Gold nanoparticle-based colorimetric platform technology as rapid and efficient bacterial pathogens detection method from various sources

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Gold nanoparticle-based colorimetric platform technology as rapid and efficient bacterial pathogens detection method from various sources

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Rapid, sensitive, and reliable bacterial pathogens detection is a chief requirement. The gold nanoparticles (AuNPs) have numerous applications such as in the detection of biomolecules for their high surface to volume ratio and unique optical property facilitating development of highly efficient AuNPs-based bio-sensing tools. Although various molecular detection methods, such as PCR, real-time PCR, and loop-mediated isothermal amplification are sensitive and convenient, these techniques need elaborate work and require special skills to increase their specificity. Smartly fabricated gold nanoparticle (GNPs) play a role as probes for selective detection of pathogens. The AuNPs-based colorimetric methods have become applicable for rapid, simple, reliable and high-efficient, sensitive, inexpensive, and easy detection of the DNA, RNA, and protein biomolecules. Colorimetric detection using AuNPs has been used for rapid and high precision and multiplex detection of a large number and of bacterial pathogens. AuNPs act in functionalized and unfunctionalized ways. AuNPs-based colorimetric methods have incredible advantages compared with many other bacterial detection methods. In spite of many molecular techniques, AuNPs-based colorimetric methods do not require additional devices, fabrication cost, signal processing and interpretation complexities, and costly and complex instruments. This simple and rapid method is suitable, especially in low-income areas and for large number of samples analysis. In this review, applications of AuNPs and AuNPs-based colorimetric methods for bacterial pathogens detection have been overviewed.

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Context

Although various molecular methods are applicable for bacterial detection, they are laborious, time-consuming, and suffer from complexities and also require elaborated techniques to increase their specificity [1–3]. In addition, loop-mediated isothermal amplification (LAMP) is a more rapid method, but labor-working in primer designing which detects target DNA in less than 1 h. On the other hand, microfluidic systems have several drawbacks and need costly and time-consuming fabrication processes. A point of care colorimetric diagnostic device could detect 30 CFU/ml of *Escherichia coli* and 200 CFU/ml of *Staphylococcus aureus* in less than 1 h [4]. Nanoparticles have unique properties such as physical strength, chemical reactivity, electrical conductivity,

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magnetism, and optical properties thanks for their small (10–100 nm) sizes [5–7]. Nanomaterials, when combined with biological and molecular tools, exhibit many potential applications in biotechnological and bioanalytical applications; therefore, nanomaterials have many applications in bioassay, biological sensors, drug delivery, and nanoscale design [5,8,9]. In recent years, great progress has been made to make nanoparticles of different materials and tough control over their size, composition and uniformity [9]. Determination of the specific properties of nanoparticles is essential for control of their synthesis and application. Gold nanoparticles (AuNPs) have many beneficial properties for high efficient bacterial detection purposes with high classification accuracy. AuNPs-based colorimetric detection of bacterial species have been demonstrated in case of *Salmonella* spp, *Helicobacter pylori* DNA (with a sensitivity of 92.5% and specificity of 95.4%) and *Mycobacterium tuberculosis* (with 94.7% sensitivity in less than 1 h) [7,10–12]. In contrast to many other techniques, colorimetric detection does not require additional complexities such as reader. A number of colorimetric dyes such as SYBR GREEN, hydroxy naphthol blue, calcein, propodium iodide have been used to this aim. Functionalized AuNPs-based biosensors can incredibly reduce bacterial incubation time which is critical for slow-growing pathogens and need no amplification time of PCR and RT-PCR [13].

Gold nanoparticles

The nanoscale gold production makes many changes in its physical and chemical properties. AuNPs maintain their activity at low temperatures, which has the advantage of being a catalyst for chemical reactions and contamination control. AuNPs have been used extensively in the diagnosis of microorganisms in the treatment of cancer, as well as in the transfer of drugs and genes [5,6,9,14].

These nanoparticles are synthesized in various sizes and in spherical, diamond, crystalline, triangular, and spiral shapes [9]. These particles have different properties due to their nanoscale size and their physical and chemical properties, including electrical, electrochemical, optical and magnetic properties, compared with golden metal in larger sizes. These features include high stability, heat resistance, unique individual optical properties, and high ability to absorb and release light [9,15]. Furthermore, they are used in the context of contamination control, such as air purification and respiratory masks [15]. In addition, due to the high activity of AuNPs and their tendency to bind to biomolecules and coarse molecules, several applications can be considered for them. It is also possible to use AuNPs and attach them to peptides, proteins, DNA and biochemical polymers, complexes and new materials (fusion) that can be used to diagnose various diseases and other medical applications [5].

