Biotechnology Advances xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Colorimetric biosensing of pathogens using gold nanoparticles

Mohit S. Verma [a,b], Jacob L. Rogowski [a,b], Lyndon Jones [a,c], Frank X. Gu [a,b,*]

[a] Department of Chemical Engineering, University of Waterloo, 200 University Avenue W, Waterloo, Ontario N2L 3G1, Canada
[b] Waterloo Institute for Nanotechnology, University of Waterloo, 200 University Avenue W, Waterloo, Ontario N2L 3G1, Canada
[c] Center for Contact Lens Research, University of Waterloo, 200 University Avenue W, Waterloo, Ontario N2L 3G1, Canada

ARTICLE INFO

Article history:
Received 25 September 2014
Received in revised form 8 February 2015
Accepted 2 March 2015
Available online xxxx

Keywords:
Bacteria
Virus
Fungus
Point-of-care
DNA
RNA
Protein
Small molecule
PCR
Color

ABSTRACT

Rapid detection of pathogens is crucial to minimize adverse health impacts of nosocomial, foodborne, and waterborne diseases. Gold nanoparticles are extremely successful at detecting pathogens due to their ability to provide a simple and rapid color change when their environment is altered. Here, we review general strategies of implementing gold nanoparticles in colorimetric biosensors. First, we highlight how gold nanoparticles have improved conventional genomic analysis methods by lowering detection limits while reducing assay times. Then, we focus on emerging point-of-care technologies that aim at pathogen detection using simpler assays. These advances will facilitate the implementation of gold nanoparticle-based biosensors in diverse environments throughout the world and help prevent the spread of infectious diseases.

Contents

* Corresponding author at: Department of Chemical Engineering, University of Waterloo, 200 University Avenue W, Waterloo, Ontario N2L 3G1, Canada. Tel.: +1 519 888 4567x38605; fax: +1 519 888 4347.
E-mail address: frank.gu@uwaterloo.ca (F.X. Gu).

http://dx.doi.org/10.1016/j.biotechadv.2015.03.003
0734-9750/

1. Introduction

Mankind has been fascinated by gold nanoparticles for centuries and the Lycurgus cup is a prime example of their unique optical properties. In the 21st century, research involving gold nanoparticles has witnessed significant growth with applications in drug delivery (Boisselier and Astruc, 2009; Ghosh et al., 2008; Paciotti et al., 2004), photothermal therapy (Gobin et al., 2007; Huang et al., 2006; Jain et al., 2007), diagnostic imaging (Eghtedari et al., 2007; Murphy et al., 2008; Popovtzer et al., 2008), and biosensors (Hutter and Fendler, 2004; Liu and Lu, 2003; Mayer and Hafner, 2011). Along with being the most stable metallic nanoparticles (Daniel and Astruc, 2004), gold nanoparticles flaunt several outstanding features, including facile reactivity with biomolecules, high surface area to volume ratios, and environment dependent optical properties, which make them the ideal candidate for use in colorimetric biosensors (Upadhyayula, 2012).

Pathogens—including bacteria, viruses, fungi, and protozoa—are a leading cause for loss of lives in the developing world, as well as rural areas of developed countries, due to lack of infrastructure and resources (Tallury et al., 2010). Since pathogens can be transmitted via plants, animals, and humans, infectious diseases can spread exponentially and lead to a pandemic if left unchecked (Kaittanis et al., 2010). The most effective method for preventing the spread of infectious diseases is early diagnosis, which is challenging using conventional methods because of expensive equipment, specialized sample preparation, and slow data output (Tallury et al., 2010). Modern biosensors have overcome these obstacles by miniaturizing devices and providing simple rapid output that can be analyzed at the point-of-care without specialized training (Lazcka et al., 2007; Mao et al., 2009; Skottrup et al., 2008).

In addition to point-of-care diagnostics and early treatment of infectious diseases in humans, microbial pathogens are also a concern at various levels of the food industry. Many bacterial genera are associated with food-borne illness such as *Salmonella*, *Listeria*, and *Escherichia*. Infections are typically caused by consumption of food or drink contaminated with these pathogens, and may lead to various inflammatory conditions including gastroenteritis, meningitis, and sepsis. Serious infections may require hospitalization and can be fatal for more vulnerable segments of the population (e.g., immunocompromised patients) (Farber and Peterkin, 1991). While low levels of bacteria and other microbial life are sometimes tolerable, high concentrations are frequently associated with food-borne illnesses (Velusamy et al., 2010). Various agencies have implemented guidelines for food production, preparation, and distribution, which aim to keep pathogen loads at acceptable levels. Often these guidelines have stringent concentration requirements and hence, screening assays require excellent detection limits.

Gold nanoparticles have been implemented for the detection of pathogens, which contaminate food, water, and hospital surfaces (Agasti et al., 2010; Azzazy et al., 2012; Bunz and Rotello, 2010; Khanna, 2008; Saha et al., 2012; Tallury et al., 2010; Upadhyayula, 2012). A major focus of research is to improve conventional genomic analysis methods using gold nanoparticles such that the assays have lower detection limits and faster response times (Fig. 1). Concurrently, novel methods of detection have been developed independent of gene amplification and the most popular strategy is based on the surface modification of gold nanoparticles with antibodies, which has led to several commercially available products for easy and timely testing of pathogens in complex samples such as plant extracts, foods, and bodily fluids. An emerging strategy is to exploit the intrinsic surface properties of gold nanoparticles and pathogens which lead to electrostatic interactions and a color change.

2. Conventional methods for pathogen detection

The importance of pathogen detection in several sectors has led to continuous improvement in detection technologies. Currently, conventional methods for pathogen detection can be roughly divided into three categories: culture and colony counting, immunological assays, and polymerase chain reaction (PCR)-based methods (Velusamy et al., 2010). These methods offer high sensitivity and specificity, providing both quantitative and qualitative information, which is often a necessity. However, some key drawbacks, chief of which being required processing times, clearly indicate a need for better solutions.

Colony counting is widely considered to be the gold standard for pathogen detection in settings ranging from clinical diagnosis to food pathogen measurement (Lazcka et al., 2007; Peters et al., 2004; Velusamy et al., 2010). This process involves isolation and growth of a suspect pathogen, followed by visual inspection. Due to the inherent amplification during colony growth, this method is good for identifying very low levels of organisms (i.e., single cells). Unfortunately, turnaround times for results are very slow using this technique due to long incubation periods and the need for intensive labor. Depending on the pathogen, initial results often require at least 2 days, with conformation after 7–10 days (Peters et al., 2004; Velusamy et al., 2010). Furthermore, the colony counting method requires a pathogen to be culturable, which may not always be the case given stringent environmental or nutritional requirements.

Immunological assays are very common for pathogen detection due to their adaptability for a wide variety of pathogens including bacteria and viruses. The enzyme-linked immunosorbent assay (ELISA) method is an example of a well-known immunological assay. These assays rely on antibody recognition of antigens and other biomolecules specific to the target. Once antibodies are identified and available, the primary advantage of immunological assays over colony counting is reduced assay time while maintaining high specificity. ELISA is capable of generating an optical response and hence is widely deployed in clinical laboratories using commercially available ELISA kits. The technique still suffers from the drawbacks of requiring multiple steps, specialized training, and several hours of runtime (Ahmed et al., 2014; Lazcka et al., 2007). Antibody-labeled gold nanoparticles have overcome these challenges by using an immunochromatographic strip (ICS) format and unique products are available for testing of foods and clinical samples. The testing of food products is facilitated by Merck Millipore's Singlepath® and Duopath® products (Billerica, MA, USA), but these products require selective enrichment of bacteria before the sample is analyzed, which is necessary because of low sensitivity and the need to detect low concentration of pathogens in food. Thus, the assay requires several hours for completion even though the ICS can respond within 20 min. In a clinical diagnostic setting, ICS-based assays have been developed by Coris Bioconcept (Gembloux, Belgium) for the detection of viruses and bacteria in stool, urine, and blood samples (Renuart et al., 2011). Current challenges faced by ICS-based assays include the variability caused by user sample preparation and cross-reactivity of analytes, but ICS has been the biggest commercially available success of colloidal gold nanoparticles because of their

Please cite this article as: Verma MS, et al, Colorimetric biosensing of pathogens using gold nanoparticles, Biotechnol Adv (2015), http://dx.doi.org/10.1016/j.biotechadv.2015.03.003

ability to analyze samples in a complex media with minimal purification. We will highlight how emerging technologies have adopted the success of antibody-labeled gold nanoparticles in later sections of this review paper.

PCR-based methods constitute a wide variety of detection schemes relying on nucleic acid amplification to increase the concentration of the detection target. Amplification of target deoxyribonucleic acid (DNA) sequences lends PCR-based conventional methods a high degree of sensitivity, even capable of detecting single gene copies. It is important to note that unlike colony counting, this sensitivity is achieved without a prolonged incubation time since bacteria do not need to be grown (Ahmed et al., 2014). Specificity is achieved through the design of primers and probes that target sequences unique to the pathogen of interest. However, interference from non-pathogenic genetic material may lead to misleading results due to mismatch or non-specific amplification (Ahmed et al., 2014; Velusamy et al., 2010). Precise genetic information is therefore required for confidence in results. Following target amplification, samples are traditionally separated by gel electrophoresis but complex sample preparations and manipulations increase labor cost and processing times (Lazcka et al., 2007). Newer technologies such as real-time PCR and fluorescent molecular probes aim to reduce these factors. Perhaps the main drawback of traditional PCR-based methods for pathogen detection is the inability to distinguish viable and non-viable cells, since both contain the amplification target (Velusamy et al., 2010). To address this issue, assays have been developed that employ reverse transcription PCR (RT-PCR) to target rapidly degrading messenger ribonucleic acid (mRNA) strands present during the cell's growth cycle (Velusamy et al., 2010; Yaron and Matthews, 2002).

3. Principles of gold nanoparticle sensing

The unique optical properties of gold nanoparticles make them very popular for pathogen detection. Most of these assays rely on the basic principle of surface plasmon resonance to detect changes in nanoparticle aggregation states (Daniel and Astruc, 2004). The peak absorbance of gold nanoparticles depends on their size and shape. Spherical nanoparticles with mean particle sizes ranging from 9 to 99 nm have been observed with absorbance peaks from 517 to 575 nm, respectively (Daniel and Astruc, 2004). Gold nanorods exhibit two absorbance peaks: one corresponding to transverse band (about 520 nm) and another corresponding to the longitudinal band (in the infrared region). The longitudinal band is typically more sensitive when gold nanorods are used in biosensors (Wang et al., 2007). Star-shaped gold nanoparticles have also been used for the colorimetric detection of pathogens, where the absorption peak is governed by the particle size and degree of branching (Verma et al., 2014a). Smaller particles are more colloidally stable but bigger particles can be more sensitive. Thus, optimization of particle size is important but rarely explored for pathogen detection (Nath and Chilkoti, 2002; Verma et al., 2014a). Most commonly, spherical gold nanoparticles in the size range of 13–20 nm with absorbance peak around 520 nm have been employed in biosensors due to ease of synthesis.

