

Rapid Optical Detection Strategy for Human Pathogens: A Brief Review

Pragati Jamdagani^{*}, JS Rana and Poonam Khatri

Department of Biotechnology, Deenbandhu Chhotu Ram University of Science and Technology, Murthal, Sonapat, Haryana, India

^{*}Corresponding authors: Pragati Jamdagani, Research Scholar, Department of Biotechnology, Deenbandhu Chhotu Ram University of Science and Technology, Murthal, Sonapat, Haryana, India, Tel: +918708318857; E-mail: pragati_318@yahoo.co.in

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Abstract

Quick, sensitive and specific detection of infectious agents can aid in better treatment of patients and for recognition of contaminants. Traditional methods suffer from drawbacks of being time consuming and tedious to perform and modern medicine advances call for better detection methods. Strategies employing nanoparticle based systems are under investigation to allow for efficient and rapid detection of pathogenic microorganisms. Nanoparticles have been conjugated with nucleic acid probes and antibodies to serve as one step detection system, based on conserved genomic DNA sequences and specific surface proteins expressed by the microorganisms, respectively. Unique size based colorimetric properties are being exploited for developing optical sensors enabling result visualization with the help of naked eye or simple spectroscopic techniques. The current communication deals with basic ideology of conjugate synthesis, strategy adopted for deduction of positive and negative interaction with the test sample and some lab tested examples employing these conjugate systems.

Keywords: Nanoparticle conjugates; Nucleic acid probes; Antibodies; Detection; Pathogens

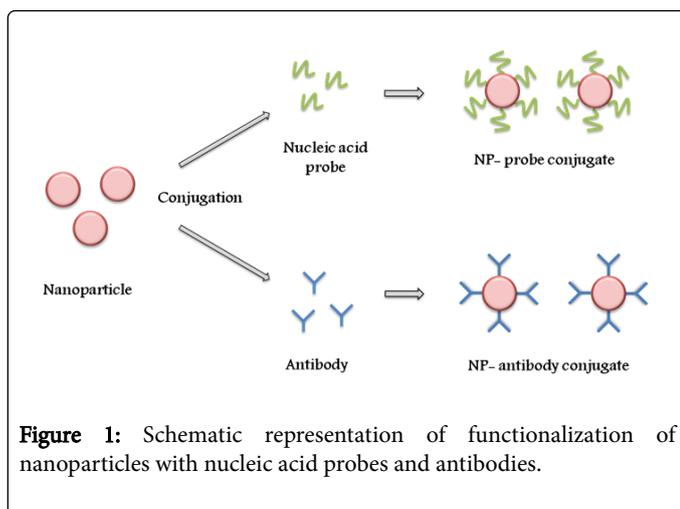
Pathogen Detection Strategy

Nanoparticles based conjugate systems

Introduction

A simple bacterial infection can lead to life threatening situations, when invading bacteria enter bloodstream of the patient, a condition known as bacteremia. Rapid detection of the causative infectious agents is the foremost necessity when it comes to effective and timely treatment for such infections. Traditionally used methods for pathogen detection have been followed since decades involving isolation and characterization of pathogens from the patients. These processes are not only time consuming but can also lead to arbitrary results since, human body has a rich flora of resident microorganisms. Hence, accurate diagnosis needs repeated culturing and specific correlation with disease symptoms observed [1]. Molecular diagnosis, based on organism specific nucleic acid sequences and proteins, has led to improvement in both sensitivity and specificity of diagnostic procedures. These methods employ the use of labeled probes or PCR amplification for more reliable detection. A pleasant development in the world of nucleic acid probes is the advent of Peptide Nucleic Acid (PNA) probes. These probes are a modification of already occurring DNA probes where the phosphodiester backbone is replaced by a peptide backbone but DNA bases remain the same and sustain their complementary binding properties. PNA probes offer the advantages of being uncharged and hydrophobic which allow for easy transport inside the cell and more efficient target binding. PNA probes have been successfully used for direct detection of microorganisms from microbiological smears [2,3]. However, these methods require expensive apparatus and trained personnel making these more or less unavailable for underprivileged creating a necessity for alternative measures that are cheap and affordable.

Nanotechnology is an emerging field with nanostructures finding extensive applications in the field of biosensing, imaging and detection of an array of biological entities that provide for point of care diagnosis of diseases and their pathogenesis examination and tracking [4]. This has been made possible owing to some extraordinary physico-chemical properties exhibited by nanoparticles (NPs), which in turn result from their ultra-fine size and high surface-to-volume ratio [5] and these size dependant properties make NPs advantageous over their bulk counterparts. Gold and silver NPs are preferred for biological applications because they are stable and non-toxic and have been coupled with nucleic acid probes and antibodies to provide reliable, fast and label free detection systems providing faster results (Figure 1) [6,7]. Nucleotide probes are modified to incorporate a thiolated moiety, preferably at 5' end, which aids in their adsorption onto the surface [8]. Antibodies, on the other hand, get readily adsorbed onto the nanoparticles with the help of electrostatic interactions, when the reaction is performed at isoelectrical point [9]. This adsorption, however, suffers from several drawbacks as the adsorption is weak, prone to replacement and completely random. To overcome these shortcomings, Kumar et al. proposed an attachment strategy protocol that ensures outward orientation of Fab region of antibodies for better interaction with target molecules in the sample. The system makes use of a linker molecule with binding sites for constant region of antibodies on one end and an alkane dithiol tether on the other end which allows for strong attachment with the NP surface [10]. Conclusively, thiol links provide extremely stable conjugation of nucleic acid probes and antibodies with nanoparticles, sturdy enough to be employed for target detection in biological samples.

