ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Aptamer Based Gold Nanoparticle In Biomedical Applications: An Overview.

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ABSTRACT

Aptamers are single-stranded deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) oligonucleotides, which are able to bind their target with high selectivity and affinity. Aptamers based gold nanoparticles are nano systems well qualified for the development of new biomedical devices for analytical, imaging, drug delivery and many other biomedical applications. Aptamers conjugated with the nanoparticles such as gold nanoparticles play vital role in the nanomedicine as the diagnostic and therapeutic tool and even in the drug delivery system. The development of biosensors is probably one of the most promising ways to solve some of the problems concerning the increasing need to develop highly sensitive, fast and economic methods of analysis in medical diagnostics. In this review, some consideration will be given to biosensors and their application in medical diagnostics, taking into account several crucial features and techniques of development of different types of Aptamers having properties such as high affinity and specificity for targets, easy chemical synthesis and modification, and rapid tissue penetration. They have become attractive molecules in diagnostics and therapeutics rivaling and, in some cases, surpassing other molecular probes, such as antibodies. In this review, we highlight the recent progress in aptamers-mediated delivery for therapeutics and disease-targeting based on aptamers integration with a variety of nanomaterials, such as gold nanorods, DNA micelles, DNA hydrogel and carbon nanotubes and their diagnostic applications.

Keywords: Aptamers, Diagnosis, Selex, Nanomedicine, Biosensor.

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ISSN: 0975-8585

INTRODUCTION

Today, in research, new drug and development of new drug delivery systems the diagnostic and therapeutic tools is the subject of interest. Most of the biological targets are proteins and nucleic acid which are considered as key source of information due to having ability to interact with amino acids, peptides and ion channel. As protein families are carriers of information, this is become a basic tool for most of the effective and successful disease diagnosis and therapeutics. Aptamers can serve as cancer diagnostic tools by detecting specific biomarkers, circulating cancer cells or imaging diseased tissue. On the other hand, aptamers can be used as therapeutic agents due to their potential antagonist activity, or as targeting agents. Applied to this, newer approaches had developed which combines the aptamers with metal nanoparticles such as gold to give a wider application in bioanalysis and nanomedicine. [1]

Aptamers

Aptamers are oligonucleotides, such as ribonucleic acid (RNA) and single-strand deoxyribonucleic acid (ssDNA) or peptide molecules that can bind to their targets with high affinity and specificity which rival antibodies in their diagnostic potential due to their ability to fold specific three-dimensional structures. Aptamers are more advantageous among other molecules because of their high stability, chemically synthesize with great accuracy and reproducibility, Low immunogenicity, low toxicity and possesses variety of target. Aptamers will apply in many field such as New drug, therapeutic tool, drug delivery, diagnosis of disease, Bio-imaging, analytical reagent, hazard detection and food inspection [2]. Systematic evolution of ligand by *ex*ponential enrichment (SELEX) is the process, developed earlier in 1990, for the discovery and isolation of aptamers. Aptamers are obtained *in vitro* by directed selection from combinatorial oligonucleotides libraries. The method enables isolating an aptamer of interest from a pool of randomized molecules by repeated steps of incubation with the target, partitioning and amplification, until the pool of molecules becomes enriched in a particular colon.[3,4]

Generally, the SELEX experiment consists of several rounds of selection of sequences that bind to a target molecule. Each round includes three main stages:

- the oligonucleotides library is incubated with the target molecule;
- the oligonucleotides complexes with the target are separated from non-bound oligonucleotides;
- The bound sequences are amplified by PCR. As a result, gradual enrichment of the oligonucleotides library with sequences exhibiting the increased affinity to the target molecule occurs[1]. Aptamers that come out of a SELEX experiment are full-length sequences containing the fixed sequences that were included to aid the amplification process [5].

Gold nanoparticles (AuNPs):

Gold nanoparticles (ranging from 1 to 100 nm) are the type of nanomaterials and becoming emerging concept in nanotechnology for their clinical aspects owing to the

subject of colorimetric, conductivity, biofunctionalization, biostability, and other physical properties such as spectral, optical, Quantum, Surface Plasmon Band (SPB), Fluorescence, Electrochemistry Electronic Properties[6].