The peak absorbance wavelength is sensitive to the distance between particles. Upon aggregation, the surface plasmon resonance of individual particles becomes coupled and shifts the absorbance spectrum (Willets and Van Duyne, 2007). This shift can be large enough to produce a visible color change, which makes the techniques favorable for

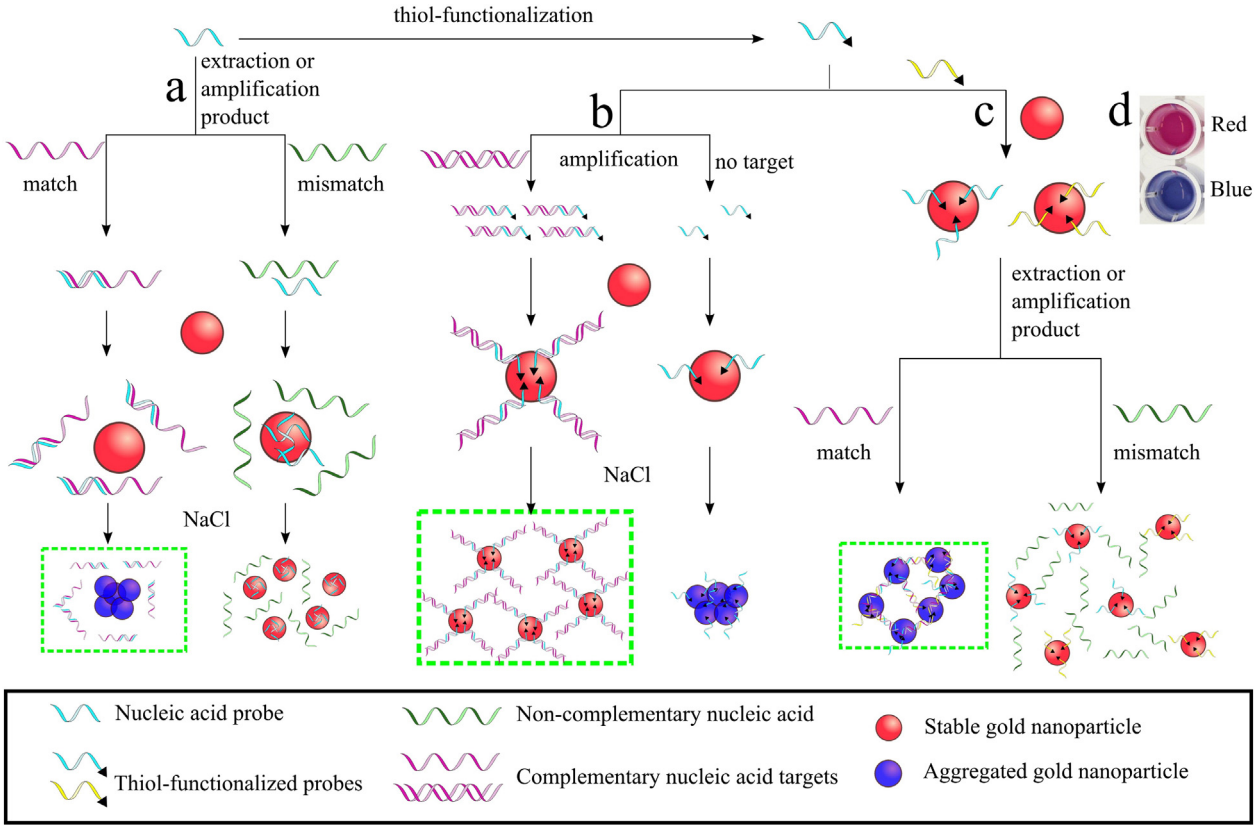

Fig. 1. Colorimetric detection of nucleic acids using non-functionalized and functionalized gold nanoparticles; a) the use of a single non-thiolated probe with non-functionalized gold nanoparticles, b) use of single thiolated probe with non-functionalized nanoparticles, c) use of a pair of thiolated probes for functionalizing gold nanoparticles, d) digital image of gold nanoparticle solutions showing typical red and blue colors.
Adapted from Jung et al. (2010), Saleh et al. (2012) and Verma et al. (2014b)

Please cite this article as: Verma MS, et al, Colorimetric biosensing of pathogens using gold nanoparticles, Biotechnol Adv (2015), http://dx.doi.org/10.1016/j.biotechadv.2015.03.003

rapid point-of-care diagnostics. Peak absorbance wavelengths exhibit a red-shift with increases in size, typically giving stable (non-aggregated) nanoparticles a red color, while aggregated nanoparticles appear blue (Fig. 1d) (Kim and Lee, 2009). Use of an ultraviolet–visible spectrophotometer can help quantify the shift in the surface plasmon resonance peak.

Gold nanoparticles are typically stabilized electrostatically, where citrate-capped nanoparticles are negatively charged and cetyltrimetylammonium bromide (CTAB)-coated nanoparticles are positively charged. The electrostatic repulsion between nanoparticles can be shielded by the addition of salts (most commonly sodium chloride), which then leads to the aggregation of nanoparticles and hence, a color change (Zhao et al., 2008).

Optical effects of surface plasmon resonance have been implemented for pathogen detection by either inducing particle aggregation or stabilization. These effects are governed by target ligands, nanoparticle functionalization, competitive binding sites, or salts. The specific combinations of these factors make up the wide variety of applications investigated in this review.

4. Gold nanoparticles for detecting nucleic acids

4.1. Gold nanoparticles for amplified nucleic acids

Amplification is a common strategy in molecular diagnostics for increasing signal strength. Various methods for the amplification of both DNA and ribonucleic acid (RNA) have been highlighted in molecular diagnostic reviews (Cenciarini-Borde et al., 2009; Lauri and Mariani, 2009; Monis and Giglio, 2006). Some of the amplification methods that have been used in conjunction with gold nanoparticles are: PCR, real-time (or quantitative) PCR, RT-PCR, asymmetric PCR, rolling circle amplification (RCA), and nucleic acid sequence-based amplification (NASBA).

4.1.1. Non-functionalized gold nanoparticles

Non-functionalized gold nanoparticles are usually used for the detection of amplified products by the addition of salt. In the presence of salt, typically gold nanoparticles will aggregate and change color from red to blue unless they can be stabilized by nucleic acids. Two primary strategies can be utilized for stabilizing the gold nanoparticles: adsorption of nucleic acids on the surface or reaction with thiol probe that has been hybridized with the target nucleic acids (Fig. 1a, b). Another approach involves the use of cationic gold nanoparticles, where the interactions between nucleic acids and the surface of gold nanoparticles lead to aggregation of the nanoparticles. This approach is similar to the one explained in Fig. 1c, except the gold nanoparticles are not functionalized with a thiol-probe but rather coated with the probe using electrostatic interactions.

DNA from bacteria and viruses has been used for detection by adsorption on the surface of gold nanoparticles. *Salmonella* spp. are troublesome for causing foodborne illnesses. Regulatory levels published by the United States Food and Drug Administration (FDA) and United States Environmental Protection Agency (EPA) for food safety require complete absence of *Salmonella* spp. in a 25 gram sample (U.S. Food and Drug Administration, 2011) It is therefore important for detection assays to have high sensitivity to very low (individual) pathogen levels. *Salmonella* spp. has been detected by targeting the *stn* gene where an oligonucleotide probe was designed to be complementary to the PCR product (Prasad et al., 2011). Here, 23 nm gold nanoparticles were able to produce a detection limit 10× more sensitive than gel electrophoresis. Also, a sensitivity (true positive rate) of 89.15% and specificity (true negative rate) of 99.04% was obtained for various food samples as compared to conventional culture methods. Detection of *Bacillus anthracis*, the causative agent of anthrax, is possible by using a similar strategy. Here, it was demonstrated that when the DNA is longer than about 100 nt (single-stranded DNA, ssDNA) or 100 bp (double-stranded DNA, dsDNA), it can prevent salt-induced aggregation of 15 nm gold nanoparticles (Deng et al., 2013) and the colorimetric response is visible by the naked eye. When considering viruses, Hepatitis B virus (HBV) is notorious for causing acute and chronic liver diseases worldwide. HBV has been detected by designing a probe targeting the *rtM204M* wild type gene (Liu et al., 2011). A colorimetric response from 13 nm gold nanoparticles was able to distinguish between target DNA and single base pair mismatched DNA. On the other hand, RCA has been used for the detection of H1N1 viral DNA, where long ssDNA curled into balls and could not stabilize 13 nm gold nanoparticles (Xing et al., 2013).

Thiol-modified probes coupled with non-functionalized nanoparticles have primarily been used for detection of bacterial DNA. *Chlamydia trachomatis* is responsible for most of the bacterial sexually transmitted diseases worldwide. The gene encoding virulence proteins was targeted with thiolated probes and detected in human urine samples using 13 nm gold nanoparticles (Jung et al., 2010). *Listeria monocytogenes* and *Salmonella enterica* are notorious for contaminating foods and causing fatalities. FDA regulations have a "zero-tolerance" policy of no detectable *L. monocytogenes* in two 25 g samples of food or beverage (Hitchins and Jinneman, 1998). The detection of these food-borne bacteria has been possible by designing thiolated probes to target the *hly* and *hut* genes for *L. monocytogenes* and *S. enterica* respectively. This assay was able to detect bacteria in contaminated milk samples using 13 nm gold nanoparticles and the specificity was confirmed by a lack of response from *Escherichia coli* (Fu et al., 2013).

The detection of DNA from human immunodeficiency virus type 1 (HIV-1) has been possible using cationic gold nanorods. The probe is designed to target sections of the *HB-hp3-LTR1.8* DNA and in the presence of the target, aggregation is induced (He et al., 2008). The specificity of this assay was confirmed by comparing results against genes from *Mycobacterium tuberculosis* and genes encoding for *Bacillus* glucanase. It was possible to perform detection under physiological conditions because the assay is tolerant to high salt concentrations. Another use of gold nanorods is for the detection of *Leishmania major*, a protozoan parasite that has led to 1.5 million cases of cutaneous leishmaniasis annually worldwide. The disease can lead to disabilities and even death. The detection of the parasite using culture-based methods is extremely slow and insensitive. Thus, molecular diagnostics can offer an improved method for detection. NASBA has been employed for the detection of 18S ribosomal RNA (rRNA) of *L. major* by designing the appropriate primer (Niazi et al., 2013). After amplification, the NASBA amplicons are incubated with gold nanorods leading to aggregation. Clinical skin biopsies were tested using this method and a sensitivity of 100% and specificity of 80% was obtained as compared to RT-PCR and gel electrophoresis.

Non-functionalized gold nanoparticles have the advantage of providing rapid response as compared to gel electrophoresis. Additionally, the equipment necessary for gel electrophoresis is not needed since a simple colorimetric response is obtained, which can be visually observed with minimal training. The synthesis of non-functionalized nanoparticles can often be executed in a single step, which simplifies the assay. The main limitation to this approach is that the conditions for detection often need to be optimized such that the appropriate concentrations of salts and reagents are used to avoid unnecessary aggregation of gold nanoparticles. The optimization of assay conditions demands extra efforts for each target in question. The studies using non-functionalized gold nanoparticles for amplified nucleic acids have been summarized in Table 1. They are divided by pathogen type: bacteria, viruses, and protozoa, and then sorted chronologically.

4.1.2. Functionalized gold nanoparticles

Gold nanoparticles can be easily functionalized with nucleic acid probes by using thiol-gold chemistry. There are two primary approaches to detection, which are governed by the number of probes used. In one scenario, salt is used to induce aggregation of probe-conjugated gold nanoparticles. Only one type of probe is used for binding to the target sequence. This approach is similar to the illustration in

Please cite this article as: Verma MS, et al, Colorimetric biosensing of pathogens using gold nanoparticles, Biotechnol Adv (2015), http://dx.doi.org/10.1016/j.biotechadv.2015.03.003

Table 1
Non-functionalized gold nanoparticles for detecting amplified nucleic acids.

Microorganisms of interest	Sample type	Analysis time	Detection limit (copies/μL DNA)	Working range (copies/μL DNA)	References
Chlamydia trachomatis	Urine	1 h post-amplification	20	20–20,000	Jung et al. (2010)
Salmonella spp.	Culture	<8 h	2×10^9	2×10^9–2×10^{11}	Prasad et al. (2011)
Listeria monocytogenes and *Salmonella enterica*	Food	3–4 h	2.1×10^4 (*L. monocytogenes*) 2.6×10^4 (*S. enterica*)	2.1×10^4–2.1×10^{11} (*L. monocytogenes*) 2.6×10^4–2.6×10^{11} (*S. enterica*)	Fu et al. (2013)
Bacillus anthracis	Nucleic acids	–	~3.9×10^6[a]	~3.9×10^6–3.9×10^8[a]	Deng et al. (2013)
HIV-1	Nucleic acids	<5 min post-amplification	4.8×10^7	1.0×10^8–7.0×10^9	He et al. (2008)
Hepatitis B virus	Serum	–	3×10^9	3×10^9–3×10^{11}	Liu et al. (2011)
H1N1 virus	Nucleic acids	3 h	6.02×10^5	6.02×10^5–6.02×10^{10}	Xing et al. (2013)
Leishmania major	Skin biopsy	–	–	–	Niazi et al. (2013)

[a] A mixture of ssDNA and dsDNA was used, molecular weight of ssDNA was used for calculations. Sample type 'culture' refers to the bacteria being grown in the lab before testing.