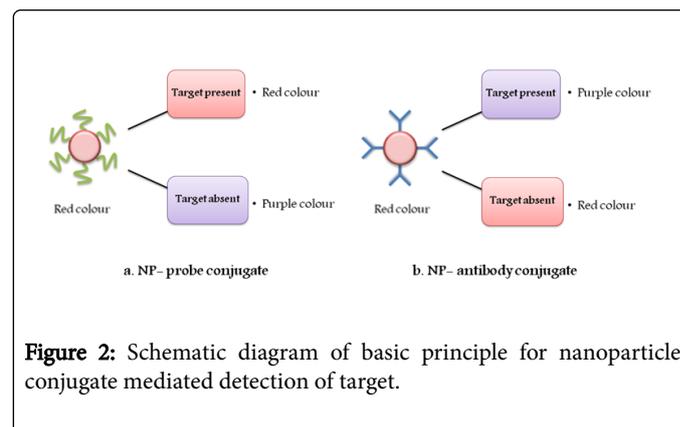


Colour based detection systems

NP-probe conjugates are used to detect complementary DNA sequences and allow for visual detection of results. Gold NP-probe conjugates are primarily, red in colour in their stable colloidal form. These are made to encounter and hybridize with genomic DNA sequences isolated from test samples. Hybridized and un-hybridized NP conjugates behave differently when subjected to analysis conditions of acidification and/or high salt concentration. Hybridization of the probe oligonucleotide to its complementary sequences encompasses a stabilizing effect and prevents aggregation of the NPs in test conditions. NPs with free probes, on the other hand, undergo rapid aggregation altering the colour of the suspension from red to purple (Figure 2a). This aggregation can also be observed as a shift in the absorption maxima towards longer wavelength in the spectrum [11-13]. Based on the above stated strategy, efficient and sensitive detection systems have been developed for methicillin-resistant *S. aureus*, *M. tuberculosis*, *M. avium* subsp. paratuberculosis, *E. coli*, *S. enteritidis*, *S. typhimurium*, *Leishmania* spp. and *P. falciparum* [14]. Recently, gold NP modified gold electrodes have been used to provide for an ultrasensitive detection system with extremely low detection limits. Moreover, the stated biosensor can distinguish single base pair mismatch and could be modified for efficient detection of pathogens [15].

NP-antibody conjugates have been used for the development of immunochromatographic strips for diagnostic applications, some which have already been commercialized. Studies aimed at detection of pathogenic bacteria have also been reported for several bacterial species. Antibody based systems are simpler as compared to nucleic acid probe conjugates as they omit the need for genomic DNA isolation and can be directed against whole cell targets. Huang developed an immunochromatographic assay for detection of *S. aureus* employing gold NPs, based on the principle of a sandwich assay. He used IgG antibodies specific for cell surface protein A of *S. aureus*, and conjugated one of them with gold NPs. Cross-linking of protein A with the two antibodies led to the development of red colour on the strip allowing for visual observation of the results with 100% sensitivity and 96-100% specificity [16]. Based on principle similar to that followed for NP-probe conjugate based detection, Wang et al. described an aggregation mediated detection of *S. typhimurium* using oval-shaped gold nanoparticles conjugated with anti-salmonella antibody. A prominent colour change from red to bluish purple was observed in

the presence of target pathogen (Figure 2b) and UV-Visible spectrum also gave characteristic absorption shift. They further reported targeted photothermal lysis of bound bacteria on exposure to near-infrared radiation making the system even more advantageous [17]. Verdoot et al. described a gold NP-antibody conjugated immunosensor for the detection of *Lactobacillus* species (spp.) and *S. aureus* [18]. Apart from antibodies, Raj et al. used cysteine capped gold NPs for direct visual detection of *E. coli* 0157:H7 in clinical samples collected from UTI patients [19].



Non colour based detection systems

Several other detection systems have also been postulated that make use of nanoparticles, but not necessarily harness their visible optical properties and focus on other optical properties of NPs, such as fluorescence and Surface-Enhanced Raman Spectroscopy (SERS). Gao et al. reported fluorescence microscopy based sensitive and rapid detection of *E. coli* and coagulase-negative *Staphylococcus* (CNS) using vancomycin-functionalized FePt nanoparticles in combination with vancomycin-conjugated fluorescent probe in blood samples [20]. Later, Wang et al. used SiO₂ spheres incorporated with CdSe/ZnS quantum dots as fluorescent probes for the detection of *S. typhimurium*. They cross-linked synthesized NPs onto bacterial surface and used fluorescence microscopy for detection [21]. SERS is a very sensitive technique and records light scattering from the surface of nanostructures. SERS based systems have allowed for ultrasensitive detection of *S. epidermidis* [22], *E. coli*, *S. aureus* [23] and foodborne *E. coli*, *S. enterica* and *S. xylosum* [24].

Conclusion and Future Prospects

Above stated NP based systems prove advantageous over traditionally used diagnostic procedures in several ways. These systems can help in detection of specific pathogen directly from test samples, without the need of isolation and culturing of the mixed microbial population. Secondly, though trained personnel are required for the synthesis of conjugates but operation and analysis is simple to perform and understand. Optical result interpretation omits the need of costly apparatus and difficult to perform analytical operations. NP-conjugates can be optimized for individual microbial strain and formulated to yield immunochromatographic strips which can further be used to form one step, ready to use diagnostic kits. Thus, these systems have the potential to serve as sensitive and easy tool for effective diagnosis.

Conflict of Interests

The authors declare that they have no competing interests.

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