahsay and Muthupandian, 2016; Sahl et al., 2015) and primates. This organism is transmitted through the fecal–oral route and also by contaminated food and water (Vongsawan et al., 2015). Clinical disease generally begins within 24–48 h of ingestion of 10–100 organisms (Sousa et al., 2013; Luo et al., 2016). The infective dose for Shigella is very low: 10 cells of *S. dysenteriae* and 500 cells of *S. sonnei* (Warren et al., 2006).

Shigella serogroups, named as *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, and *Shigella sonnei* are believed to be highly infectious due to their low infectious dose (10–100 colony-forming unit (CFU) (Kahsay and Muthupandian, 2016). Shigellosis can be detected via traditional microbiological and molecular biology assays. Traditional microbiological assays such as enrichment, culture, and biotype- and serotype identification steps are time consuming, requiring several days to obtain results (Zhao et al., 2014). The conventional methods such as immunological (methods based on antibody–antigen interactions, e.g., ELISA)(Amani et al., 2015; Bhardwaj et al., 2017) and molecular biology assay such as PCR (Alipour et al., 2012), multiplex PCR (Binet et al., 2014; Koziel et al., 2013; Ojha et al., 2013), multiplex real-time PCR(Barletta et al., 2013; Ma et al., 2014; Wiemer et al., 2011), LAMP (Loop-mediated isothermal amplification) (Soli et al., 2013) are some of the nucleic-acid-based techniques that are employed for *Shigella* detection.

In spite of being predominately used in clinical laboratories, the immunological methods rely on bacteria concentrations that require 7–24-h culture-based enrichment of samples to bring the number of bacteria to the detectable level. The nucleic acid based methods are generally specific but require expensive instrumentation and skills (Luo et al., 2016; Kant et al., 2018; Richter et al., 2017). A model for optical forward scattering by a bacterial colony based on scalar diffraction

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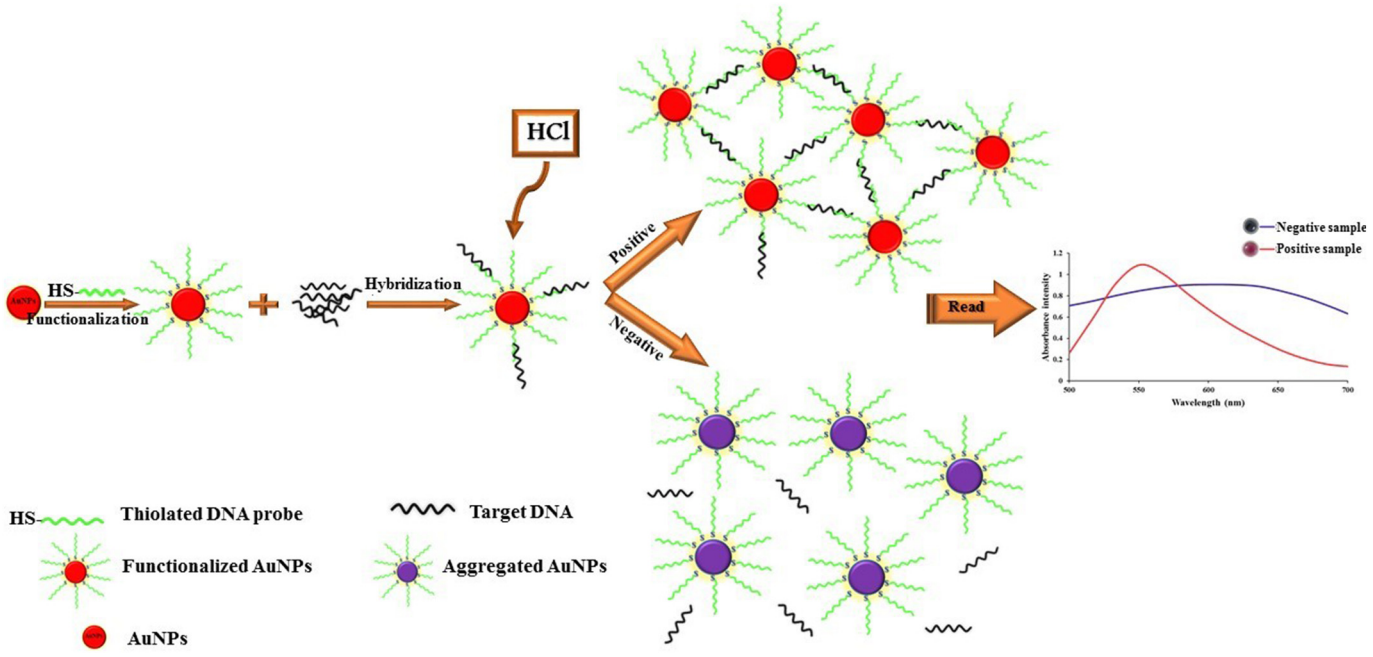

Fig. 1. Schematic presentation of DNA hybridization detection with AuNPs-based optical genosensor.