Fig. 1b, except the gold nanoparticles are conjugated to the thiolated probe before hybridization. In this situation, binding of the target to the probe results in double helix formation and particles can remain stable under higher salt conditions. Consequently, absence of the target would lead to particle aggregation at similar salinity. In another scenario, two probes are used such that each probe can bind to the same nucleic acid strand. There are two main methods within the two-probe approach. In one method, gold nanoparticles are functionalized with each of the two probes separately and then mixed together. The presence of the target causes particle aggregation by cross-linking gold nanoparticles together. In the absence of target sequence or the presence of a mismatched sequence, aggregation does not occur and particles remain stable in suspension (Fig. 1c). Another method using two probes is called gold label silver stain. Here, one probe is immobilized on a glass slide and another on the gold nanoparticles. The target nucleic acid binds to the glass slide first, followed by the addition of the gold nanoparticles and then silver for signal enhancement (Taton et al., 2000). In recent studies, the probe immobilized on gold nanoparticle has been replaced by streptavidin and the PCR product has been functionalized with biotin for facilitating binding via streptavidin–biotin interactions instead of hybridization.

Using the one-probe approach, *M. tuberculosis* has been detected by designing probes targeting the *rpoB* gene and immobilizing them on 14 nm gold nanoparticles (Veigas et al., 2010). The design is able to discriminate against the non-tuberculosis causing *Mycobacterium kansasii*. This design has also been implemented in a paper format, by using wax-based ink for making a 384-well paper microplate (Veigas et al., 2012). The assay has been adapted for differentiating between *Mycobacterium bovis* and *M. tuberculosis* by targeting the *gyrB* gene (Costa et al., 2010). Three probes were designed to target specific segments of the *gyrB* gene and immobilized on gold nanoparticles. Each strain of *Mycobacterium* interacted differently with the probes and hence allowed accurate identification. Another notorious pathogen methicillin-resistant *Staphylococcus aureus* (MRSA) has been responsible for numerous persistent infections. It has been possible to detect MRSA by using probes towards 23S rRNA and *mecA* genes (Chan et al., 2014). In this study, the sensitivity and specificity were comparable to real-time PCR assays but with a lower cost per reaction. RNA has also been targeted using the one-probe approach. One example is the detection of *dnaK* messenger RNA of *S. enterica* serovar Typhimurium after amplification by NASBA (Mollasalehi and Yazdanparast, 2012). The probe was immobilized on 17 to 23 nm gold nanoparticles and the assay was able to distinguish between RNA from *S.* Typhimurium and *Bacillus firmus*.

The two-probe approach has also gained popularity for a variety of bacterial and viral targets. *Helicobacter pylori* is responsible for several gastric conditions such as chronic gastritis, gastric adenocarcinoma and gastric ulcers. Detection of *H. pylori* is possible by designing probes towards the *ureC* gene and immobilizing them on gold nanoparticles. Target DNA was amplified using thermophilic helicase-dependent isothermal amplification and the assay was able to distinguish between *H. pylori*, *E. coli*, and human DNA (Gill et al., 2008). While some strains of *E. coli* can be harmless, Shiga toxin producing *E. coli* O157:H7 can cause disease outbreaks when it gets transmitted via food or water. FDA and EPA regulations for clams, mussels, oysters, and scallops require *E. coli* levels to be below 330/100 g as determined by the Most Probable Number method, which translates to approximately 3.3 colony forming unit (CFU)/g (U.S. Food and Drug Administration, 2011). The detection of *E. coli* O157:H7 has been achieved by designing a pair of probes targeting the *stx2* gene and immobilizing them on gold nanoparticles for a visible color change (Jyoti et al., 2010). Another food-borne pathogen *S.* Typhimurium can be detected by targeting the invasion (*inv* A) gene (Majdinasab et al., 2013). The specificity of this assay was confirmed by comparing response to PCR products of other non-*Salmonella* spp. bacteria. The assay can provide better sensitivity compared to gel electrophoresis (Majdinasab et al., 2013). While most studies have focused on detection of a single species of bacteria, it is also possible to design gold nanoparticles for the detection of multiple bacteria, including the non-pathogenic ones. This is especially important in blood components because of the zero-tolerance policy. The 16S rDNA sequence is present in most bacteria and hence can be used as a target for detection (Wang et al., 2012b). A pair of 12-mer probes have been designed to target the 16S rDNA sequence and immobilized on gold nanorods. This method was tested for detection of the following species of bacteria in platelet concentrates: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Serratia marcescens* and *Bacillus cereus*. It was found that the assay was most sensitive for the detection of *S. marcescens*. The assay provides a simple method for giving a yes/no result in contamination of blood components, but it does not identify the species of contamination.

A slightly different two-probe approach has been adapted for the detection of human papillomavirus (HPV) type 16 (HPV-16) and type 18 (HPV-18). These viruses are responsible for over 70% of cervical cancer cases and hence fall under the "high risk" category. Two pairs of thiolated oligonucleotide probes have been designed to target the L1 gene of HPV-16 and HPV-18. These probes were immobilized on 13 nm gold nanoparticles, which aggregate in the presence of asymmetric PCR products. In the presence of the target, gold nanoparticles remain stable under high salt conditions because they are spaced apart by the target DNA (Chen et al., 2009).

Modifications of gold label silver stain method have been implemented for detection of viruses and bacteria. HIV-1 and *Treponema pallidum* are prominent causes of sexually transmitted diseases and their prevalence has been rising. Amino-terminated oligonucleotide probes have been designed to target the *gag* gene for HIV-1 and 47k Ag gene for *T. pallidum* and immobilized on glass surfaces. The target genes were amplified and biotinylated by multiplex asymmetric PCR and then detected (Tang et al., 2009). A similar approach has been deployed for the detection of *Acinobacter baumannii*, which is responsible for a high incidence of bacteremia in hospitals. The specificity of the assay has been determined by comparing the response to other strains within the species (positive control), other species within the same genus (negative control), and bacteria from other genera (negative

Please cite this article as: Verma MS, et al, Colorimetric biosensing of pathogens using gold nanoparticles, Biotechnol Adv (2015), http://dx.doi.org/10.1016/j.biotechadv.2015.03.003

Table 2
Functionalized gold nanoparticles for detecting amplified nucleic acids.

Microorganisms of interest	Sample type	Analysis time	Detection limit	Working range	Sensitivity	Specificity	References
Helicobacter pylori	Gastric biopsy	<1 h	10 CFU/mL	10–10,000 CFU/mL	92.5% (culture) 100% (histology)	95.4% (culture) 98.8% (histology)	Gill et al. (2008)
Escherichia coli O157:H7	Culture	–	2.2×10^5 copies/μL DNA	2.2×10^5–2.2×10^7 copies/μL	–	–	Jyoti et al. (2010)
Mycobacterium tuberculosis	Respiratory samples	15 min post-amplification	4.5×10^{10} copies/μL DNA	–	84.7% (*AccuProbe*®)	100% (*AccuProbe*®)	Costa et al. (2010), Veigas et al. (2010), Veigas et al. (2012)
Acinetobacter baumannii	Culture	~4 h post-amplification	1.07×10^7 copies/μL DNA	1.07×10^7–3.57×10^{10} copies/μL	–	–	Yeh et al. (2012)
Salmonella enterica serovar Typhi	Culture	~1 h post-amplification	10^3 CFU/mL	10^3–10^5 CFU/ml	–	–	Qi et al. (2012)
Pseudomonas aeruginosa, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Kelbsiella pneumoniae*, *Serratia marcescens* and *Bacillus cereus*	Spiked platelet concentrates	0.8 h post-amplification	~3×10^6 copies/μL DNA	~3×10^6–6×10^9 copies/μL	–	–	Wang et al. (2012b)
Salmonella enterica serovar Typhimurium	Culture	~8 h	~3.6×10^{11} copies/μL	–	–	–	Mollasalehi and Yazdanparast (2012), Majdinasab et al. (2013)
Staphylococcus aureus (methicillin-resistant)	Blood culture, urine, respiratory samples, wound swabs, pus and body fluids	~20 min post-amplification	~8×10^{10} copies/μL DNA	–	97.14% (culture)	91.89% (culture)	Chan et al. (2014)
HIV-1 and *Treponema pallidum*	Serum	~5 h post-amplification	10 copies/μL DNA	–	100% (ELISA & real-time PCR)	100% (ELISA & real-time PCR)	Tang et al. (2009)
HPV-16 and HPV-18	Ectocervical/endocervical cell samples	20 min post-amplification	8.4×10^7 copies/μL DNA	8.4×10^7–8.4×10^{11} copies/μL	95% (real-time PCR)	90% (real-time PCR)	Chen et al. (2009)
Influenza H1N1 virus	Nucleic acids	2.5 h	2.58×10^8 copies/μL RNA	2.58×10^8–2.58×10^9 copies/μL	–	–	Kim et al. (2012), Nagatani et al. (2012)

Sample type 'culture' refers to the bacteria being grown in the lab before testing.

control) (Yeh et al., 2012). In order to test a large number of samples simultaneously, the assay has been incorporated in microarrays. Typical biotin-tyramine microarray designs do not provide sufficient accumulation of gold nanoparticles and hence 1.4 nm gold nanoparticles have been modified with 3,3′-diaminobenzidine (DAB), which is a substrate for horseradish peroxidase (HRP) (Qi et al., 2012). Here, the HRP is modified with streptavidin, which binds to biotinylated PCR products that are immobilized on the glass surface via a probe. The presence of DAB promotes the accumulation of gold nanoparticles and simplifies the assay by reducing an incubation step compared to biotin-tyramine based microarrays. This approach was deployed for the detection of *S. enterica* serovar Typhi, which is responsible for causing typhoid fever (a life-threatening infection, especially in developing countries) (Qi et al., 2012).

Finally, biotin–streptavidin interactions have also been exploited for implementing gold nanoparticles in an ICS format for the detection of influenza H1N1 virus. An ICS format is ideal for detection because of its portability and easy readout. In this design, gold nanoparticles were functionalized with anti-hapten antibodies and added to the conjugate pad. RT-PCR products labeled with biotin and Texas Red (a hapten) are added to the conjugate pad, where they attach to the gold nanoparticles. The test line contains streptavidin while the control line contains anti-mouse IgG and thus, the gold nanoparticles attach to test line only if the biotin labeled RT-PCR products are present (Kim et al., 2012).

Functionalized gold nanoparticles share the advantage of eliminating the need for gel electrophoresis as was the case with non-functionalized nanoparticles. Additionally, functionalization widens the scope of formats in which the assays are implemented ranging from solution-based methods to strip-based methods. The major limitation of functionalization is that the gold nanoparticles need to be modified for each analyte of interest and then purified before use. These additional processing steps can require additional time and technical expertise and also lead to loss of nanoparticle yield. The studies employing functionalized gold nanoparticles for amplified nucleic acids have been summarized in Table 2.

4.2. Gold nanoparticles for unamplified nucleic acids

4.2.1. Non-functionalized gold nanoparticles

As-synthesized gold nanoparticles can exert surface charges and hence be used directly for detection without specific functionalization. Most of the studies that incorporate this strategy depend on the color change of gold nanoparticles from red to blue due to their electrostatic aggregation behavior. Two common coatings are present on as-synthesized nanoparticles: citrate for providing a net negative charge and CTAB for providing a net positive charge.

Citrate capped nanoparticles have been used for the detection of nucleic acids in a manner similar to the illustration in Fig. 1a, where nanoparticles aggregate when the target is present. This approach has been implemented in the detection of hepatitis C virus RNA by designing probes targeting the 5′UTR region and using them with 15 nm gold nanoparticles (Shawky et al., 2010).

CTAB-coated gold nanoparticles have been used for detecting DNA where the idea is similar to Fig. 1c, except instead of using thiolated probes, the probes are electrostatically adsorbed. Detection of HIV-1 and *B. anthracis* has been possible by designing probes to target the U5 long terminal repeat sequence of HIV-1 and cryptic protein and protective antigen precursor genes of *B. anthracis*. The probes were adsorbed on 16–30 nm gold nanoparticles for obtaining a color change from red to purple (Pylaev et al., 2011).

Eliminating nucleic acid amplification provides major advantages in the required analysis time and equipment. Specifically, the use of non-functionalized nanoparticles simplifies the synthesis of gold nanoparticles and thus the entire assay.

4.2.2. Functionalized gold nanoparticles

Unamplified nucleic acids can also be detected by functionalizing gold nanoparticles with specific thiolated probes. Three main strategies have been employed for implementing this method: functionalizing with a single probe (Fig. 1b), functionalizing with two probes (Fig. 1c), and the use of DNA enzymes (DNAzymes). As compared to amplification-based methods, these assays are simpler and faster.

