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Gold Nanoparticles Based Strategy for Detection of Microbial Gene Targets

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Gold nanoparticles (AuNPs) plasmon changes, as a result of additives and their follow up reaction in solution around zeta space of AuNPs, have been extensively used for analyte detection. Biomolecules like protein or nucleic acid, in conjugated or non-conjugated forms with AuNPs, when allowed to react with complimentary molecule such as antibody or nucleic acid target, lead to visual colour changes and may offer diagnostic test. Using synthetic nucleic acid probes, specific to complimentary target gene in a microbe, conjugated probe-AuNPs were exploiting Au-S bond chemistry. Thiolated nucleotide probes were successfully conjugated on AuNPs to prepare gold reagents that reacted specifically with complimentary gene target and not with non-complimentary sequence, the later failed to stabilise the probe-AuNPs and gave red to blue colour changes in solution after addition of salt. This rapid colour change of probe-AuNPs solution is able to differentiate the presence of complimentary and non-complimentary gene target in test solution, thus offer a strategy for detection of microbial genes.

Keywords: Gold nanoparticle, visual detection, *Brucella,* surface plasmon resonance.

Rapid detection of pathogen causing infectious disease become very important for early diagnosis, successful treatment and to reduce the cost of treatment. Conventional methodologies like isolation of organism, serological assays, though of extreme relevance, may be time consuming, hazardous and laborious, which lead to delayed diagnosis and treatment[1]. Different approaches have been adapted to increase the sensitivity, specificity for detection of pathogens[2]. Among these approaches, nanoparticles based detection of pathogens in various formats has been showing great success and most promising in last decade[3,4,5]. The unique physical and chemical properties of AuNPs have allowed various sensing platform to be developed with higher sensitivity and specificity[6]. The unique surface chemistry of AuNPs facilitates functionalisation of AuNP surface with biomolecules like nucleic acid or protein[7]. This ease the utilisation of AuNP in nanotechnology based detection of pathogens. AuNPhas characteristic optical property with surface plasmon resonance band (SPR) in visible range[8]. This SPR band changes with particle shape, size and inter particle distance[9]. For example, aggregation of AuNPs leads to visual colour change from red to blue along with its characteristic band at 520 (13nm size)shifts to 650nm[5,10]. Base on this, different calorimetric strategieshave been adapted for detection of pathogens. These strategies are basically based on two principles: cross-linking induced aggregation of AuNPs where two sets of oligo-probes complementary to the adjacent sequences at the target gene are designed.

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Presence of complementary sequences with these probes conjugated with AuNPs (Probes-AuNP) promotes aggregation of gold nanoprobes (Au-nanoprobes). This triggers visual colour change of AuNP solution to blue[3]. Another approach is based on non cross-linking or salt induced aggregation of AuNPs where only one oligo probe complementary to a target gene need to be designed. In this strategy, hybridisation of probes-AuNPs with target gene prevents aggregation of Au-nanoprobes induced by increasing ionic strength[11,12].

Various studies on nanodiagnostics using AuNPs for detection of different pathogens have been done. AuNP based nanodiagnostics was first introduced by Mirkin et al, 1996[13]. This led to development of first pathogen detection system by Baily et al, 2003[14]. Further studies have been done for detection of Mycobacterium, Brucella, E.coli etc[15,16,17].In the present study, two oligoprobes complementary to IS711 insertion sequence and BCSP31 gene of brucella have been designed and synthesized. Here we are presenting preliminary study showing visual colour change of Au-nanoprobe after allowing interaction of Probe-AuNPs with complementary as well as non-complementary sequences. This may be extended to detection of gene from bacterial genome after further study.

MATERIALS AND METHODS

Reagents and apparatus

Gold (III) chloride hydrate, $HAuCl_4$; Sodium citrate tribasic; Tris (2-carboxyethyl)-phosphine; Sodium dodecyl sulphate; di sodium hydrogen phosphate; sodium di hydrogen phosphate; sodium hydroxide; potassium di hydrogen phosphate; Hydrochloric acid; Sodium Chloride. The reagents used in this study were of analytical grade. All the chemical solutions were prepared using sterile ddH_2O. All glasswares were scrupulously clean. Glass and plastic containers and stirrers for AuNP preparation were cleaned in aqua-regia and thoroughly washed in MilliQ water. Oligo probes and complementary sequences were ordered from IDT.

Thiol-modified oligonucleotide probes sequences and complimentary sequences were as follow:

i) IS711 probe: 5'SH $-(CH_2)_9$CTTAAG GGCCTTCATTGCCAGCAA-3'

ii) BCSP31 probe: 5'SH$-(CH_2)_9$GGGCAA GGTGGAAGATTTGCGCCT-3'

iii) IS711 complementary sequence: 5'-TTGC TGGCAATGAAGGCCCTTAAG-3'

iv) BCSP31 complementary sequence: 5'-AGGC GCAAATCTTCCACCTTGCCC-3'

Preparation of AuNPs

AuNPs were prepared by citrate reduction method describe earlier[18]. Briefly, 200ml of chloroauric acid (1mM) was boiled with vigorous stirring. While boiling 20ml of sodium citrate (38.8mM) was poured at once and refluxed for 15-20min. The colloidal solution of gold nanoparticles was allowed to cool at room temperature (RT) overnight. The nanoparticle solution then then stored in dark at 4^0C until used. The prepared nanoparticle solution was characterized by spectrophotometry.