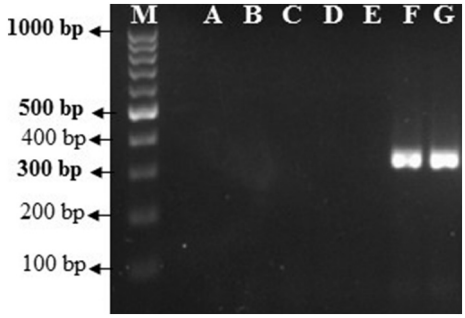

Fig. 2. Agarose gel electrophoresis photos, PCR products of 320 bp sequence of *Spa* gene *Shigella spp.* (F and G), negative control: *S. aureus*, *E. coli*, *P. aeruginosa,* and *V. cholera* (B-E), blank (A) respectively and 100 bp size marker (M).

theory was introduced by Bae et al. for detection of *Listeria* species (Bae et al., 2007). An optical forward-scattering system, called 'scatterometer' was utilized for identification of bacteria colonies such as *Listeria* species (Banada et al., 2007). Podbielska et al. presented an optical system for bacteria classification by means of the statistical analysis of Fresnel diffraction bacteria colonies pattern (Podbielska et al., 2012). Although, these methods are introduce as a non-invasive, accurate and simple systems but they are culture-dependent method which time-consuming, special storage conditions, the requirement for sample pre-enrichment steps limit their usage in practical applications.

Overall, the aforementioned methods are labor-intensive, low-sensitivity and low-specificity based time-consuming processes that need professional skills as well as long assay times (Mokhtari et al., 2013; Singh et al., 2013; Xiao et al., 2014).

Recent advances in nanotechnology have empowered the

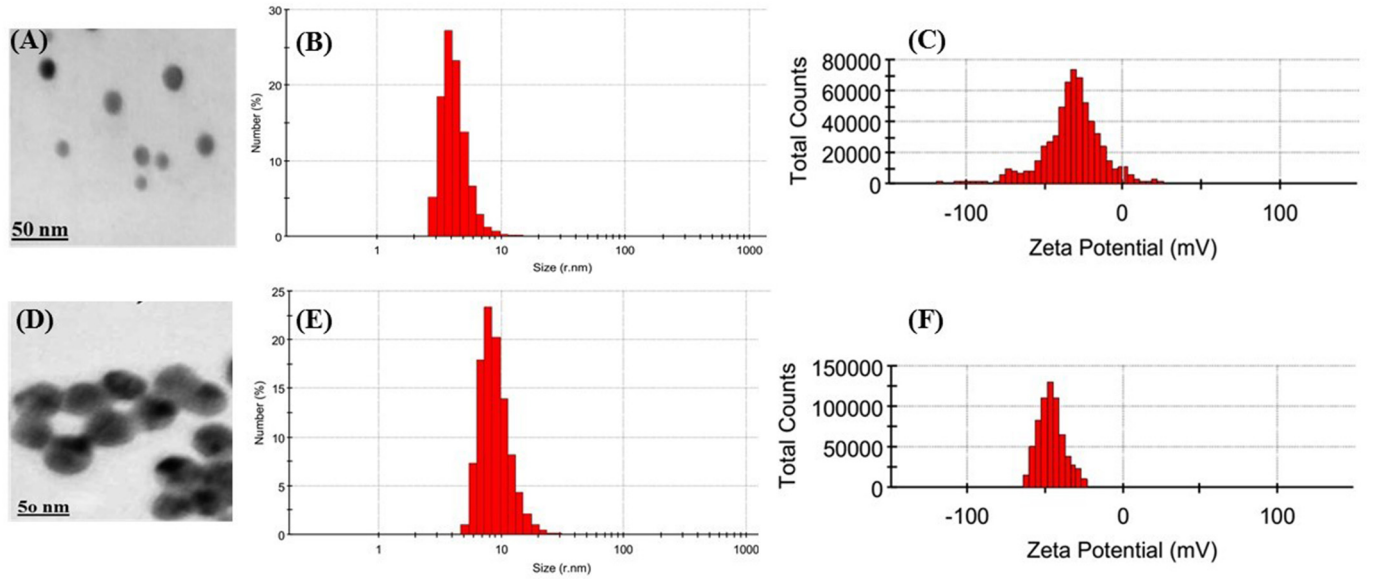

Fig. 3. Morphology, size, and potential distribution analysis of AuNPs and nanomaterial's system by TEM and dynamic light scattering (DLS): pure AuNPs (A, B, C), AuNPs-Probes (D, E, F).

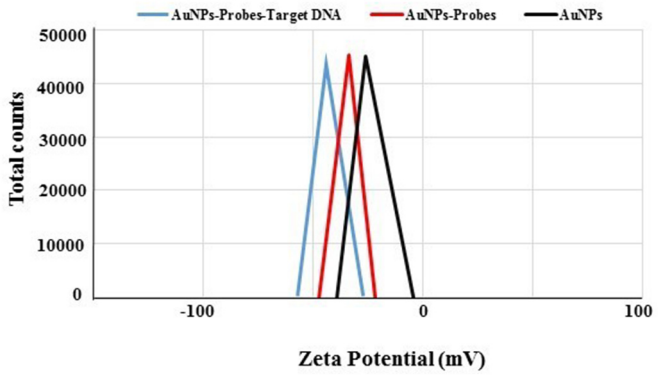

Fig. 4. Comparison of the potential distribution of the prepared nanomaterials.

improvment of new diagnostic platforms for sensitive and fast detection of various pathogens. Optical biosensor detections as the modern generation of detection have attracted much attention due to their simplicity, and practicality, and cost-effective (Briese et al., 2005; Verma et al., 2015; Ajay Piriya et al., 2017).