A thiolated nucleic acid probe has been designed for the detection of *Mycobacterium* spp. by targeting the 16s–23s DNA region of mycobacterial species. The probe was immobilized on 15–20 nm gold nanoparticles and the presence of target DNA stabilized the nanoparticles upon addition of HCl (Fig. 1a). Specificity of the assay was confirmed by comparing the response from non-mycobacterial species (Liandris et al., 2009). Detection of *E. coli* genomic DNA has been possible by targeting the *malB* gene and immobilizing the obtained probe on 20 nm gold nanoparticles. In this assay, the enzymatic degradation of DNA before hybridization improved the detection limit of the assay by 5 times. Specificity was confirmed by comparing the response to other pathogenic bacteria (Bakthavathsalam et al., 2012).

Aggregation of nanoparticles by target DNA can also be used for the colorimetric detection if a pair of appropriate probes is designed (Fig. 1c). One example of this approach is the detection of Kaposi's sarcoma-associated herpesvirus (KSHV). KSHV is responsible for Kaposi's sarcoma, an infectious cancer most commonly occurring in HIV positive patients. The detection of KSHV is challenging because several other diseases present similar symptoms and histopathological features. One such confounding disease is bacillary angiomatosis, which can be caused by *Bartonella quintana* and *Bartonella henselae*. Thus, distinction between these pathogens is necessary and has been achieved by designing pairs of thiolated oligonucleotide probes targeting the DNA that codes for vCyclin in KSHV and conserved regions of *Bartonella* strains. The probes for KSHV and *Bartonella* were then immobilized on 15 nm gold and 20 nm silver nanoparticles respectively to obtain different color changes (Mancuso et al., 2013). Another study has demonstrated the detection of genomic DNA of *S. enterica* by the use of probes targeting the *invA* gene. Here, the mechanism of detection was unclear because detection of genomic DNA was possible using both one-probe and two-probe approaches. Additionally, the thiolated probes were first incubated with the genomic DNA and then incubated with 15 nm gold nanoparticles. In this study, the absence of target DNA allows gold nanoparticles to maintain stability, which is most likely because of high coverage of the probe molecules on the surface of the nanoparticles. In the presence of the target, the probes hybridize with the target DNA and hence, are probably unable to cover the gold nanoparticles sufficiently to stabilize them. This leads to the aggregation of gold nanoparticles and hence detection of the target DNA. This assay allowed the detection of dsDNA at room temperature (Kalidasan et al., 2013).

DNAzymes are nucleic acids that can catalyze the cleavage of other nucleic acids with multiple turnovers and hence are capable of providing amplification in an assay. Multicomponent nucleic acid enzyme (MNAzyme) is a type of DNAzyme that can be designed to perform catalysis specifically in the presence of the target DNA. Gold nanoparticle cross-linkers can be used as MNAzyme substrates such that aggregation of gold nanoparticles can be modulated by the presence of target DNA. This approach has been applied for the detection of AF-1 and genetic sequences from *Neisseria gonorrhoeae*, *Treponema pallidum*, *Plasmodium falciparum*, and HBV. In the absence of target DNA, the cross-linker remained intact and led to aggregation of 13 nm gold nanoparticles. Designing the appropriate MNAzymes allows this method to detect multiple targets, which is useful for diagnosing co-infections (Zagorovsky and Chan, 2013). Another example of DNAzymes is the detection of dengue viruses. Dengue viruses cause periodic explosive epidemics and can lead to 50–100 million infections annually. These viruses are typically carried by mosquitoes and can lead to dengue fever or potentially fatal dengue hemorrhagic fever. DNAzymes have

Please cite this article as: Verma MS, et al, Colorimetric biosensing of pathogens using gold nanoparticles, Biotechnol Adv (2015), http://dx.doi.org/10.1016/j.biotechadv.2015.03.003

Table 3
Gold nanoparticles for detecting unamplified nucleic acids.

Microorganisms of interest	Sample type	Analysis time	Detection limit	Working range	References
Hepatitis C virus[a,b]	Serum	30 min	2.5 copies/μL RNA	~2.5–100 copies/μL	Shawky et al. (2010)
HIV-1 and *Bacillus anthracis*[a]	Nucleic acids	~30 min	6×10^7 copies/μL DNA	6×10^7–3×10^9 copies/μL	Pylaev et al. (2011)
Mycobacterium spp.[c]	Goat feces	~15 min post-extraction	18.8 ng/μL mycobacterial DNA	18.8–1,200 ng/μL	Liandris et al. (2009)
Escherichia coli[d]	Spiked urine	<30 min post-extraction	5.4 ng/μL genomic DNA	5.4–43 ng/μL	Bakthavathsalam et al. (2012)
Kaposi's sarcoma-associated herpes virus and *Bartonella*	Nucleic acids	2 h post-extraction	1×10^9 copies/μL DNA	$1–10 \times 10^9$ copies/μL	Mancuso et al. (2013)
Neisseria gonorrhoeae, *Treponema pallidum*, *Plasmodium falciparum* and hepatitis B virus	Nucleic acids	~1.5 h post-extraction	3×10^7 copies/μL model DNA	3×10^7–6×10^8 copies/μL	Zagorovsky and Chan (2013)
Salmonella enterica	Nucleic acids	~15 min post-extraction	2.2×10^4 copies/μL genomic DNA	2.2×10^4–3.8×10^5 copies/μL	Kalidasan et al. (2013)
Dengue virus	Culture	5 min post-extraction	4×10^7 copies/μL RNA	4×10^7–4×10^{12} copies/μL	Carter et al. (2013)

[a] Non-functionalized gold nanoparticles.
[b] Sensitivity 92%, specificity 88.9% (RT-PCR).
[c] Sensitivity 87.5%, specificity 100% (real-time PCR).
[d] Specificity 100% (PCR); most studies used functionalized gold nanoparticles, except where indicated otherwise. Sample type 'culture' refers to the microorganism being grown in the lab before testing.

been designed and immobilized on 15 nm gold nanoparticles to cleave dengue virus RNA in the presence of magnesium ions. The cleaved RNA leads to aggregation of gold nanoparticles in the presence of salt and heat (Carter et al., 2013).

Functionalizing gold nanoparticles with DNAzymes has allowed the incorporation of signal amplification during detection and hence provided excellent detection limits. The major limitation of this approach has been the requirement of nucleic acid extraction, since it can increase the assay time by several hours. The studies employing gold nanoparticles for detection of unamplified nucleic acids are summarized in Table 3.

5. Emerging biosensors for detecting non-nucleic acid analytes

While several strategies have been presented for the detection of nucleic acids, it is possible to detect pathogens by targeting other analytes of interest. Non-functionalized gold nanoparticles can use the native surface charges of nanoparticles and bacteria for producing a color change. Functionalizing gold nanoparticles with proteins or small molecules can facilitate the detection of proteins, lipopolysaccharides, or even whole cells.

5.1. Non-functionalized gold nanoparticles

One approach for using citrate-capped nanoparticles is to design aptamers for specific targets and allow them to adsorb on the surface of gold nanoparticles. In the presence of the target, the aptamers get stripped from the surface of gold nanoparticles and bind to the target, which destabilizes the gold nanoparticles in high salt conditions. This strategy has been applied for the detection of *E. coli* O157:H7 and *S.* Typhimurium, where aptamers were selected against these bacteria and adsorbed on 15 nm gold nanoparticles. The specificity of the assay was confirmed by testing the interaction with seven other species of bacteria and a significant response was observed only when the desired target was present (Wu et al., 2012).

In addition to whole cells, citrate-capped nanoparticles have also been used to detect proteins. β-Lactamases are bacterial enzymes that cleave β-lactam antibiotics and hence render them ineffective towards bacterial infections. The detection of β-lactamase activity can assist in designing better antibiotics. *Enterobacter cloacae* is a pathogen responsible for producing class C P99 β-lactamase, which can cleave cephalosporin derivatives and produce products with free thiols and positively charged amino groups. These products can replace some citrate ions on the surface of gold nanoparticles and then lead to their aggregation due to electrostatic interactions. With the help of 16 nm citrate-capped gold nanoparticles, P99 β-lactamase could be detected (Liu et al., 2010). The same method has also been used for detection of class A β-lactamases, which are produced by *E. coli*, *B. cereus* and *K. pneumoniae* (Jiang et al., 2009). Another notorious enzyme is the immunoglobulin A1 protease (IgA1P) produced by *Streptococcus pneumoniae*, which allows the bacterium to infect the lower respiratory tract, ear, or bloodstream and lead to diseases such as pneumonia, otitis media, sepsis, and meningitis. The protease cleaves human IgA1 and coats the bacterium with Fab fragments to act as a shield against the immune response and also to assist invasion into epithelial cells. Thus, IgA1P serves as a promising antibacterial target to curb the infection. The detection of IgA1P has been achieved using IgA1 and 20 nm citrate-adsorbed gold nanoparticles. In the presence of IgA1P, the IgA1 is cleaved to produce positively charged Fab regions, which is detected by the aggregation of the anionic nanoparticles. The specificity of the assay was confirmed by the lack of response in the presence of IgA2, which is not cleaved by IgA1P (Garner et al., 2013).

Whole cell detection has also been achieved using CTAB-coated gold nanostars with a size range of 31 nm to 113 nm (Verma et al., 2014a). Here, the positive charges on gold nanostars interact with the negative charges on bacterial cell walls presented by teichoic acids, lipopolysaccharides, and phospholipids. This strategy produced a unique degree of color change for different species of bacteria when testing the ocular pathogens: *S. aureus*, *Achromobacter xylosoxidans*, *Delftia acidovorans*, and *Stenotrophomonas maltophilia*. An accuracy of 99% was obtained for identifying randomized samples of the four bacteria (Verma et al., 2014b).

ELISA has been used in a variety of applications for highly specific and sensitive detection of target molecules. Typically, a color change is obtained at the end of the assay because of enzymatic conversion of the substrate into a colored molecule, which is then detected by a spectrophotometer. The color change could also be obtained using growth of gold nanoparticles such that it would be visually detectable. In the absence of target molecules, a high concentration of hydrogen peroxide is present, which rapidly reduces gold ions and forms spherical non-aggregated nanoparticles, producing a red color. In the presence of target molecules, hydrogen peroxide is consumed by the enzyme and hence growth of the gold nanoparticles is slower, which results in aggregated particles with a blue color. This approach has been used for detection of HIV-1 capsid antigen p24 with the naked eye. This method presents an extremely sensitive assay, which performs better than existing established methods based on nucleic acid detection (de la Rica and Stevens, 2012).

As compared to conventional methods for pathogen detection, the non-functionalized gold nanoparticles provide a dramatic colorimetric output, which can often be visualized by the naked eye. The most important limitation of this strategy is that various interferents from the environment can cause aggregation of nanoparticles and hence a false positive response, since the target analyte is often very general. The

Please cite this article as: Verma MS, et al, Colorimetric biosensing of pathogens using gold nanoparticles, Biotechnol Adv (2015), http://dx.doi.org/10.1016/j.biotechadv.2015.03.003

Table 4
Non-functionalized gold nanoparticles for detection without nucleic acid amplification.

Microorganisms of interest	Sample type	Analysis time	Detection limit	Working range	References
Escherichia coli, *Bacillus cereus*, *Klebsiella pneumoniae*	Culture	~1 h	~10^8 CFU/mL	–	Jiang et al. (2009)
Enterobacter cloacae	β-Lactamase	~35 min	16 fmol/mL of P99 β-lactamase	15–80 fmol/mL	Liu et al. (2010)
Escherichia coli O157:H7 and *Salmonella enterica* serovar Typhimurium	Culture	20 min	10^5 CFU/mL	10^5–10^8 CFU/mL	Wu et al. (2012)
Streptococcus pneumoniae	Culture	~20 h	–	–	Garner et al. (2013)
Staphylococcus aureus, *Achromobacter xylosoxidans*, *Delftia acidovorans*, *Stenotrophomonas maltophilia*	Culture	~5 min	~1.5×10^6 CFU/mL	–	Verma et al. (2014a,b)
HIV-1	Serum	~21 h	10^{-15} g/μL capsid antigen p24	10^{-15}–10^{-18} g/μL	de la Rica and Stevens (2012)

Sample type 'culture' refers to the microorganism being grown in the lab before testing.

studies using non-functionalized gold nanoparticles for detection have been summarized in Table 4.