Among the conventional methods of synthesis of AuNPs by reduction of gold (III) derivatives, the most popular one for a long time has been that using citrate reduction of HAuCl4 in water, which was introduced by Turkevitch in 1951[7]

Gold nanoparticles exhibit in different form according to the size, shape and physical properties such Gold nanospheres, Gold nanorods, Gold nanoshells, Gold nanocages [8]

The assortment of GNPs exercise in contemporary medical and biological studies is enormously spacious. In particular, it comprises genomics, DNA and antigens[9,10,11] biosensorics,[12,13] immunoanalysis,[14] clinical chemistry, detection and photothermolysis of microorganisms and cancer cells; the targeted delivery of drugs, optical bioimaging and the monitoring of cells and tissues using modern registration systems[15-17].

It has been argued that gold nanoparticles could be used in almost all medical applications: diagnostics, therapy, prevention, and hygiene [18]. AuNPs functionalized with oligonucleotides (ODN-AuNPs) have emerged as a kind of novel nanomaterials for diagnosis, therapy [19] and materials design [20].

APPLICATION

Aptamer conjugated with the nanoparticles such as gold nanoparticles play vital role in the nanomedicine as the diagnostic and therapeutic tool and even in the drug delivery system also. The Characterization of Aptamer−Gold Conjugates becomes easy due to advanced analytical techniques. In addition, the interior volumes of nanoparticles can be used to store large quantities of drug molecules, thereby enhancing loading capacity [21].

In vitro Assay

Detection of Pathogens

Traditional method for detection of pathogens takes long time which becomes tiresome in initiating treatment. Because of this reason it is important to detect pathogens as early, before they can speedily multiply and persuade harmful effects. Previously antibodies were used for the detection of pathogen since antibodies are the majority accepted class of molecules for molecular recognition purpose. However it is inconvenient due to the *in vivo* system for generation and their restriction to only working under physiological conditions. These weaknesses of antibodies overcome by use of Aptamers conjugated with nanoparticles.

Aptamer conjugated gold nanoparticle are readily useful for the detection of pathogens such as salmonella, Escher coli and staphylococcus aureus.

In year 2012 group of Elizabeth I. Maurer and coworker developed novel Biosensor to detect bacteria. Their experiment based on carbon nanotubes functionalized with gold nanoparticles with a ribonucleic acid (RNA) sequence attached as a capture element and an UV-Vi measurements were conducted on the *E. coli* samples containing uncoated and RNA-coated gold nanoparticles. These RNA coated gold nanoparticles were shown to enhance *E. coli* capture by 189% when compared to bare gold nanoparticles. Thus they are able to create the electrochemical multi-array sensor to determine the strain of bacteria as well as their aqueous solution concentration[22].

For detection of staphylococcus aureus, a rapid, ultra-sensitive, low cost, and non-PCR–based method that combines aptamer-conjugated GNPs and a resonance light-scattering detection system has been developed. Two of the isolated aptamers, SA17 and SA61, recognized S. aureus with high specificity and nanomolar affinity. The number of SA17 and SA61 aptamers (selected from SELEX) or aptamer-conjugated GNPs when incubated with S. aureus cells, bound to single S. aureus cells and this is quantified by quantitative Polymerase chain reaction (qPCR). For ultrasensitive detection of S. aureus cells, aptamers are conjugated onto GNPs followed by bead-based amplification. After amplification, one bacterial cell was capable of generating more than 10^4 GNPs, and amplified GNPs could be detected by the light-scattering–sensing system. Single cell detection was reached within 1.5 hours without expensive equipment such as thermal cyclers or centrifuges[23].