The best-sized nanoparticles in the medical applications range from 10 to 100 nm, where those smaller than 10 nm are released through the kidneys, and those larger than 200 nm, will be eliminated by the body's defense system as a foreign material or antigen [16–18]. AuNPs have the highest optical absorption among known nanoparticles, and their absorption spectra can be set at any desired wavelength in the near-infrared domain. Strong optical absorption properties of nanoparticles make them as intermediates for raising the temperature, so that these nanoparticles absorb light energy and convert it into thermal energy. Studies have shown that AuNPs-based biosensors are nontoxic for host cells and can detect various pathogens rapidly [19,20].

Applications of gold nanoparticle-based biosensors

The AuNPs has numerous applications such as in the detection of various biomolecules, high resolution live cells imaging, in photoacoustic imaging and accurate determination of bacterial pathogens [21–24]. AuNPs in different sizes have different curvatures and differential binding interactions leading to different color shifts due to the aggregation of AuNPs. In addition, microorganisms have differential bindings to surface charged AuNPs, allowing their differentiation [25]. The positively charged polyethyleneimine-coated gold nanoparticles (PEI-AuNPs) has been used for colorimetric detection of bacterial pathogens (negatively charged) via hydrolysis of the chromogenic substrate chlorophenol red β-D-galactopyranoside (CPRG). This method could detect gram-positive and gram-negative pathogens *E. coli* and *S. aureus* as low as 10 CFU/ml within 10 min using an optical reader and 2–3 h with naked eye [26]. It is noteworthy that in Xizhe' study, a colorimetric method using AuNPs could identify 15 microorganisms based on diverse surface charges within 5 s with naked eye [27].

Common methods of DNA detection are mainly based on PCR and RT-PCR which need more than 1 h time, elaborate works to increase specificity, and require expensive and complex equipment detecting low numbers of bacterial cells within several hours [11]. On the other, proteins detection by use of enzyme-linked immunosorbent assay (ELISA) technique requires large amounts of proteins and has low sensitivity and specificity [11]. Thus, there is an unmet demand to develop more accurate platforms overcoming this problems [11,28–30]. Over the past decade, many improvements have been made in the use of nanoscale methods for molecular detection, so that most efforts have been focused on designing bioassays for accurate, sensitive, selective, and applied biomolecule detection [12]. AuNPs increase the sensitivity and rapidity of pathogens detection with large number of samples both *in vivo* and *in vitro* [12]. In a study by Marisca *et al.*, collagen-coated AuNPs showed lower toxicity and higher uptake rate compared with polymer-coated AuNPs [31].

AuNPs-based platform technologies have been developed as rapid, ultra-sensitive, low-cost, and efficient technologies for bacterial identification in less than 2 h. Antibodies and aptamers are commonly used to detect specific proteins and DNA sequences, respectively [32,33].

DNA detection using gold nanoparticles

In this method, the DNA sequence of interest is prepared and its complementary sequence is synthesized and activated on the AuNPs [10]. Furthermore, another DNA strand complementary to the target DNA is fixed to nanoparticles after activation [34]. Then, the target sequence is allowed to be hybridized with complementary DNA. On the other hand, the third strand of DNA, which complements another part of the target DNA, is stabilized upon activation on magnetic particles [34]. By placing these two particles in the solution, the target DNA existence even in very small amounts, will interact or bind to the two particles together [34]. In the next step, due to the magnetic properties of the second particle, they can be separated from the solution, and then DNA is separated from the complementary sequence by using the denaturing agents. In this way, very low amounts of a DNA sequence, without the need for a PCR, are recognizable and measurable [34]. The biological code load is a biologically constructed and selective DNA sequence for a DNA target that is used to detect DNA or proteins in biological samples [35]. For example, in a study by Shi, a fluorescence resonance energy transfer (FRET) biosensor based on graphene quantum dots was developed for *S. aureus mec A* gene sequence detection. After immobilization of capture and reporter probes on graphene quantum dots and AuNPs respectively, target DNA co-hybridized with probes to trigger the FRET effect. The detection limit of 1 nmol/l was achieved in this method [33]. In another study, a detection platform based on FRET was applied for rapid, ultrasensitive and specific bacteria detection, where AuNPs were conjugated with aptamers while upconversion nanoparticles (UCNPs, donor) were functionalized with corresponding complementary DNA (cDNA). This method could detect *E. coli* ATCC 8739 in water and food at 20 min with a detection limit of 3 CFU/ml [36]. In a study by Wang and Alocilja, AuNPs were conjugated with polyclonal Abs, and were then introduced to the MNP-target complex to form a sandwich magnetic NP-target-AuNP and detected O157H7 *E. coli* at 10^1 CFU/ml in less than 1 h [37]. Moreover, using sandwich hybridization assays in AuNPs-based paper platform, as little as 0.5 pg/μl genomic RNA from a food-born pathogen *Listeria monocytogenes* (20 CFU/ml) was detected in several hours [38]. In another study, Raman signal probe and the capture probe were designed and Fe_3O_4 AuNPs immobilized with both aptamer of *S. typhimurium* and *S. aureus* were used as the capture probe. The limit of detection was 35 CFU/ ml for *S. aureus* and 15 CFU/ml for *S. typhimurium* [39].