5.2. Gold nanoparticles functionalized with proteins

Gold nanoparticles are often functionalized with antibodies that can target specific sites on the surface of pathogens. This antibody–antigen association leads to aggregation of gold nanoparticles around the pathogen of interest and can thus generate a colorimetric response (Fig. 2). Another common approach is to use aggregation of antibody-functionalized gold nanoparticles as a labeling method followed by amplification of the signal using the growth of silver or gold around the initial seeds. Finally, these nanoparticles have been widely implemented in an ICS format as a replacement for ELISA.

The aggregation of gold nanoparticles around bacteria has been used for the detection of multi-drug resistant *S.* Typhimurium DT104. The bacterium presents a great challenge in health care because of its persistent survival. The detection was possible by functionalizing 30 nm popcorn-shaped gold nanoparticles with monoclonal M3038 antibody against *S.* Typhimurium DT104. The response was specific to the drug resistant *S.* Typhimurium as compared to other *Salmonella* or *E. coli* strains (Khan et al., 2011).

Colorimetric response from the aggregation of nanoparticles can often have insufficient sensitivity. Thus, the growth of gold or silver is used for signal amplification. This strategy has been deployed for the detection of protozoa and bacteria. The detection of intestinal protozoan *Giardia lamblia* is possible by first separating it from solution using centrifuge filtration (0.45 μm pore size) and then incubating it with a solution of anti-*G. lamblia* antibody-coated 15 nm gold nanoparticles. The unbound gold nanoparticles are removed by centrifuge filtration followed by the addition of a gold growth solution, which changes color depending on the concentration of gold nanoparticles. Since the assay uses centrifugation for concentration, it is possible to implement this assay in large sample volumes (Li et al., 2009). The filtration approach can be combined with magnetic nanoparticles to allow detection in complex media. This approach has been used for the detection of *S. aureus* in milk. Magnetic nanoparticles were first coated with bovine serum albumin (BSA) and then with 10 nm gold nanoparticles. Anti-*S. aureus* antibodies were then adsorbed on the surface of gold nanoparticles. This hybrid system of nanoparticles was incubated with the sample contaminated with bacteria, magnetically separated, and then filtered through a 0.8 μm cellulose acetate membrane. The magnetic separation retained all the nanoparticles and bacteria that were attached to the nanoparticles. The filter retained bacteria and attached nanoparticles while allowing free nanoparticles to pass through. Finally, the color of nanoparticles on the filter was enhanced by a gold growth solution. The specificity of the assay was confirmed by comparing the response to samples contaminated with other pathogenic bacteria (Sung et al., 2013).

In addition to nucleic acid detection, gold label silver staining has also been implemented for antibody-functionalized nanoparticles. This method has been used for the detection of *Campylobacter jejuni* by using monoclonal antibodies against the bacterium and coating them on 18 nm gold nanoparticles. In order to implement this method, a glass slide functionalized with streptavidin is first conjugated with biotinylated polyclonal antibodies against *C. jejuni*. This is followed by the addition of the bacteria and then the functionalized gold nanoparticles. Then, the gold growth solution is added followed by silver enhancement. The silver enhancement is stopped by immersing the slide in deionized water. Using this method, specificity was confirmed by comparing the response obtained from *C. jejuni* to that of *Salmonella enteritidis* and *E. coli* (Cao et al., 2011).

Immobilization of antibodies has also been extended to nitrocellulose paper, which is followed by the addition of the target and then the protein-functionalized gold nanoparticles. This has been used for the detection of Vi antigen of *S.* Typhi by adsorbing anti-*Salmonella* antibodies on 30 nm gold nanoparticles. This assay has a potential of detecting typhoid early because it can not only detect the whole bacterial cell, but also just the Vi antigen (Pandey et al., 2012). Similarly, ICS-based assays have been developed for the detection of *P. aeruginosa* and *S. aureus* by using polyclonal antibodies against the bacteria and conjugating them to ~20 nm gold nanoparticles. The test line in these assays had monoclonal antibodies against the bacteria and produced a red color in the presence of the target bacteria (Li et al., 2011). Another example of ICS is the detection of toxic metabolites produced by the microscopic fungi *Aspergillus*. These metabolites, such as ochratoxin A, can lead to nephrotoxicity, hepatotoxicity, and carcinogenicity in humans. In this scenario, a competitive assay was developed by

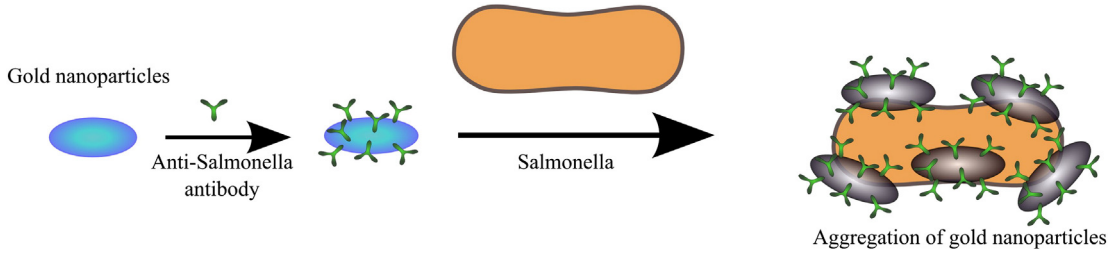

Fig. 2. Gold nanoparticle functionalized with antibodies aggregate around bacteria and lead to color change.
Adapted from Wang et al. (2010).

Table 5
Gold nanoparticles functionalized with proteins.

Microorganisms of interest	Sample type	Analysis time	Detection limit	Working range	References
Campylobacter jejuni	Culture	Overnight	10^6 CFU/mL	10^6–10^9 CFU/mL	Cao et al. (2011)
Salmonella enterica serovar Typhimurium DT104	Culture	<5 min	10^3 CFU/mL	10^3–10^4 CFU/mL	Khan et al. (2011)
Pseudomonas aeruginosa and *Staphylococcus aureus*	Culture	3 min	5×10^2 CFU/mL	5×10^2–5×10^3 CFU/mL	Li et al. (2011)
Salmonella enterica serovar Typhi	Spiked blood	~1 h	10^2 CFU/mL	10^2–10^7 CFU/mL	Pandey et al. (2012)
Escherichia coli	Culture	–	10^2 CFU/mL	10^2–10^6 CFU/mL	Lim et al. (2012)
Staphylococcus aureus	Spiked milk	40 min	1.5×10^7 CFU/mL (milk) 1.5×10^5 CFU/mL (PBS)	1.5×10^7–1.5×10^8 CFU/mL (milk) 1.5×10^5–1.5×10^8 CFU/mL (PBS)	Sung et al. (2013)
Aspergillus	Plant extracts	10 min	5 ng/mL ochratoxin A	5–50 ng/mL	Urusov et al. (2011)
Giardia lamblia cysts	Culture	–	1.088×10^3 cells/mL	10^3–10^4 cells/mL	Li et al. (2009)

Sensitivity and specificity were not reported for any of the studies. Sample type 'culture' refers to the microorganism being grown in the lab before testing.

immobilizing a BSA conjugate of ochratoxin A on the test zone and immobilizing monoclonal antibodies against ochratoxin A on 27 nm gold nanoparticles. In the presence of target ochratoxin A, gold nanoparticles do not bind to the test line and hence there is no color (Urusov et al., 2011).

A unique strategy using switchable linkers has been deployed for detection by functionalizing gold nanoparticles with streptavidin. The switchable linker specifically binds to the target of interest and also contains biotin, which would lead to aggregation of streptavidin-coated gold nanoparticles. Changing the concentration of the target will change the number of free switchable linkers available and hence change the degree of aggregation of gold nanoparticles. If there is a high concentration of the switchable crosslinker, they occupy all the binding sites on the gold nanoparticles and prevent crosslinking. Therefore, there is a specific concentration of crosslinker and target within which the color changes. When biotinylated anti-*E. coli* polyclonal antibodies are used as the switchable crosslinker, *E. coli* can be easily detected at low concentrations (Lim et al., 2012).

Antibody-labeled gold nanoparticles have facilitated the detection of whole cells, which minimizes the efforts required for sample preparation and yet provides faster response compared to culture-based methods. As compared to ELISA, methods employing functionalized gold nanoparticles immobilized on paper substrates (e.g. ICS) are simpler to deploy in the field since the strips can be easily transported and require minimal training. Two major limitations exist for gold nanoparticles functionalized by proteins: the assays often require antibodies for specific targets, which can increase the cost of the assay, and many assays require centrifugation or filtration, which is often only available in laboratories. The studies that utilize gold nanoparticles functionalized with proteins have been summarized in Table 5.

5.3. Gold nanoparticles functionalized with small molecules

Besides proteins and nucleic acids, small molecules can also be used for detection of pathogens by exploiting the electrostatic, covalent, or receptor-mediated interactions. In a typical case, the small molecule is immobilized on gold nanoparticles, which allows their aggregation around the pathogen of interest and hence leads to a color change. Electrostatic interactions have been possible by modifying the surface of nanoparticles to make them cationic. Covalent interactions have been exploited by using phenylboronic acid and its ability to bind to diol groups in bacterial polysaccharides. Receptor-mediated interactions are possible by functionalizing gold nanoparticles with sialic acids, which exhibit binding to haemagglutinin present on the surface of viruses.

Cationic nanoparticles have been used for the detection of lipopolysaccharides and whole cells. Lipopolysaccharides are present on the surface of Gram-negative bacteria and provide a high negative charge to these surfaces. The detection of lipopolysaccharides is important because they can lead to sepsis or septic shock. When gold nanoparticles are modified with cysteamine, they aggregate in the presence of lipopolysaccharides and hence allow their detection as compared to other biological anions. These nanoparticles could also interact with lipopolysaccharides on the surface of *E. coli* 055:B5, which was confirmed by observing their aggregation using transmission electron microscopy (Sun et al., 2012). This modification has also been used for colorimetric detection of *E. coli* O157:H7 (Su et al., 2012). Whole cells can be detected by using cationic gold nanoparticles obtained by using a variety of small molecules with varying alkyl chain lengths and hydrophobicity. This approach was used for detecting *E. coli* XL1. An enzyme (β-galactosidase) is first adsorbed on the gold nanoparticles by electrostatic interactions. Then, in the presence of *E. coli*, gold nanoparticles aggregate around the bacteria and release the enzyme, which catalyzes the hydrolysis of chlorophenol red β-D-galactopyranoside and causes a color change (Miranda et al., 2011).

Covalent interactions have been used for the detection of a variety of bacteria. In one of the studies involving *E. coli* O157:H7, gold nanoparticles were first coated with platinum and then functionalized using 4-mercaptophenylboronic acid. The platinum on the surface of gold nanoparticles acts as a peroxidase mimic and can catalyze oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) by hydrogen peroxide. Thus, when functionalized gold nanoparticles are mixed with *E. coli* O157:H7, they aggregate around the bacteria. After purification by centrifugation, the bound nanoparticles were mixed with hydrogen peroxide and TMB, which led to a color change depending on the concentration of bacteria present. The specificity of this method was shown by demonstrating the lack of response from *S. aureus* (Su et al., 2013). In contrast to this study (Su et al., 2013), another group functionalized 13 nm gold nanoparticles with dithiodialiphatic acid-3-aminophenylboronic acid and achieved the detection of *S. aureus*. In this case, the functionalized gold nanoparticles were allowed to interact with *S. aureus* and then the bacteria were separated by centrifugation. The separated bacteria had a red color characteristic of the gold nanoparticles. The specificity was confirmed by comparing the response from *S. aureus* to that from *E. coli*, *Bacillus subtilis*, and *E. cloacae*. The difference between the two studies is most likely because of the different configurations of phenylboronic acid used and also because of additional functionalization of gold nanoparticle with a pentapeptide for stabilization in the detection of *S. aureus* (Wang et al., 2012a).