Other Methicillin-resistant *Staphylococcus aureus* aptamers developed that can be easily modified as molecular probes for bioanalysis or antibiotics-free therapy as the staphylococcus aureus get easily developed the antibiotic resistant strain[24]. Novel Point-of-care molecular diagnostics by simple colorimetric detection developed for *Cryptosporidium parvum* oocysts responsible for cryptosporidiosis. *Cryptosporidium parvum* oocysts using oligonucleotide-functionalized gold nanoparticles targeted against heat shock protein 70 (HSP70) DNA and RNA. One advantage of targeting HSP70 is the ability to induce mRNA amplification by the use of a simple heating process that does not involve more complicated PCR-based amplification. We hypothesize that simple amplification (via the heat shock process) of targets present at very low copies in biological samples will facilitate the detection of the desired targets that are present at levels within the limit of detection of the gold aggregation assay [25].

Cocaine and other small molecule detection

With novel bioassay approach, gold nanoparticle and engineered aptamers are useful in cocaine and small molecule detection. In this tactic, an aptamer is cut into two pieces of random coil-like ssDNA which are separated in the absence of the ligand. The surface Plasmon resonance (SPR) color property of red AuNPs is utilized to identify ligand-free ssDNA and ligand-induced aptamer structures. The ssDNA used are anticocaine aptamer (ACA), ACA-1 and ACA-2. It was observed that AuNPs retained the red color upon in the presence of cocaine-free ACA-1 and ACA-2 upon addition of NaCl saltThis because of, highly negatively charged ssDNA spontaneously binds to AuNPs through interactions between gold and nitrogen containing bases, and effectively stabilizes AuNPs against salt-induced aggregations. Though, in the presence of cocaine, AuNPs solution readily turned blue. This color change implies that ACA-1 and ACA-2 form a tertiary structure along with

cocaine, which does not possess an affinity to AuNPs strong enough to resist salt-induced aggregation though the effect of cocaine on AuNPs in the absence of aptamers found to be remains the same as as-prepared AuNPs (red color). Thus, Juan Zhang et. Al., have successfully developed method for selectively detect micromolar quantities of cocaine within minutes. This approach is also shown to be generic and applicable to the assay of small molecules such as both adenosine and potassium[26].

Protein analysis

Human thrombin, is an important endolytic serine protease found in blood and it serve as the target protein.

In support of, rapid detection of thrombin (qualitative and Quantitative) various experiments were successfully developed. Such as

- Dry-Reagent Strip Biosensor which able to qualitative (visual)/quantitative detection of protein within minutes by avoiding the multiple incubation and washing steps in regular aptamer based protein detection [27].
- Dye-labeled DNA hybridized with aptamer that is immobilized on GNPs as Ap–Im–GNPs sensor based on fluorescence quenching were developed for the thrombin detection. Three strategies were tested for protein detection by using thrombin and thrombin aptamer such as Ap–Im–GNPs, Ap–Hy–GNPs (DNA hybridized with GNPs), and Ap–Ad–GNPs (DNA adsorbed on surface). Among these Ap–Im–GNP strategy had the highest affinity constant and, subsequently, the most sensitive detection limit hence considered as best for protein detection [28].
- A promising detection approach for proteins with two binding sites is possible due to the Dual-signal enhancement of aptamer-functionalized gold nanoparticles (Apt-GNPs) on quartz crystal microbalance with dissipation monitoring (QCM-D) sensing with good selectivity and repeatability in complex matrix. A highly sensitive QCM-D biosensor for protein detection was developed using Apt-GNPs. Human α-thrombin, captured by immobilized aptamers, thrombin was determined on-line using Apt-GNPs to enhance both frequency and dissipation signals. The fabricated sandwich of aptamer/thrombin/Apt-GNPs on chip surface was confirmed by atomic force microscopy (AFM)[29].
- A label-free, aptamer-based chemiluminescent biosensor developed, capable to detect thrombin with low detection limit, and higher sensitivity than AuNP-based colorimetric methods. The biosensor relies upon the catalytic activity of unmodified gold nanoparticles (AuNPs) on the luminol–H2O2 chemiluminescence (CL) reaction, and the interaction of unmodified AuNPs with the aptamer. The unmodified AuNPs can effectively differentiate unstructured and folded aptamer. During the assay, no covalent functionalization of the AuNPs or aptamer is required [30].
- Gold nanoparticle (NP) enhanced surface sandwich assays for the detection of proteins is developed in conjunction with a surface enzyme reaction. As a model protein, immunoglobulin E (IgE) possessing two different epitopes for anti-IgE and IgE specific aptamer is used. A surface sandwich was first formed via the adsorption of IgE onto IgE aptamer coated Au NP-modified gold electrodes followed by the specific interaction of alkaline phosphatase (ALP) conjugated anti-IgE onto the