Among the different types of nanomaterials, gold nanoparticles (AuNPs) have been extensively used for biomolecule detection due to intrinsic features such as unique optical phenomenon called surface plasmon resonance (SPR) (responsible for characteristic color of colloidal AuNPs) unique optical property, ease of synthetic manipulation and functionalization, strong binding affinity to thiols, disulfides and amines which provide the opportunity of being remarkable scaffolds for various applications like coloriometric assay (Elahi et al., 2018; Narmani et al., 2018). The basic of AuNPs-based colorimetric assay is the aggregation of AuNPs, which is detectable by the naked eye and/or spectrophotometry.

Herein, we demonstrate an AuNPs-based optical genosensing system for detection of *Shigella* spp. pathogen infection, which is the continuance of Narmani et al. study (Narmani et al., 2018) for detection of pathogens. In this study, the possibility of *Spa* genes for detection of four species of *Shigella* was described for the first time by AuNP–DNA probes. The *Spa* (surface presentation of antigen) genes, which belong to the Mxi-*Spa* T3SS (type III secretion system) virulence factors group specific in these bacteria, was selected as the hallmark target. For this purpose, the synthesized AuNPs were conjugated with oligonucleotide probes at first. Due to the surface plasmon resonance of AuNPs, their initial color is deep red. After functionalization of AuNPs with thiolated probes, a red-shift in the surface plasmon band would be to the larger wavelength. In the absence of complimentary DNA, AuNPs–probes precipitate under acid environment and the color of solution turns from red to purple. The aggregation causes an obvious red shift to larger wavelengths from the λ_{max} of the initial particles. While in the presence of target DNA, the color of the solution remains red. This phenomenon can be detected by visual observation and absorption spectrum. The suggested process in the proposed method have enough simplicity, practicality, sensitivity and cost-effective compared to conventional method and can be a significant method for substitution with the conventional ones.

2. Materials and methods

2.1. Materials

Staphylococcus aureus (ATCC 27217), *Pseudomonas aeruginosa* (ATCC27853), *Shigella spp*. (ATCC12022), *Vibrio cholerae* (ATCC 51394) and *Shigella dysenteria*e (PTCC 1188) (as the positive and negative control), were purchased from Pasteur Institute, Tehran, Iran. All oligonucleotides (primers and probe) were synthesized by Bioneer, Korea. DNA purification kit (PureLink® Genomic DNA Mini Kit) and ultra-pure agarose were purchased from (Invitrogen, USA). Taq DNA Polymerase Master Mix RED, 2× and 1.5 mM $MgCl_2$ and DNA Ladder were purchased from (Ampliqon, Denmark). DNA safe stain dye was purchased from (GeneTeks BioScience, Inc. Taiwan). Thermal cycler C1000 (Bio Rad, USA), gel documentation system (Bio Rad, USA), nanodrop 2000c UV–vis spectrophotometer (Thermo scientific), and ultraviolet transiluminator (Bio Rad. USA) were used for characterization, detection and performance of the project. DLS Nano (Malvern, USA) was used for characterization of size and zeta potential of nanoparticles. In the characterization experiments of AuNPs, a UV–vis absorption spectroscopy was used to determine the absorbance of AuNPs, and transmission electron microscope (TEM) (Zeiss EM10C, Carl Zeiss AG, Oberkochen, Germany) was used for characterization of the nanoparticle dimension. Ultrasonic Homogenizer (Development of Ultrasonic Technology, Iran) was used for homogenization of nanoparticles. A centrifuge (Micro12, Fisher Scientific, USA) was used for separation and purification of AuNPs. An incubator (HS-101, Amerax Instrument Inc., USA) was used to enrich the bacteria and hybridization reaction.

2.2. Bacteria species, culture and DNA preparation

The *Shigella* spp. as reference species as well as other species, including *S. aureus*, *E. coli*, *P. aeruginosa,* and *V. cholerae* bacteria as negative controls, were utilized in the diagnostic specificity of assay. The bacteria were cultured in LB-Agar for 18–24 h at 37 °C. Then, one of the colonies was cultured in LB-broth for 18 h at 37 °C.

For target DNA extraction, 5 mL of the bacteria culture was centrifuged at 1400*g* for 5 min. Then, the Genomic DNA was extracted

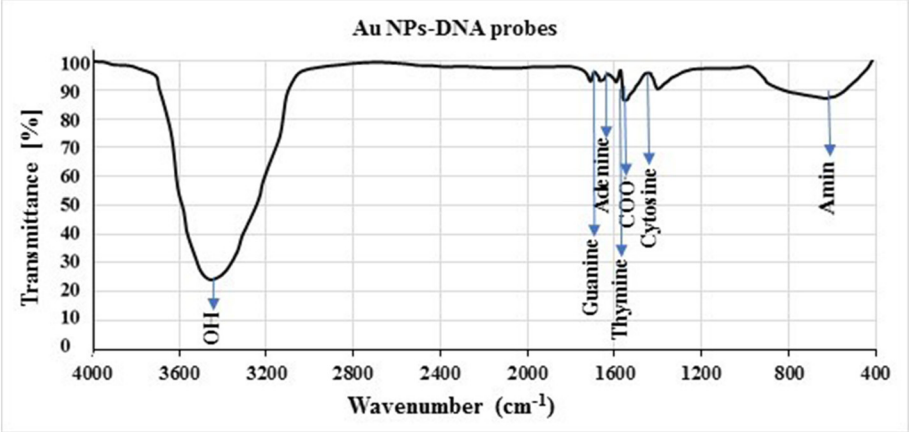

Fig. 5. Evaluation of the conjugation efficiency of AuNPs-Probes measured by FT-IR spectroscopy.