In addition to bacteria, influenza viruses can be detected using gold nanoparticles functionalized with sialic acids. Influenza viruses present haemagglutinin on the surface, which recognizes sialic acids on host cells for infecting the cells. Haemagglutinin has been used as a target for detecting viruses because they can facilitate aggregation of functionalized gold nanoparticles. To achieve detection, 16 nm gold nanoparticles were functionalized with trivalent α2,6-thio-linked sialic acid and mixed with human influenza virus X31 (H3N2) to observe a color change. This method was able to distinguish between human influenza virus and avian influenza virus (H5N1) because the human strain binds to α2,6 residues, whereas the avian strain binds to α2,3 residues. Detection was also possible in influenza allantoic fluid, which demonstrates the possibility of detection in clinical samples (Marin et al., 2013). A similar method has been employed for the detection of influenza B/Victoria and influenza B/Yamagata, where 20 nm gold nanoparticles

Please cite this article as: Verma MS, et al, Colorimetric biosensing of pathogens using gold nanoparticles, Biotechnol Adv (2015), http://dx.doi.org/10.1016/j.biotechadv.2015.03.003

Table 6
Gold nanoparticles functionalized with small molecules.

Microorganisms of interest	Sample type	Analysis time	Detection limit	Working range	Small molecule used	References
Escherichia coli XL1	Culture	~10 min	10^2 CFU/mL (solution) 10^4 CFU/mL (test strip)	10^2–10^7 CFU/mL (solution) 10^4–10^8 CFU/mL (test strip)	Several different cationic molecules	Miranda et al. (2011)
Staphylococcus aureus	Spiked milk, urine, lung fluid	~2 h	50 CFU/mL	5×10^2–5×10^6 CFU/mL	Dithiodialiphatic acid-3-aminophenylboronic acid	Wang et al. (2012a)
Escherichia coli O157:H7	Culture	<40 min	7 CFU/mL	$7–6 \times 10^6$ CFU/mL	4-Mercaptophenylboronic acid	Su et al. (2012, 2013)
Escherichia coli 055:B5	Lipopolysaccharides	~5 min	330 fmol/mL lipopolysaccharides	5–90 pmol/mL	Cysteamine	Sun et al. (2012)
Human influenza virus X31 (H3N2)	Allantoic fluid	~30 min	~1 μg/mL virus	~1–2 μg/mL	Trivalent α2,6-thio-linked sialic acid	Marin et al. (2013)
Influenza B/Victoria and Influenza B/Yamagata	Culture	~10 min	0.156 vol.% dilution of hemagglutination assay titer 512 virus	0.156–1.25 vol.%	Sialic acid (N-acetylneuraminic acid)	Lee et al. (2013)

Sensitivity and specificity were not reported for any of the studies. Sample type 'culture' refers to the microorganism being grown in the lab before testing.

were synthesized and stabilized using sialic acid using a one-pot method (Lee et al., 2013).

Gold nanoparticles modified with small molecules have typically provided some of the fastest response times while maintaining excellent detection limits. Small molecules are typically cheaper than proteins or nucleic acids and hence the overall cost of the assay is lower. The major limitation of this approach is that small molecules target general components of the pathogens and hence cross-reactivity is likely. Thus, the assay might provide a false positive response if a closely related pathogen was present instead of the targeted one. All the studies using gold nanoparticles functionalized with small molecules have been summarized in Table 6.

6. Comparison of gold nanoparticles to conventional methods

Conventional and gold nanoparticle-based pathogen detection assays can be compared using a variety of metrics reflecting assay performance. The main criteria by which we will be evaluating the advantages and disadvantages of the previously mentioned assays are time, limit of detection, specificity, technical complexity, and specific limitations. These parameters have been grouped by detection principle, and are summarized in Table 7.

6.1. Analysis time

Analysis times were generally much longer for conventional methods than those using gold nanoparticles. Colony counting was by far the most time-consuming method, due to the need for colonies to be grown on selective media prior to visual identification (de Boer and Beumer, 1999). Of the organisms presented, the longest culture time was reported for *Campylobacter*, where culture methods require 4–9 days for negative results and 14–16 days for positive confirmation (Brooks et al., 2004; Velusamy et al., 2010). In contrast, protein-functionalized gold nanoparticles have been used to detect *Campylobacter* following overnight incubation (Cao et al., 2011).

The fastest conventional methods are typically PCR-based assays, which can deliver results in 5–24 h, depending on the mode of analysis and pathogen of interest (Lazcka et al., 2007). This processing time is heavily dependent on the time required for sample enrichment and nucleic acid amplification, and is related to the detection limit (Che et al., 2001; Ng et al., 1997). Amplification-based techniques with gold nanoparticles can improve upon conventional PCR-based methods by generating rapid color changes in response to pathogens, thereby simplifying the detection of target amplicon, and reducing the time required to obtain a result. Furthermore, emerging biosensors that do not involve the time-consuming step of nucleic acid amplification reported the shortest processing times with several groups reporting results within an hour (Tables 4, 5, and 6).

6.2. Limit of detection

Despite advances in analysis time, reducing detection limits remains a key challenge for gold nanoparticle-based assays. Conventional methods of colony counting and PCR are typically capable of detecting pathogens at concentrations in the range of 1 CFU/mL or 10 copies/μL DNA (Lazcka et al., 2007; Velusamy et al., 2010). Nanoparticle-based methods reported a wide variety of detection limits, ranging from 7 to 10^8 CFU/ml or 10^1 to 3×10^{11} copies/μL DNA depending on the target (Majdinasab et al., 2013; Mollasalehi and Yazdanparast, 2012; Tang et al., 2009). While some groups reported detection limits much higher than those for conventional methods, particularly those assays without target amplification, other nanoparticle-based assays were comparable in terms of detection limit.

Please cite this article as: Verma MS, et al, Colorimetric biosensing of pathogens using gold nanoparticles, Biotechnol Adv (2015), http://dx.doi.org/10.1016/j.biotechadv.2015.03.003

Table 7
Comparing conventional and nanoparticle-based assays.

Category	Detection principle	Analysis time	Detection limit	Specificity	Technical requirements	Limitations	References
Conventional	Colony counting	1–16 d	10^0–10^1 CFU/mL	Good	Basic microbiology lab equipment and training	Only culturable strains are detected	de Boer and Beumer (1999), Brooks et al. (2004)
	Immunological assay	1–5 d	10^3–10^6 CFU/mL	Good	Specific antibodies for pathogen	Sample enrichment is often necessary for high sensitivity	de Boer and Beumer (1999), Lazcka et al. (2007)
	PCR	5–48 h	<10 copies/μL	Excellent	Thermal cycling or isothermal amplification, gel electrophoresis equipment	Distinguishing live and dead cells, presence of inhibitors in complex media	Che et al. (2001), Drosten et al. (2002), Lazcka et al. (2007)
Gold nanoparticle assays for amplified nucleic acids	Non-functionalized	3–8 h	2×10^1–3×10^9 copies/μL DNA	Excellent	Thermal cycling or isothermal amplification equipment	Need to design specific probes for every pathogen of interest	Jung et al. (2010), Liu et al. (2011), Prasad et al. (2011)
	Functionalized (nucleic acid)	1.5–8 h	10^1–3×10^{11} copies/μL DNA	Excellent	Thermal cycling or isothermal amplification equipment	Functionalization requires purification, can affect stability and yield of nanoparticles	Tang et al. (2009), Mollasalehi and Yazdanparast (2012), Majdinasab et al. (2013)
Gold nanoparticle assays for unamplified nucleic acids	Non-functionalized	~30 min	2.5–6×10^7 copies/μL RNA/DNA	Good	Minimal equipment	Need to design specific probes for targets of interest	Shawky et al. (2010), Pylaev et al. (2011)
	Functionalized (nucleic acid)	5 min–2 h	2.2×10^4–1×10^9 copies/μL DNA	Excellent	Basic lab equpiment for nucleic acid extraction	Nucleic acid extraction can consume considerable time compared to assay time	Carter et al. (2013), Kalidasan et al. (2013), Mancuso et al. (2013)
Gold nanoparticle assays for non-nucleic acid targets	Non-functionalized	5 min–21 h	10^5–10^8 CFU/mL	Good	Minimal equipment	Nanoparticle stability in detection media can be limited	Jiang et al. (2009), Wu et al. (2012)
	Functionalized (protein)	3 min–overnight	10^2–1.5×10^7 CFU/mL	Good	Often need filtration or centrifugation equipment	Throughput limited by filtration/centrifugation	Cao et al. (2011), Li et al. (2011), Pandey et al. (2012), Sung et al. (2013)
	Functionalized (small molecule)	5 min–2 h	7–10^2 CFU/mL	Poor	Minimal equipment	Cross-reactivity is often present	Miranda et al. (2011), Su et al. (2013)

6.3. Specificity

Specificity of colony counting methods is dependent on the ability to selectively isolate and culture particular pathogen strains. Due to the use of morphological and physiological characteristics for pathogen identification, specificity may be lower for closely related strains which are less distinguishable based on phenotypic traits. Similarly, immunological assays may suffer from low specificity if antibodies are selected for target analytes that are present on more than one pathogen variety. However, with proper antibody selection and species enrichment, immunological assays have good specificity. PCR-based methods achieve specificity by targeting nucleic acid sequences with selected primers and/or probes. Excellent assay specificity can be achieved when the sequences targeted by PCR are unique to the strain of interest since single base pair mismatches can often be discriminated.

Specificity of gold nanoparticle-based assays is determined by either nucleic acids or antibodies in most cases. Thus, the specificity of these assays is comparable to the methods based on PCR and immunological assays. In the case of small molecule modified nanoparticles and non-functionalized nanoparticles, the assays detect general targets and hence, the specificity suffers. One method for overcoming this specificity challenge is to adopt a "chemical nose" type system, where each analyte presents a unique set of responses and hence can be distinguished (Miranda et al., 2011; Verma et al., 2014b). The limitation of a "chemical nose" approach is that the system needs to be trained for each analyte of interest before attempting the detection.

6.4. Technical requirements

Procedures for bacterial plating, colony counting, and species identification vary according to the target organism. Generally, the first step involves serial dilution of a sample or automatic plating (de Boer and Beumer, 1999) onto agar plates with selective media. Plates must then be incubated to allow for colony growth to a visually detectable level. This incubation period is dependent on the bacterial species and growth conditions. The number of resulting colonies is counted to infer pathogen concentration in the original sample. This is a time consuming step which can be done by hand or using automated systems (de Boer and Beumer, 1999). Pathogen identity is determined using various morphological and biochemical tests. The colony counting method is good for workers in microbiology laboratories due to its reliability and use of common laboratory equipment and reagents, however the laborious process is not adequate for rapid diagnostics and requires specialized training.

Immunological assays rely primarily on specificity of antibodies to antigens from the target pathogen. A wide variety of characterized antibodies and kits are available for most pathogens and complexity is dependent on the particular detection strategy (de Boer and Beumer, 1999). While common immunological methods (e.g., ELISA) do not require specialized lab equipment, they typically require some form of sample enrichment due to decreased sensitivity (Brooks et al., 2004).

PCR-based methods are typically less laborious and time-consuming than previously mentioned conventional methods (Lazcka et al., 2007). Specific DNA or RNA sequences amplified using PCR can be subsequently visualized using a number of ways, depending on the type of PCR. The most common methods are sample separation using gel-electrophoresis and fluorescence observation during real-time PCR with probes. Primer and probe selection is dependent on the target pathogen being investigated. While traditional PCR-based methods require access to a thermal cycler, advances in lab-on-a-chip and isothermal amplification techniques are reducing this barrier to out-of-laboratory field applications.

Some of the main aims of nanoparticle assays are to simplify assay procedure, reduce the need for complex lab equipment, and minimize labor. Nucleic acid amplification-based techniques require either thermal cycling or isothermal amplification equipment which is a significant issue for point-of-care or field applications. However, emerging amplification-free techniques require only basic laboratory equipment. In these cases, the primary technical requirement remains the ability to extract and purify the target analyte (i.e., nucleic acids, proteins, or whole cells) from the sample.

7. Conclusions

Gold nanoparticles with a variety of surface features have been used for the colorimetric detection of pathogens either by detecting nucleic acids, surface proteins, or whole cells. While the majority of the literature has focused on the use of gold nanoparticles as a replacement for gel electrophoresis after nucleic acid amplification, there is a growing body of work in detecting unamplified targets. There is a growing drive towards developing methods or devices that could be used at the point-of-care or in the field by providing a simple visual output. Overall, although gold nanoparticles have facilitated the development of simple and sensitive assays that are replacing conventional methods of pathogen detection, current technologies are not yet ready to be translated directly to the point-of-care or field use because the current methods require extensive sample processing before analysis. Additionally, current biosensors with gold nanoparticles suffer from lower sensitivity when complex media are involved because of non-specific adsorption, which can be mitigated in the future by modifying the surface of gold nanoparticles with non-fouling coatings.

