Rapid Optical Detection Strategy for Human Pathogens: A Brief Review

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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 systems, 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

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 form 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.

Pathogen Detection Strategy

Nanoparticles based conjugate systems

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 dependent 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.

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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]. Verdoet et al. described a gold NP-antibody conjugated immunosensor for the detection of *Lactobacillus* species (ssp.) 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].

**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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