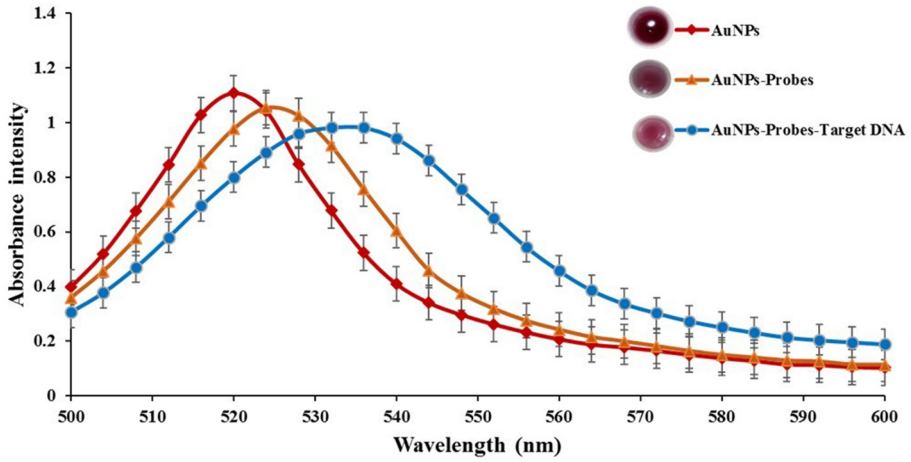

Fig. 6. UV–vis spectrophotometry analysis of AuNPs and its conjugates.

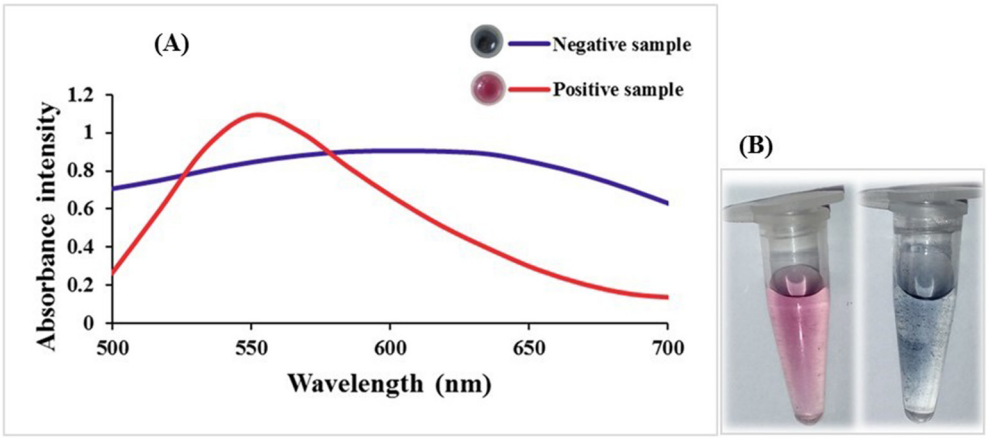

Fig. 7. (A) Absorption peak of a positive and a negative sample. (B) Test tubes with positive (pink color) and negative (purple color) clinical samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