In order to bring gold nanoparticles to the point-of-care or field use, it will be necessary to simplify the detection process and make it user friendly. A few possibilities exist for achieving this goal: the use of strip-based detection, cellphone-based detectors, and disposable microfluidic chips. Strip-based sensors can be cheap and easy to use, where the color of the strip is compared to a reference. Some of these strategies have already been highlighted in this review, but in order to broaden the scope of detection, we recommend the use of multiple shapes and sizes of gold nanoparticles such that a unique response could be obtained for each pathogen of interest. This color output could be combined with the use of powerful cellphones for colorimetric analysis. Cellphone spectrometers are currently under development (Smith et al., 2011) and could be used in the field for sensitive assays. Since cellphones are widely used, such spectrometers could be easily adapted by the general public. Another possibility of portable detectors includes the use of disposable microfluidic devices such that all components of detection are included within the chip. This is possible by incorporating gold nanoparticles in paper-based microfluidic devices (Liana et al., 2012) such that their sensitivity and accuracy is improved. Reducing assay time and simplifying the biosensors for direct use in complex media is the most promising avenue for future research. The optimal biosensor will be designed to be simple such that the user can obtain results with the push of a single button.

Abbreviations

BSA	bovine serum albumin
CTAB	cetyltrimethylammonium bromide
DAB	3,3′-diaminobenzidine
DNA	deoxyribonucleic acid
DNAzymes	deoxyribonucleic acid enzymes
dsDNA	double-stranded deoxyribonucleic acid
ELISA	enzyme linked immunosorbent assay
EPA	United States Environmental Protection Agency
FDA	United States Food and Drug Administration
HBV	hepatitis B virus
HIV-1	human immunodeficiency virus type-1
HPV	human papillomavirus
HPV-16	human papillomavirus type 16
HPV-18	Human papillomavirus type 18
HRP	horseradish peroxidase

Please cite this article as: Verma MS, et al, Colorimetric biosensing of pathogens using gold nanoparticles, Biotechnol Adv (2015), http://dx.doi.org/10.1016/j.biotechadv.2015.03.003

ICS	immunochromatographic strip
IgA1P	immunoglobulin A1 protease
KSHV	Karposi's sarcoma-associated herpes virus
MNAzyme	multicomponent nucleic acid enzyme
mRNA	messenger ribonucleic acid
MRSA	methicillin-resistant *Staphylococcus aureus*
NASBA	nucleic acid sequence-based amplification
PCR	polymerase chain reaction
RCA	rolling circle amplification
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
ssDNA	single-stranded deoxyribonucleic acid
TMB	3,3′,5,5′-tetramethylbenzidine

Acknowledgments

This work was financially supported by the Natural Sciences and Engineering Research Council of Canada (NSERC). M.S.V. is grateful for the M. Moo-Young Biochemical Engineering Scholarship and the NSERC Vanier Canada Graduate Scholarship. J.L.R. is grateful for the Waterloo Institute for Nanotechnology (WIN) Nanofellowship.

References

Agasti SS, Rana S, Park MH, Kim CK, You CC, Rotello VM. Nanoparticles for detection and diagnosis. Adv Drug Deliv Rev 2010;62:316–28.
Ahmed A, Rushworth JV, Hirst NA, Millner PA. Biosensors for whole-cell bacterial detection. Clin Microbiol Rev 2014;27:631–46.
Azzazy HM, Mansour MM, Samir TM, Franco R. Gold nanoparticles in the clinical laboratory: principles of preparation and applications. Clin Chem Lab Med 2012;50:193–209.
Bakthavathsalam P, Rajendran VK, Ali BMJ. A direct detection of *Escherichia coli* genomic DNA using gold nanoprobes. J Nanobiotechnol 2012;10:8. [3155-10-8].
Boisselier E, Astruc D. Gold nanoparticles in nanomedicine: preparations, imaging, diagnostics, therapies and toxicity. Chem Soc Rev 2009;38:1759–82.
Brooks BW, Devenish J, Lutze-Wallace CL, Milnes D, Robertson RH, Berlie-Surujballi G. Evaluation of a monoclonal antibody-based enzyme-linked immunosorbent assay for detection of *Campylobacter fetus* in bovine preputial washing and vaginal mucus samples. Vet Microbiol 2004;103:77–84.
Bunz UH, Rotello VM. Gold nanoparticle–fluorophore complexes: sensitive and discerning "noses" for biosystems sensing. Angew Chem Int Ed Engl 2010;49:3268–79.
Cao C, Gontard LC, Thuy Tram le L, Wolff A, Bang DD. Dual enlargement of gold nanoparticles: from mechanism to scanometric detection of pathogenic bacteria. Small 2011;7:1701–8.
Carter JR, Balaraman V, Kucharski CA, Fraser TS, Fraser Jr MJ. A novel dengue virus detection method that couples DNAzyme and gold nanoparticle approaches. Virol J 2013;10:201. [422X-10-201].
Cenciarini-Borde C, Courtois S, La Scola B. Nucleic acids as viability markers for bacteria detection using molecular tools. Future Microbiol 2009;4:45–64.
Chan WS, Tang BS, Boost MV, Chow C, Leung PH. Detection of methicillin-resistant *Staphylococcus aureus* using a gold nanoparticle-based colourimetric polymerase chain reaction assay. Biosens Bioelectron 2014;53:105–11.
Che Y, Li Y, Slavik M. Detection of *Campylobacter jejuni* in poultry samples using an enzyme-linked immunoassay coupled with an enzyme electrode. Biosens Bioelectron 2001;16:791–7.
Chen SH, Lin KI, Tang CY, Peng SL, Chuang YC, Lin YR, et al. Optical detection of human papillomavirus type 16 and type 18 by sequence sandwich hybridization with oligonucleotide-functionalized Au nanoparticles. IEEE Trans Nanobioscience 2009;8:120–31.
Costa P, Amaro A, Botelho A, Inacio J, Baptista PV. Gold nanoprobe assay for the identification of mycobacteria of the *Mycobacterium tuberculosis* complex. Clin Microbiol Infect 2010;16:1464–9.
Daniel MC, Astruc D. Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. Chem Rev 2004;104:293–346.
de Boer E, Beumer RR. Methodology for detection and typing of foodborne microorganisms. Int J Food Microbiol 1999;50:119–30.
de la Rica R, Stevens MM. Plasmonic ELISA for the ultrasensitive detection of disease biomarkers with the naked eye. Nat Nanotechnol 2012;7:821–4.
Deng H, Zhang X, Kumar A, Zou G, Zhang X, Liang XJ. Long genomic DNA amplicons adsorption onto unmodified gold nanoparticles for colorimetric detection of *Bacillus anthracis*. Chem Commun (Camb) 2013;49:51–3.
Drosten C, Gottig S, Schilling S, Asper M, Panning M, Schmitz H, et al. Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR. J Clin Microbiol 2002;40:2323–30.
Eghtedari M, Oraevsky A, Copland JA, Kotov NA, Conjusteau A, Motamedi M. High sensitivity of *in vivo* detection of gold nanorods using a laser optoacoustic imaging system. Nano Lett 2007;7:1914–8.
Farber JM, Peterkin PI. *Listeria monocytogenes*, a food-borne pathogen. Microbiol Rev 1991;55:476–511.
Fu Z, Zhou X, Xing D. Rapid colorimetric gene-sensing of food pathogenic bacteria using biomodification-free gold nanoparticle. Sensors Actuators B Chem 2013;182:633–41.
Garner AL, Fullagar JL, Day JA, Cohen SM, Janda KD. Development of a high-throughput screen and its use in the discovery of *Streptococcus pneumoniae* immunoglobulin A1 protease inhibitors. J Am Chem Soc 2013;135:10014–7.
Ghosh P, Han G, De M, Kim CK, Rotello VM. Gold nanoparticles in delivery applications. Adv Drug Deliv Rev 2008;60:1307–15.
Gill P, Alvandi AH, Abdul-Tehrani H, Sadeghizadeh M. Colorimetric detection of *Helicobacter pylori* DNA using isothermal helicase-dependent amplification and gold nanoparticle probes. Diagn Microbiol Infect Dis 2008;62:119–24.
Gobin AM, Lee MH, Halas NJ, James WD, Drezek RA, West JL. Near-infrared resonant nanoshells for combined optical imaging and photothermal cancer therapy. Nano Lett 2007;7:1929–34.
He W, Huang CZ, Li YF, Xie JP, Yang RG, Zhou PF, et al. 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. Anal Chem 2008;80:8424–30.
Hitchins AD, Jinneman K. Detection and enumeration of *Listeria monocytogenes* in foods. Anonymous bacteriological analytical manual. U.S. Food and Drug Administration; 1998.
Huang X, El-Sayed IH, Qian W, El-Sayed MA. Cancer cell imaging and photothermal therapy in the near-infrared region by using gold nanorods. J Am Chem Soc 2006;128:2115–20.
Hutter E, Fendler J. Exploitation of localized surface plasmon resonance. Adv Mater 2004;16:1685–706.
Jain PK, El-Sayed IH, El-Sayed MA. Au nanoparticles target cancer. Nano Today 2007;2:18–29.
Jiang T, Liu R, Huang X, Feng H, Teo W, Xing B. Colorimetric screening of bacterial enzyme activity and inhibition based on the aggregation of gold nanoparticles. Chem Commun (Camb) 2009(15):1972–4. [doi:1972-4].
Jung YL, Jung C, Parab H, Li T, Park HG. Direct colorimetric diagnosis of pathogen infections by utilizing thiol-labeled PCR primers and unmodified gold nanoparticles. Biosens Bioelectron 2010;25:1941–6.
Jyoti A, Pandey P, Singh SP, Jain SK, Shanker R. Colorimetric detection of nucleic acid signature of shiga toxin producing *Escherichia coli* using gold nanoparticles. J Nanosci Nanotechnol 2010;10:4154–8.
Kaittanis C, Santra S, Perez JM. Emerging nanotechnology-based strategies for the identification of microbial pathogenesis. Adv Drug Deliv Rev 2010;62:408–23.
Kalidasan K, Neo JL, Uttamchandani M. Direct visual detection of *Salmonella* genomic DNA using gold nanoparticles. Mol Biosyst 2013;9:618–21.
Khan SA, Singh AK, Senapati D, Fan Z, Ray PC. Targeted highly sensitive detection of multidrug resistant *Salmonella* DT104 using gold nanoparticles. Chem Commun (Camb) 2011;47:9444–6.
Khanna VK. Nanoparticle-based sensors. Def Sci J 2008;58:608–16.
Kim JY, Lee JS. Synthesis and thermally reversible assembly of DNA-gold nanoparticle cluster conjugates. Nano Lett 2009;9:4564–9.
Kim YT, Chen Y, Choi JY, Kim WJ, Dae HM, Jung J, et al. Integrated microdevice of reverse transcription-polymerase chain reaction with colorimetric immunochromatographic detection for rapid gene expression analysis of influenza A H1N1 virus. Biosens Bioelectron 2012;33:88–94.
Lauri A, Mariani PO. Potentials and limitations of molecular diagnostic methods in food safety. Genes Nutr 2009;4:1–12.
Lazcka O, Del Campo FJ, Munoz FX. Pathogen detection: a perspective of traditional methods and biosensors. Biosens Bioelectron 2007;22:1205–17.
Lee C, Gaston MA, Weiss AA, Zhang P. Colorimetric viral detection based on sialic acid stabilized gold nanoparticles. Biosens Bioelectron 2013;42:236–41.
Li XX, Cao C, Han SJ, Sim SJ. Detection of pathogen based on the catalytic growth of gold nanocrystals. Water Res 2009;43:1425–31.
Li CZ, Vandenberg K, Prabhulkar S, Zhu X, Schneper L, Methee K, et al. Paper based point-of-care testing disc for multiplex whole cell bacteria analysis. Biosens Bioelectron 2011;26:4342–8.
Liana DD, Raguse B, Gooding JJ, Chow E. Recent advances in paper-based sensors. Sensors (Basel) 2012;12:11505–26.
Liandris E, Gazouli M, Andreadou M, Comor M, Abazovic N, Sechi LA, et al. Direct detection of unamplified DNA from pathogenic mycobacteria using DNA-derivatized gold nanoparticles. J Microbiol Methods 2009;78:260–4.
Lim S, Koo OK, You YS, Lee YE, Kim MS, Chang PS, et al. Enhancing nanoparticle-based visible detection by controlling the extent of aggregation. Sci Rep 2012;2:456.
Liu J, Lu Y. A colorimetric lead biosensor using DNAzyme-directed assembly of gold nanoparticles. J Am Chem Soc 2003;125:6642–3.
Liu R, Teo W, Tan S, Feng H, Padmanabhan P, Xing B. Metallic nanoparticles bioassay for *Enterobacter cloacae* P99 beta-lactamase activity and inhibitor screening. Analyst 2010;135:1031–6.
Liu M, Yuan M, Lou X, Mao H, Zheng D, Zou R, et al. Label-free optical detection of single-base mismatches by the combination of nuclease and gold nanoparticles. Biosens Bioelectron 2011;26:4294–300.
Majdinasab M, Aminlari M, Sheikhi MH, Niakousari M, Shekarforoosh S. Detection of inv A gene of *Salmonella* by DNA-gold nanoparticles biosensor and its comparison with PCR. J Exp Nanosci 2013;8:223–39.
Mancuso M, Jiang L, Cesarman E, Erickson D. Multiplexed colorimetric detection of Kaposi's sarcoma associated herpesvirus and *Bartonella* DNA using gold and silver nanoparticles. Nanoscale 2013;5:1678–86.