Functionalization of AuNPs

AuNPs were conjugated with thiol modified oligo probes following the protocol reported earlier[19]. Briefly, 4nmol of thiol modified oligo probes were reduced by using TCEP (10mM). This reduced the disulfide present in the modified probe to sulfhydryl which enhanced the conjugation of probes to nanoparticle surface. The reduced probes were incubated with 1ml of prepared AuNP solution overnight at RT with gentle shaking. The solution was brought to a final concentration of 9mM sodium phosphate buffer by using 100mM phosphate buffer, SDS (0.1%) and gentle shaken for 30 min at RT. The salting buffer (2 M NaCl in 10 mM PBS, pH 7) was added in 6 equal doses to the above solution during 2 days to bring final concentration of 0.3M NaCl. The solution was incubated overnight at RT. After incubation, the solution was centrifuge 15000rpm for 15min. Supernatant was discarded and washed the pellet with washing buffer (10 mM PBS, pH 7.4, 150 mMNaCl, 0.1% SDS). The solution was again centrifuged as above and finally suspended the pellet with 500µl of the above same buffer.

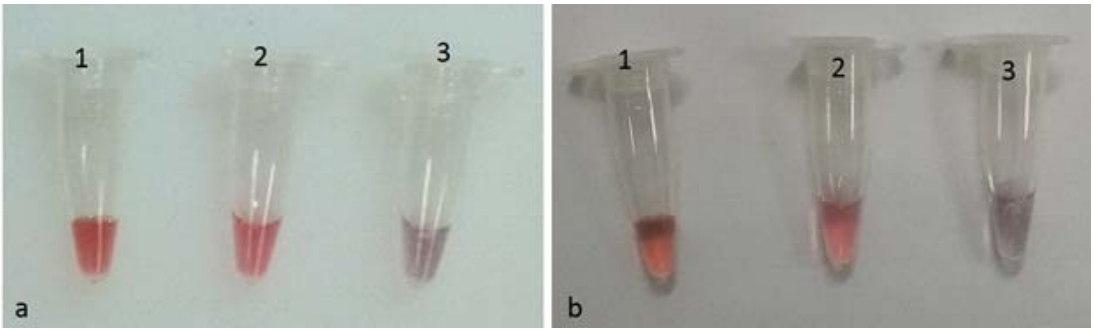
Hybridization assay

The hybridization reaction consists of four components: 10mM sodium phosphate buffer (pH 5), probe-AuNPs, complementary oligo sequence and 0.1M HCl. At first, different reactions having different volume combination of these components at different temperature and duration

in each step were assayed for standardisation. 1µl of complementary sequence was mixed with 9µl of buffer in a 0.2ml tube. The mixture was heated at 95^0C for 5 min to denature any secondary structure in the complementary sequence. After denaturation, 15µl of probe-AuNP solution was added and kept immediately at 57^0C (IS711) and 60^0C (BCSP31) maintained in water bath for 16min to allow hybridization. Hybridization of probe with specific template was confirmed by adding 6µl HCl. The colour change was then observed visually.

RESULTS AND DISCUSSION

Visual detection of nucleic acid using specific nucleotide probe and gold nanoparticles (AuNPs) can provide simple and rapid screening for infectious disease or environmental microbes. Sensitivity of the test is continuing bottleneck and therefore require amplification for gene targets normally available in low abundance and require costly setup for their detection. But using conjugation of the nuceotidesAuNPs and subsequent hybridization with target gene provide additional stability to the AuNP colloidal solution against charge neutralization as compare to the single stranded nucleotide conjugated to the gold. This phenomenon was exploited in the present experiments by two different nucleotide probes specific to *Brucella* gene targets. These probes were covalently conjugated to AuNPs through S-Au bond and were allowed to react with complementary gene target sequence, prepared synthetically. Hybridization and reaction conditions are provided in the material methods. The results for visual plasmonchanges of nucleotide conjugated AuNPs with synthetic gene target are shown in the fig. a and fig. b respective complementary gene sequences were used as non-complementary sequence for different probes in the present exercise. In fig. a IS711, probe-AuNPs were allowed to react with both the complementary



a. IS711 b.BCSP-31

Fig. 1. Visual colour changes after addition of HCl: tube 1 – AuNP solution without HCl; tube 2 – probe-AuNPs with complementary sequence; tube 3 – probe-AuNPs with non-complemenatry sequence

(tube 2) and non-complemenatary (tube 3) oligo nucleotides. Similarly fig. b BCSP-31 depicts the reaction of BCSP-31 probe-AuNPs with complementary target sequence (tube 2) and non-complementary (tube 3). Bonding of probe to gene target increases the net negative charge on AuNPs as compare to non-binding of AuNPs-probe to non-complementary sequence, thus lead to stabilization of AuNPs (red colour) as compare to non-binding stage leading to aggregation (blue colour) on decreasing the pH with addition of HCl. This corolary using specific nucleotide conjugated AuNPs is able to distinguish rapidly the complementary (microbial gene) and non complemenatary (no microbial gene) targets in sample and may be extended for devising visual colorimetric biosensor platform for detection of microbial gene targets.

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