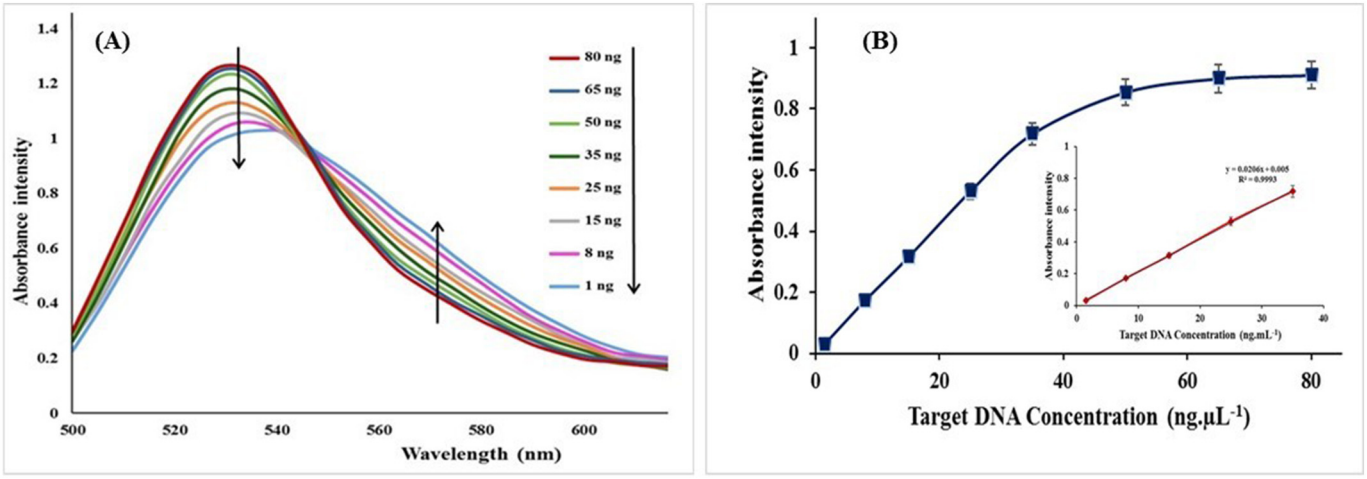

Fig. 8. UV–vis absorption spectroscopy and calibration curve of *Shigella spp.* (A) Determination sensitivity of designed biosensor at different concentrations of the target DNA (1.5, 8, 15, 25, 35, 50, 65 and 80 ng ml^{-1}); (B) Linear relationship between the different concentrations of *Spa* gene and absorbance changes from 1.5–80 ng ml^{-1}.

using DNA purification kit. The extraction was performed according to the manufacturer's instructions (PureLink® Genomic DNA Mini Kit). The target DNA concentration was determined using by the UV–vis absorption spectroscopy at 260 nm and it was stored at −20 °C.

2.3. Primers and probe designing

The *Spa* gene was selected as a target DNA for the detection of *Shigella spp*. (GenBank, NCBI). The *Spa* gene nucleotide sequence was extracted from the gene bank database. It is a 320 bp gene. The primer

Table 1
The applied gene for the determination of *Shigella* spp. with conventional methods.

Method	Bacteria strains	Target	Ref
Multiple endonuclease restriction real-time loop-mediated isothermal amplification technology (MERT-LAMP)	*Shigella spp.*	*ipaH*	(Wang et al., 2015a)
LAMP	*Shigella spp.*	*ipaH*	(Wang et al., 2015a)
Quantitative PCR	*Shigella spp.*	*ipaH*	(Wang et al., 2015a)
PCR	*Shigella spp.*	*ipaH*	(Wang et al., 2015a)
Pentaplex PCR	*Shigella spp.*	*invC*	(Ojha et al., 2013)
	S. flexneri	*rfc*	
	S. sonnei	*wbgZ*	
	S. dysenteriae	*rfpB*	
Real-time PCR	*Shigella spp.*	*ipaH*	(Bae et al., 2007)
Quantitative PCR	*Shigella spp.*	*ipaH*	(Lindsay et al., 2013)
Multiplex-PCR	*S. dysenteriae*	*stx1*	(Amani et al., 2015)
		stx2	
Multiplex real-time PCR	*Shigella spp.*	*ipaH*	(Barletta et al., 2013)
PCR-microplate	*S. dysenteriae*		(Woubit et al., 2013)
Real-time multiplex PCR	*Shigella spp.*	*ipaH*	(Van Lint et al., 2015)
Multiplex PCR	*Shigella spp.*	*ipaH*	(Jiménez et al., 2010)
Conventional PCR	*Shigella spp*	*ipaH*	(Jiménez et al., 2010)
Culture	*Shigella spp.*		(Jiménez et al., 2010)
Multiplex PCR	*S. boydii*	*wzy*	(Radhika et al., 2014)
Multiplex PCR	*Shigella Spp.*	*ipaH1*	(Radhika et al., 2014)
Multiplex PCR	*S. flexneri*	*ipaH*	(Radhika et al., 2014)
Multiplex PCR	*S. sonnei*	*wbgZ*	(Radhika et al., 2014)
Multiplex PCR	*Shigella spp.*	*ipaH*	(Binet et al., 2014)
Multiplex PCR	*S. flexneri 2457 M*	*virB*	(Binet et al., 2014)
IC-PCR (Immunocapture-PCR)	*S. flexneri*	*ipaH*	(Fakruddin et al., 2017)
IC-Culture((Immunocapture- Culture)	*S. flexneri*	*ipaH*	(Fakruddin et al., 2017)
Culture	*S. flexneri*		(Fakruddin et al., 2017)
PCR	*S. flexneri*	*ipaH*	(Fakruddin et al., 2017)
Multiplex PCR	*Shigella spp.*	*stx1*	(Taniuchi et al., 2012)
		stx2	
Multiplex PCR assay with dual priming oligonucleotide system (DPO system-based MPCR)	*Shigella spp.*	*ipaH*	(Xu et al., 2017)
Multiplex real-time PCR	*Shigella spp.*	*ipaH*	(He et al., 2017)
Endonuclease restriction-mediated real-time multiple cross displacement amplification (ET-MCDA)	*Shigella spp.*	*ipaH*	(Wang et al., 2016)
Multiple cross displacement amplification (MCDA)	*Shigella spp.*	*ipaH*	(Wang et al., 2016)
Quantitative PCR	*Shigella spp.*	*ipaH*	(Wang et al., 2016)
PCR	*Shigella spp.*	*ipaH*	(Wang et al., 2016)
PCR	*S.dysenteriae*	*stx*	(Gupta and Dhaked, 2017)
Multiplex PCR	*Shigella spp.*	*ipaH*	(Wang et al., 2015b)
Real-time multiplex PCR	*Shigella spp.*	*ipaH*	(Wiemer et al., 2011)