Please cite this article as: Verma MS, et al, Colorimetric biosensing of pathogens using gold nanoparticles, Biotechnol Adv (2015), http://dx.doi.org/10.1016/j.biotechadv.2015.03.003

Mao X, Ma Y, Zhang A, Zhang L, Zeng L, Liu G. Disposable nucleic acid biosensors based on gold nanoparticle probes and lateral flow strip. Anal Chem 2009;81:1660–8.

Marin MJ, Rashid A, Rejzek M, Fairhurst SA, Wharton SA, Martin SR, et al. Glyconanoparticles for the plasmonic detection and discrimination between human and avian influenza virus. Org Biomol Chem 2013;11:7101–7.

Mayer KM, Hafner JH. Localized surface plasmon resonance sensors. Chem Rev 2011;111: 3828–57.

Miranda OR, Li X, Garcia-Gonzalez L, Zhu ZJ, Yan B, Bunz UH, et al. Colorimetric bacteria sensing using a supramolecular enzyme-nanoparticle biosensor. J Am Chem Soc 2011;133:9650–3.

Mollasalehi H, Yazdanparast R. Non-crosslinking gold nanoprobes for detection of nucleic acid sequence-based amplification products. Anal Biochem 2012;425:91–5.

Monis PT, Giglio S. Nucleic acid amplification-based techniques for pathogen detection and identification. Infect Genet Evol 2006;6:2–12.

Murphy CJ, Gole AM, Stone JW, Sisco PN, Alkilany AM, Goldsmith EC, et al. Gold nanoparticles in biology: beyond toxicity to cellular imaging. Acc Chem Res 2008;41:1721–30.

Nagatani N, Yamanaka K, Ushijima H, Koketsu R, Sasaki T, Ikuta K, et al. Detection of influenza virus using a lateral flow immunoassay for amplified DNA by a microfluidic RT-PCR chip. Analyst 2012;137:3422–6.

Nath N, Chilkoti A. A colorimetric gold nanoparticle biosensor: effect of particle size on sensitivity. Second joint EMBS–BMES conference 2002, vols. 1–3, conference proceedings: bioengineering — integrative methodologies. New Technologies; 2002. p. 574–5.

Ng LK, Kingombe CI, Yan W, Taylor DE, Hiratsuka K, Malik N, et al. Specific detection and confirmation of *Campylobacter jejuni* by DNA hybridization and PCR. Appl Environ Microbiol 1997;63:4558–63.

Niazi A, Jorjani ON, Nikbakht H, Gill P. A nanodiagnostic colorimetric assay for 18S rRNA of *Leishmania* pathogens using nucleic acid sequence-based amplification and gold nanorods. Mol Diagn Ther 2013;17:363–70.

Paciotti GF, Myer L, Weinreich D, Goia D, Pavel N, McLaughlin RE, et al. Colloidal gold: a novel nanoparticle vector for tumor directed drug delivery. Drug Deliv 2004;11:169–83.

Pandey SK, Suri CR, Chaudhry M, Tiwari RP, Rishi P. A gold nanoparticles based immunobioprobe for detection of Vi capsular polysaccharide of *Salmonella enterica* serovar Typhi. Mol Biosyst 2012;8:1853–60.

Peters RP, van Agtmael MA, Danner SA, Savelkoul PH, Vandenbroucke-Grauls CM. New developments in the diagnosis of bloodstream infections. Lancet Infect Dis 2004;4: 751–60.

Popovtzer R, Agrawal A, Kotov NA, Popovtzer A, Balter J, Carey TE, et al. Targeted gold nanoparticles enable molecular CT imaging of cancer. Nano Lett 2008;8:4593–6.

Prasad D, Shankaracharya, Vidyarthi AS. Gold nanoparticles-based colorimetric assay for rapid detection of *Salmonella* species in food samples. World J Microbiol Biotechnol 2011;27:2227–30.

Pylaev TE, Khanadeev VA, Khlebtsov BN, Dykman LA, Bogatyrev VA, Khlebtsov NG. Colorimetric and dynamic light scattering detection of DNA sequences by using positively charged gold nanospheres: a comparative study with gold nanorods. Nanotechnology 2011;22:285501. [4484/22/28/285501. Epub 2011 May 31].

Qi H, Chen S, Hao R, Shi H, Zhang M, Wang S. Introduction of nanogold-DAB as a HRP substrate for simplifying detection in visual DNA microarrays. Anal Methods 2012;4: 1178–81.

Renuart I, Mertens P, Leclipteux T. One step oligochromatographic device and method of use; 2011 [US 10/555,154].

Saha K, Agasti SS, Kim C, Li X, Rotello VM. Gold nanoparticles in chemical and biological sensing. Chem Rev 2012;112:2739–79.

Saleh M, Soliman H, Schachner O, El-Matbouli M. Direct detection of unamplified spring viraemia of carp virus RNA using unmodified gold nanoparticles. Dis Aquat Organ 2012;100:3–10.

Shawky SM, Bald D, Azzazy HM. Direct detection of unamplified hepatitis C virus RNA using unmodified gold nanoparticles. Clin Biochem 2010;43:1163–8.

Skottrup PD, Nicolaisen M, Justesen AF. Towards on-site pathogen detection using antibody-based sensors. Biosens Bioelectron 2008;24:339–48.

Smith ZJ, Chu K, Espenson AR, Rahimzadeh M, Gryshuk A, Molinaro M, et al. Cell-phone-based platform for biomedical device development and education applications. PLoS One 2011;6:e17150.

Su H, Ma Q, Shang K, Liu T, Yin H, Ai S. Gold nanoparticles as colorimetric sensor: a case study on *E. coli* O157:H7 as a model for Gram-negative bacteria. Sensors Actuators B Chem 2012;161:298–303.

Su H, Zhao H, Qiao F, Chen L, Duan R, Ai S. Colorimetric detection of *Escherichia coli* O157: H7 using functionalized Au@Pt nanoparticles as peroxidase mimetics. Analyst 2013; 138:3026–31.

Sun J, Ge J, Liu W, Wang X, Fan Z, Zhao W, et al. A facile assay for direct colorimetric visualization of lipopolysaccharides at low nanomolar level. Nano Res 2012;5:486–93.

Sung YJ, Suk HJ, Sung HY, Li T, Poo H, Kim MG. Novel antibody/gold nanoparticle/magnetic nanoparticle nanocomposites for immunomagnetic separation and rapid colorimetric detection of *Staphylococcus aureus* in milk. Biosens Bioelectron 2013;43: 432–9.

Tallury P, Malhotra A, Byrne LM, Santra S. Nanobioimaging and sensing of infectious diseases. Adv Drug Deliv Rev 2010;62:424–37.

Tang J, Zhou L, Gao W, Cao X, Wang Y. Visual DNA microarrays for simultaneous detection of human immunodeficiency virus type-1 and *Treponema pallidum* coupled with multiplex asymmetric polymerase chain reaction. Diagn Microbiol Infect Dis 2009; 65:372–8.

Taton TA, Mirkin CA, Letsinger RL. Scanometric DNA array detection with nanoparticle probes. Science 2000;289:1757–60.

U.S. Food and Drug Administration. Fish and fishery products hazards and controls guidance; 2011.

Upadhyayula VK. Functionalized gold nanoparticle supported sensory mechanisms applied in detection of chemical and biological threat agents: a review. Anal Chim Acta 2012;715:1–18.

Urusov AE, Kostenko SN, Sveshnikov PG, Zherdev AV, Dzantiev BB. Immunochromatographic assay for the detection of ochratoxin A. J Anal Chem 2011;66: 770–6.

Veigas B, Machado D, Perdigao J, Portugal I, Couto I, Viveiros M, et al. Au-nanoprobes for detection of SNPs associated with antibiotic resistance in *Mycobacterium tuberculosis*. Nanotechnology 2010;21:415101. [4484/21/41/415101. Epub 2010 Sep 16].

Veigas B, Jacob JM, Costa MN, Santos DS, Viveiros M, Inacio J, et al. Gold on paper-paper platform for Au-nanoprobe TB detection. Lab Chip 2012;12:4802–8.

Velusamy V, Arshak K, Korostynska O, Oliwa K, Adley C. An overview of foodborne pathogen detection: in the perspective of biosensors. Biotechnol Adv 2010;28:232–54.

Verma MS, Chen PZ, Jones L, Gu FX. Branching and size of CTAB-coated gold nanostars control the colorimetric detection of bacteria. RSC Adv 2014a;4:10660–8.

Verma MS, Chen PZ, Jones L, Gu FX. "Chemical nose" for the visual identification of emerging ocular pathogens using gold nanostars. Biosens Bioelectron 2014b;61:386–90.

Wang C, Chen Y, Wang T, Ma Z, Su Z. Biorecognition-driven self-assembly of gold nanorods: a rapid and sensitive approach toward antibody sensing. Chem Mater 2007; 19:5809–11.

Wang S, Singh AK, Senapati D, Neely A, Yu H, Ray PC. Rapid colorimetric identification and targeted photothermal lysis of *Salmonella* bacteria by using bioconjugated oval-shaped gold nanoparticles. Chemistry 2010;16:5600–6.

Wang J, Gao J, Liu D, Han D, Wang Z. Phenylboronic acid functionalized gold nanoparticles for highly sensitive detection of *Staphylococcus aureus*. Nanoscale 2012a;4:451–4.

Wang X, Li Y, Wang J, Wang Q, Xu L, Du J, et al. A broad-range method to detect genomic DNA of multiple pathogenic bacteria based on the aggregation strategy of gold nanorods. Analyst 2012b;137:4267–73.

Willets KA, Van Duyne RP. Localized surface plasmon resonance spectroscopy and sensing. Annu Rev Phys Chem 2007;58:267–97.

Wu WH, Li M, Wang Y, Ouyang HX, Wang L, Li CX, et al. Aptasensors for rapid detection of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium. Nanoscale Res Lett 2012;7: 658. [276X-7-658].

Xing Y, Wang P, Zang Y, Ge Y, Jin Q, Zhao J, et al. A colorimetric method for H1N1 DNA detection using rolling circle amplification. Analyst 2013;138:3457–62.

Yaron S, Matthews KR. A reverse transcriptase-polymerase chain reaction assay for detection of viable *Escherichia coli* O157:H7: investigation of specific target genes. J Appl Microbiol 2002;92:633–40.

Yeh C, Chang Y, Lin H, Chang T, Lin Y. A newly developed optical biochip for bacteria detection hybridization. Sensors Actuators B Chem 2012;161:1168–75.

Zagorovsky K, Chan WC. A plasmonic DNAzyme strategy for point-of-care genetic detection of infectious pathogens. Angew Chem Int Ed Engl 2013;52:3168–71.

Zhao W, Brook MA, Li Y. Design of gold nanoparticle-based colorimetric biosensing assays. Chembiochem 2008;9:2363–71.

Please cite this article as: Verma MS, et al, Colorimetric biosensing of pathogens using gold nanoparticles, Biotechnol Adv (2015), http://dx.doi.org/10.1016/j.biotechadv.2015.03.003