surface IgE complex. The selective electrochemical signal was then achieved by measuring released electrons from the reaction of the substrate, 4-aminophenylphosphate (APP) with the surface IgE-aptamer-NPs/IgE/anti-IgE-ALP complex. The signal enhancement effect of NPs in ALP amplified assays was also studied using the IgE aptamer/IgE/antiIgE-ALP complex. The use of aptamer coated NPs with the enzymatically amplified sandwich assay resulted in an excellent enhancement for IgE detection and a significant reduction of non-specific adsorption events[31].

- Low-cost Thrombin-binding aptamer-conjugated gold nanoparticles (TBA29-Au NPs) show their potential in biomedical applications for treating various diseases related to blood clotting disorders as highly effective control of thrombin activity towards fibrinogen[32].
- Novel assay for selective determination of polynucleotides using atomic force microscopy in conjunction with the formation of the probe/target/DNA-gold nanoparticle sandwich structure at a gold surface is described. A 17-mer probe was attached to the surface for subsequent hybridization with a polynucleotide target [33].

Cancer Cell detection

Development of easy and rapid methods for detection of cancer cells becomes significant in medical field to initiate the proper treatment as early.

Molecular recognition of disease-specific biomarkers, especially the recognition of proteins or other biological molecules that differentiate between normal and abnormal cells is a fundamental challenge in cancer cell biology [34].

Computed tomography (CT) is one of the most useful diagnostic tools which include magnetic resonance imaging (MRI), positron emission tomography (PET), and ultrasound. However, currently available CT contrast agents, which are based on small iodinated molecules, possess a number of limitations, including a lack of targeted molecular imaging, short imaging time, and renal toxicity. Here, we report a multifunctional nanoparticle for targeted molecular CT imaging and therapy of prostate cancer. By functionalizing the surface of gold nanoparticles (GNPs) with a prostate-specific membrane antigen (PSMA) RNA aptamer that binds to PSMA, we established a targeted molecular CT imaging system capable of specific imaging of prostate cancer cells that express the PSMA protein. The resulting PSMA aptamer-conjugated GNP showed more than 4-fold greater CT intensity for a targeted LNCaP cell than that of a non targeted PC3 cell.

- Gold nanoparticles functionalized with prostate-specific membrane antigen (PSMA) aptamers was demonstrated to target to PC3 prostate epithelial cells which express PSMA more than surrounding other kind of cells[35] .
- The gold nanoparticle-aptamer nanocomposites were shown to be targeting epidermoid carcinoma cells rather than Epidermal Growth Factor Receptor (EGFR) -free breast cancer cells. EGFR is known to be internalized and gold nanoparticles were demonstrated to enter into the cells after binding on the

surface of cells. The human epithelial carcinoma culture cell line A431 cells express EGFR and the anti-EGFR aptamer was used to identify that cell type [36] .

CURRENT TRENDS

Aptamer based biosensor:

MUC1 protein is an attractive target for anticancer drug delivery owing to it's over expression in most adenocarcinomas. In this study, a reported MUC1 protein aptamer is exploited as the targeting agent of a nanoparticle-based drug delivery system. Paclitaxel (PTX) loaded poly (lactic-co-glycolic-acid) (PLGA) nanoparticles were formulated by an emulsion/evaporation method, and MUC1 aptamers (Apt) were conjugated to the particle surface through a DNA spacer. The aptamer conjugated nanoparticles (Apt-NPs) are about 225.3 nm in size with a stable *in vitro* drug release profile. Using MCF-7 breast cancer cell as a MUC1-overexpressing model, the MUC1 aptamer increased the uptake of nanoparticles into the target cells as measured by flow cytometry. Moreover, the PTX loaded Apt- NPs enhanced *in vitro* drug delivery and cytotoxicity to MUC1+ cancer cells, as compared with nontargeted nanoparticles that lack the MUC1 aptamer ($P<0.01$). The behavior of this novel aptamernanoparticle bioconjugates suggests that MUC1 aptamers may have application potential in targeted drug delivery towards MUC1-overexpressing tumors[37].