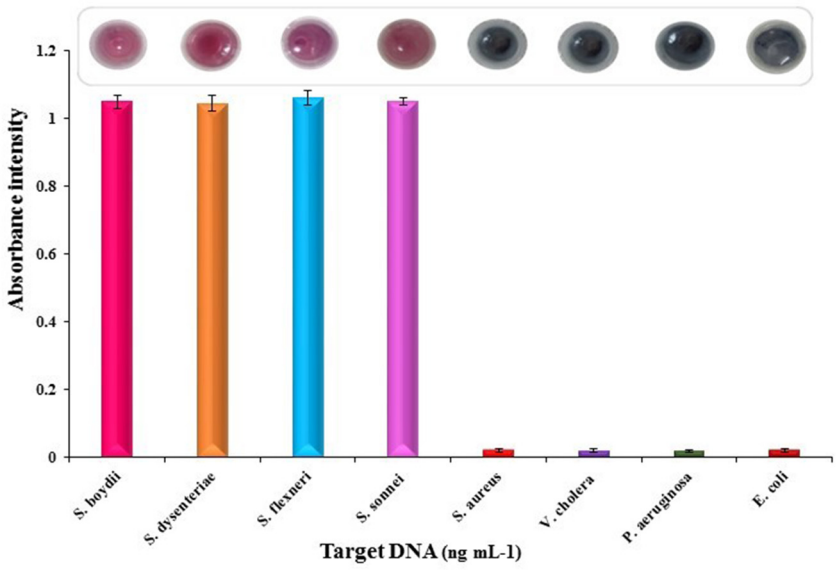

Fig. 9. Selectivity evaluation of the proposed probes for the detection of target DNA. The visual detection and the absorbance spectrum obtained after adding AuNPs-probe to target DNA isolated from bacterial strains selected as positive and negative control including *Shigella spp.*(*S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*), *S. aureus, V. cholera, P. aeruginosa*, and *E. coli* at 65 (ng ml^{-1}) of their extracted DNA, respectively.

(version 3) and oligo (version 7.56) softwares designed the sequences of primers targeting the *Spa* gene of *Shigella*. The sequences of 320 bp product primer pair (fw: 18 bp 5′CTGGATGGTATGGTGAGG3′) and (rv: 20 bp 5′GGAGGCCAACAATTATTTCC3′) and the probe (5′-thiol-TTAA GAGTGGGGTTTGATG-3′) were manufactured by Bioneer R&D center, suspended in sterile water to a 100 pmol/μl concentration each, and stored at −20 °C.

2.4. PCR assays

The PCR amplifications were conducted in a 25 µl reaction containing 2 µl of DNA sample, 1.5 µl (10 pmol) of forward and reverse primers, 12.5 µl of 2× master mix 1.5 mM $MgCl_2$ and 7.5 µl of DDW. The samples were put in a thermocycler and were subjected to the following thermocycling process in the given order: an initial denaturation step at 95 °C for 10 min; 30 cycles secondary denaturation was carried out at 95 °C for 60 s; annealing the primers to the target DNA was carried out at 50 °C for 60 s; the extension temperature was set at 72 °C for 60 s; and the final incubation temperature was 72 °C for 5 min. The PCR products were detected by 2% (*w/v*) agarose gel electrophoresis, stained with DNA SafeStain dye, along with a 100 bp Plus DNA Ladder (80 V at 45 min) in 0.5 X TBE Buffer at pH 8.0 at room temperature. Electrophoresis patterns of PCR products were visualized by erythro-gel safe staining and detected with Bio-Rad Gel-Doc device.

2.5. Synthesis of AuNPs

Colloidal AuNPs with diameters of 15 nm were prepared by the citrate reduction method described by Narmani et al. and Shi et al. (Narmani et al., 2018; Shi et al., 2017). Typically, the aqueous solution of $HAuCl_4$ (1 mM, 100 ml) was brought to a reflux while stirring. Then, 5 mL of 38.5 mM of sodium citrate was added dropwise to mixture. The color of solution first turned from pale yellow to purple within seconds and after about 2 min changed to deep red, indicating the formation of AuNPs. The solution was heated for another 20 min. Subsequently, the heating source was removed and the solution was continuously stirred until it had cooled to room temperature. The colloidal AuNPs were stored at 4 °C. The typical solution of these 15-nm-diameter gold particles exhibited the characteristic surface plasmon band centered at 520 nm. After sonication of nanoparticles using Ultrasonic Homogenizer (Development of Ultrasonic Technology, Iran), were determined by transmission electron microscopy (TEM) and zeta seizer were utilized to characterize the morphology, size and potential distribution of the AuNPs. The absorption spectra of AuNPs were measured by UV–vis absorption spectroscopy.