Surface chemistry and activation of gold nanoparticles

AuNPs can be functionalized with a wide range of functional materials for specific binding to bacterial surface. Polymers such as polyvinylpyrrolidone pvp, and tannic acid are coating agents particularly used to stabilize nanoparticles [40]. AuNPs used for biological applications are commonly coated with polyethylene glycol, bovine serum albumin or other proteins, peptides, and oligonucleotides [40]. AuNPs can be functionalized by molecules converting surface charge from negative charge to a positive charge, leading to various pathogens detection [40]. AuNPs can also be functionalized to form reactive groups (e.g., a surface with a carboxylamino terminus) for further conjugation [40]. Nonconductive coating materials such as silica, aluminum oxide, and titanium oxide which their thickness is precisely controlled, can be used to encapsulate, change the optical properties, or bind fluorescent colors to AuNPs [40].

Gold nanoparticle-based colorimetric bacterial detection methods

In most of biosensors for selectivity, the target DNA hybridization is used with complementary probes which can incredibly decrease detection time [10]. In addition, AuNPs are used to detect specific DNA sequences in the form of DNA sensor [19]. In this method, a colorimetric sensor based on the target DNA hybridization is generated with its complement oligonucleotides, in which the level of the AuNPs is altered using the thiol oligonucleotide probes, hybridized with the target DNA, leading to change in the color of the suspension [19,41]. This method is rapid, efficient, and highly sensitive for bacterial pathogens detection. In addition, it is inexpensive and comfortable to handle. Colorimetric method has been used to detect *Salmonella* spp 10 times more sensitive than gel agarose analysis with the same DNA concentration. It was applied in the detection of *Helicobacter pylori* DNA with a sensitivity of 92.5% and specificity of 95.4% and *Mycobacterium tuberculosis* with 94.7% sensitivity in less than 1 h [7,10–12].

The PEI-AuNPs could detect Gram-positive and Gram-negative pathogens *E. coli* and *S. aureus* as low as 10 CFU/ml within 10 min using an optical reader and 2–3 h with naked eye [26]. It is noteworthy that in Xizhe' study, a colorimetric method using AuNPs could identify 15 microorganisms based on diverse surface charges within 5 s with naked eye [27]. Various microorganisms can be rapidly detected (multiplex sensing of pathogens) with AuNPs-based colorimetric methods which other methods lack this potential [42]. This simple and rapid diagnostic platform technology is high efficient, especially in low-income areas and for large number of samples analysis.

Conclusion

Compared with fluorescence sensor arrays, AuNPs-based colorimetric sensor arrays are simple, fast and label-free with visualization detection. It is applicable in clinical diagnostics and environmental monitoring and for online identification of various microorganisms (multiplex sensing of pathogens). AuNPs-based colorimetric methods are a simple, reliable and sensitive easy-to-fabricate, easy-to assemble diagnostic device capable of bacteria and pathogens detection with high sensitivity and specificity. In spite of many molecular techniques, AuNPs-based colorimetric methods do not require additional devices, fabrication cost, signal processing, and interpretation complexities and costly or complex instruments. Recent findings have highlighted that this simple and rapid method is suitable, especially in developing areas and for large number of samples analysis.

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Conflicts of interest

There are no conflicts of interest.

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