A selective kanamycin-binding single-strand DNA (ssDNA) aptamer (TGGGGGTTGAGGCTAAGCCGA) was discovered through in vitro selection using affinity chromatography with kanamycin-immobilized sepharose beads. The selected aptamer has a high affinity for kanamycin and also for kanamycin derivatives such as kanamycin B and tobramycin. The dissociation constants (K(d) [kanamycin]=78.8 nM, K(d) [kanamycin B] =84.5 nM, and K(d) [tobramycin]=103 nM) of the new aptamer were determined by fluorescence intensity analysis using 5'-fluorescein amidite (FAM) modification. Using this aptamer, kanamycin was detected down to 25 nM by the gold nanoparticle-based colorimetric method. Furthermore, the selected new aptamer has many potential applications as a bioprobe for the detection of kanamycin, kanamycin B, and tobramycin in pharmaceutical preparations and food products[38] .

Herein, we present a simple signal-off electrochemical lysozyme aptasensor with gold nanoparticles modification were anchored onto a gold electrode surface which was previously modified with self-assembled monolayers of p-aminothiophenol which will be used in promising stratergy for protein detection. In this work, p-aminothiophenol, gold nanoparticles amplification, and the DNA duplex in which one strand was covalently attached with thiol and the other was tagged with Fc, were three main protocols employed in lysozyme detection. The SWV peak current decreased with increasing concentration of lysozyme. A lower detection limit of 0.1 pM lysozyme has been obtained. The aptasensor also presents high specificity for lysozyme, which is not affected by the coexistence of other proteins[39].

Neatly arranged gold nanoparticles (AuNPs) were directly electrodeposited on an electrochemically polymerized self-assembled monolayer (SAM) of thiol-functionalized 3, 4-ethylenedioxythiophene (EDOT) derivative, EDTMSHA. A thiolated single-stranded DNA

(ssDNA) aptamer with high specificity to LPS was immobilized on the AuNPs/conducting polymer composite film, serving as sensing platform for LPS detection. Electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), scanning electron microscope (SEM), and atomic force microscopy (AFM) were utilized to characterize the modification and detection processes. The electron transfer resistance was found to have a linear relationship with LPS concentration from 0.1 pg/mL to 1 ng/mL[40].

A DNAzyme was designed to recognize the 5' cyclization sequence (5' CS) that is conserved among all DENV, and conjugated to AuNPs(the salt induced aggregation of gold nanaoparticles). DDZ-AuNP has demonstrated the ability to detect the genomic RNA of our model dengue strain, DENV-2 NGC, isolated from infected Aedes albopictus C6/36 cells. These targeting events lead to the rapid aggregation of AuNPs, resulting in a red to clear color transition of the reaction mixes, and thus positive detection of the DENV RNA genome. The inclusion of SDS in the reaction mixture permitted the detection of DENV directly from cell culture supernatants without additional sample processing. Specificity assays demonstrated detection is DENV-specific, while sensitivity assays confirm detection at levels of 1 × 101 TCID50 units. These results demonstrate DDZ-AuNP effectively detects DENV genomes in a sequence specific manner and at concentrations that are practical for field use.

CONCLUSIONS

We have developed an effective detection assay using DNAzyme catalysis coupled with AuNP aggregation for the detection of DENV genomes in a sequence specific manner. Full development of our novel DDZ-AuNP detection method will provide a practical, rapid, and low cost alternative for the detection of DENV in mosquito cells and tissues, and possibly infected patient serum, in a matter of minutes with little to no specialized training required [41].

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