2.6. Conjugation of AuNPs with the probe

In order to form a self-assembled monolayer on the surface of AuNPs, an active group such as sulfhydryl (-SH) is required. Thus, 15 µL (1 µM) of the probe was dissolved in 100 µL of 1.0 N dithiothreitol solution (1.0 N DTT: 0.01 M Sodium acetate (pH = 5) 20 ml + 1.545 g DTT dissolve) at room temperature for 15 min. Then, the excess DTT and unwanted thiol fragments were removed from the thiol-modified oligonucleotide mixture by extracting with 200 µL ethyl acetate 3 times. After stirring for 2 min, the upper layer was discarded. After purification, the DNA concentration was determined by Nano drop UV–vis spectrophotometer. The purified thiolated DNA probes were immediately added to 1 mL of the AuNPs synthesized previously. After 14 h in a dark place at room temperature and then a serial salt addition, the particles were stabilized for long-time storage at room temperature (Narmani et al., 2018; Andreadou et al., 2014; Liandris et al., 2009). AuNPs-probes complex was centrifuged twice at 13,000 rpm for 20 min, precipitated, washed with 500 µl of 10 mM PBS buffer, 0.15 M NaCl, 0.1% SDS, (pH 7.4) to remove the unreacted materials, and re-dispersed in 500 ml of the same buffer. The efficiency of AuNPs-probes complex was analyzed by FT-IR spectroscopy.

2.7. Hybridization and color detection

Hybridization of AuNPs-probes complex with the target DNA was performed based on the described method of Andreadou et al. (Andreadou et al., 2014). Typically, different serial dilution of the complementary oligonucleotide were put in PCR tube in the thermocycler at 95 °C for 10 min for the dehybridization the target dsDNA into ssDNA. After denaturation, the solution was cooled to 45 °C and 50 µl of the prepared and purified AuNPs-probes complex was added. The hybridization reaction was accomplished at the temperature of 45 °C for 2 h in shaking incubator. On the completion of this step, 20 µl of 0.1 N HCl was added to the mixture reaction. The solutions was kept for 5–15 min at room temperature, by which time its content appears respectively red or purple in the presence (positive samples) or absence (negative samples) of the target sequence. The color change of the environment was detected visually, and it was confirmed with UV–vis absorption spectroscopy Fig. 1.

2.8. Sensitivity

The sensivity of described method was determined by using different concentrations of the genomes of *Shigella spp.* (1.5, 8, 15, 25, 35, 50, 65, and 80 ng ml^{-1}) isolated of standard sample. The results were investigated with the naked eye, and UV–vis absorption spectroscopy.

2.9. Specificity

To determine the specificity of the colorimetric assay, *Shigella* spp. as reference species as well as other species, including *S. aureus*, *E. coli*, *P. aeruginosa,* and *V. cholerae* bacteria as negative controls, were utilized. After forming the AuNPs-probes complex, the genomic DNA of each bacteria was added to the complex based on the described method of section 2.7. Afterwards, the results were analyzed visually and by means of UV–vis spectrophotometry.

3. Results and discussion

3.1. PCR amplification

The presence of the *Spa* gene on the chromosomal DNA of *Shigella* species was determined by using PCR assay. The PCR amplification with forward and reverse primers and DNA extracted from *Shigella* spp. and *S. dysentery* was performed in triplicate, which produced a fragment with an approximate size of 320 bp (Fig. 2). The lack of amplification in the bacteria selected as negative control including *S. aureus*, *E. coli*, *P. aeruginosa,* and *V. cholerae* (lane B-E) suggests the specificity of designed primers for the detection of *Shigella* species. The result showed the applied primers were specific for *Shigella* species (lane F and G), and no amplification was observed with the other strains.

3.2. Characterization of the AuNPs

The morphology, size, and potential distribution of synthesized AuNPs were investigated by the transmission electron microscopy (TEM) and the zeta seizer. The Fig. 3. (A, D) indicates the TEM images of AuNPs before and after the DNA probe conjugation. These results indicate the well-defined spherical shape for AuNPs and AuNPs-Probes. The size and potential distribution of the AuNPs before and after DNA probe conjugation are represented in Fig. 3. (B, C, E, F). The AuNPs have an average dimension of 15 nm with a surface charge of −23.3 mV, which changes after conjugation. The AuNPs-Probes and AuNPs-Probes-Target DNA have the surface charge of −32.5 mV and −42.5 mV respectively(Fig. 4.). The conjugation efficiency of AuNPs-Probes was evaluated by FT-IR spectroscopy. Fig. 5. shows the FT-IR spectra of AuNPs-DNA probes over the range of 400–4000 cm^{-1}. In the region of 3400–3600 cm^{-1}, the wide absorbing peak of the O—H was shown. The absorbing peaks observed at 1700, 1612, 1666, and 1495 cm^{-1} corresponded to guanine, adenine, thymine, and cytosine, respectively.

3.3. UV–vis absorption spectra

The absorbance ratio of the AuNPs, AuNPs-Probes and AuNPs-Probes-target DNA in aqueous solutions were measured by UV–vis spectrophotometry (Fig. 6.).The prepared AuNPs had a maximum absorbance wavelength of 520 nm and were wine red in color. After functionalization of AuNPs with thiolated probes, a red-shift in the surface Plasmon band from 520 to 526 nm was obtained. It is well corroborated that AuNPs can provide colorimetric contrast, which is induced by surface plasmon resonance that originated from their quantum-size effect. As shown in Fig. 6. another significant red-shift to larger wavelength was determined near 534 nm after hybridization of AuNPs-Probes with target DNA. Overall, the attachment of functionalized molecules onto the surface of AuNPs caused the red-shift in the surface Plasmon band from the λ_{max} which was due to the large number of particles chromophores. So, as the attachments increase, the larger wavelength is admissible.

3.4. Detection of target DNA hybridization

The AuNPs-probes solution represent a red color primarily due to the surface plasmon resonance at an absorbance peak of ~526 nm. In the absence of the specific target DNA sequence, the AuNPs-probes precipitate after the addition of HCl and the color turns from red to purple. The absorbance peak shift toward the longer wavelength is the result of this phenomenon. The electrostatic properties of double and single-stranded oligonucleotides are different. When hybridization occurs, the ssDNA forms dsDNA that has double-helix geometry. In comparison with ssDNA, the dsDNA is disable of uncoiling adequately to expose its bases toward the AuNP-probes. Consequently, the aggregation of AuNPs-probes take place in an acidic environment. As shown in Fig. 1., the presence of target DNA that is complementary to the designed probes, prevents the aggregation of AuNPs-probes. Thus, the solution remains red. Fig. 7. indicates the results of UV–vis spectra and visual observation of the positive and negative samples.

3.5. Sensitivity analysis of the AuNPs-based optical genosensor

Development of a colorimetric biosensor with proper efficiency is in need of high sensitivity. To assess sensitivity, minimum amount of DNA detectable by the AuNPs–probes assay, the AuNPs-probes were conjugated with different concentrations of target DNA (1.5, 8, 15, 25, 35, 50, 65 and 80 ng ml^{-1}). After forming the AuNPs-probes-target DNA and addition of HCl, the results were recorded by UV–vis spectrophotometry as shown in Fig. 8A. In this way, the spectrophotometric analysis for *Spa* detection was accomplished. The results demonstrate that absorbance intensity increased gradually with the concentration of target DNA from 1.5 to 80 ng ml^{-1} and the changes were obtained by drawing the calibration curve. According to the Beer-Lambert law ($A = \varepsilon \times B \times C$), there is a linear relationship between absorbance and concentration at low concentration of an absorbing species. This law is disaffirm at high concentration of the sample, so the graph would be nonlinear. The regression equations was $y = 0.0377\times + 0.9858$ (x represented the concentration of target DNA), with the correlation coefficient of $R^2 = 0.9821$ target DNA (Fig. 8B).

3.6. Detection range and limit of detection

Limit of detection (LOD) and limit of quantification (LOQ) have been employed as two significant implementation characteristics in method validation. LOD and LOQ are terms utilized to describe the smallest concentration of an analyte that can be reliably measured by an analytical method (Shrivastava and Gupta, 2011; Armbruster and Pry, 2008). The limit of detection of the AuNPs-probes assays was confirmed by genomic DNA amount of the template. LOD can be expressed in a model such as $y = a + bx$ because of the linear relationship between the target-DNA concentration and the absorbance intensity of our detection (y). Thus, it can be determined as $LOD = 3S_a/b$, where S_a is the standard deviation of blank measurements ($n = 6$) and b is the slope of the calibration curve. Under optimal condition, the evaluated LOD and LOQ of our detection was 8.14 and 26.6 ng ml^{-1}, with correlation coefficients of $R^2 = 0.9821$ target DNA, respectively. Table 1. shows a list of various genes of *Shigella* which have been utilized for assays. Compared to our proposed gene, the *Spa* gene is capable of recognizing all the species while the others not. Therefore, due to the suitable features of AuNPs-based colorimetric assay such as simplicity, rapidity, ease of preparation and novelty, selectivity and sensivity of DNA-base probes collectively, the designed system is as a good choice for modern detection of *Shigella* species compared to conventional and formerly reported methods.

3.7. Specificity analysis of the AuNPs-based optical genosensor

The specificity of expressed method depends exceedingly on the exact part of the *Shigella* genome used for the hybridization of the AuNPs–probes. In order to determine the specificity of the designed biosensor, *Shigella spp.* (*S. dysenteriae, S. flexneri, S. boydii, and S. sonnei*), *P. aeruginosa*, *S. aureus, E. coli*, and *V. cholerae* were chosen as positive and negative controls, respectively. The DNA of the bacteria strains were extracted and the conjugation of AuNPs with the probes and the same concentration of target DNA was constructed as described in previous section. As shown in Fig. 9, there was no significant absorbance change upon the addition of the aforementioned bacteria except *Shigella* species. Furthermore, in the presence of the complementary target (*Shigella* spp. DNA), the color of mixture reaction remained red, whereas in the other tubes it turn to purple.

4. Conclusion

This study has led to the development of a novel and improved AuNPs-based optical genosensing method for detection of *Shigella* species. The proposed strategy had several significant features. First, the method relied on the utilization of AuNPs for fast and specific detection of *Shigella* spp. exempting from the need for DNA amplification. The self-assembly conjugation of AuNPs with thiolated probes, and then with target DNA were performed, respectively. Afterwards, the results were analyzed visually and by means of UV–vis spectrophotometry comparison of solutions before and after acid induced AuNPs-probes aggregation. Second, the designed colorimetric biosensor had simple construction and cost-effective operation So that the need for complicated experimental techniques and equipments could be omitted. Third, the limit of detection (LOD and LOQ: 8.14 and 26.6 ng ml^{-1}, respectively) was lower than those of previously reported conventional methods. In conclusion, it is suggested that the proposed method had the potential to be used as a sensitive, simple, rapid, and of low cost method with appropriate efficiency for detection of the amount of the *Spa* gene of *Shigella*.

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