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Aptamers-The Alternative To Antibodies Conjugated Gold Nanoparticles For Detection Of Staphylococcus Aureus

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***Abstract-Staphylococcus Aureus* (*S. Aureus*) pathogenic bacteria remain a challenge for the struggle against food safety. However, the detection become the overall tool extensively used in different bioanalytical applications. Aptamers are artificial functional DNA/RNA oligonucleotides selected in vitro from random-sequence nucleic acid libraries through an in vitro evolution process called systematic evolution of ligands by exponential enrichment (SELEX). Besides, Aptamers also known as chemical antibodies, and possess a number of advantages over antibodies. however, aptamers conjugated gold nanoparticles are nano systems well qualified for the development of detecting platforms for different analytical applications including nanomedicine as well as the diagnostic and therapeutic tool and even in the food stuffs for detecting different materials. Well, Gold nanoparticles with its properties, enhance aptasensor sensitivity as well as amplification process. Consequently, biosensors based aptamers 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 of different targets molecules. Thus, this review aim to focus on aptamer biosensors as alternative over antibody modified gold nanoparticles for detection of *S. Aureus* in food stuffs.**

Keywords—aptamers, antibody, Gold nanoparticles, biosensors.

I. INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a Gram-positive, coagulase positive bacterium that is generally found in the natural flora of the human nasal passage, skin and gastrointestinal tract of humans and animals [1, 2]. Literature citations reported that about 30% to 50% of the population have been carriers of *S. Aureus* at one time in their lives, and about 20% are long-term carriers[3]. Moreover, Food handlers naturally carrying enterotoxin-producing *S. aureus* in their noses or on their hands are responsible to cause the main source of food contamination, through manual contact or also via respiratory secretions [4]. In addition, a list of Foods that are frequently associated with staphylococcal food poisoning including meat and egg products, milk and dairy products, and various other products that may contain these food ingredients [5]. Some strains of this organism can cause food poisoning by production of enterotoxins (SEs) when

growing in foods [6], over an extensive range of temperature, pH, sodium chloride concentration and water activity [7]. Nevertheless, it is well proved that the consumption of foods contaminated with *S. aureus* can cause severe toxin-mediated illness including gastroenteritis, nausea, vomiting, diarrhea, and abdominal pain within 1-6 h post-consumption of contaminated foods [8]. Meanwhile, in recent years, it has been cited that *S. aureus* food poisoning can be caused by as little as 20-100 ng of enterotoxin [7] , which is able to cause a significant range of food-borne diseases, causing an estimated of 241.000 illness per year in the united states [9]. The U.S. Centers for Disease Control and Prevention has published that 48 million Americans get unwell because of contaminated food, 128,000 are hospitalized and 3000 pass on due to foodborne infections [10] [11]. Consequently, world health organization also reported that almost 2.2 million people, mostly children in developing countries die because of food borne diseases transmitted through consumption of unsafe food containing pathogenic bacteria including *S. Aureus* [12]. In order to eliminate foodborne diseases and safeguard consumer health, detection is the most crucial measure for food safety as in quality assurance and product conformity assessment process [13]. Therefore, development of specific, selective and sensitive sensors for detection of *S. Aureus* in food stuffs is of a great interest.

Aptamers are synthetic single strand DNA or RNA, which are selected from large combinational libraries according to their ability to bind to the target of interest through the process of systematic evolution of ligands by exponential enrichment (SELEX) [14, 15]. Moreover, aptamers are an alternative to antibodies and can be deployed in many application in which you might use an antibody [16]. As an alternative strategy, aptamers possessing high recognition ability toward specific molecular targets which have a strong potential application as bioprobes for targeting drug, developing new drugs, and biosensing [17]. The enzyme-linked immunosorbent assay (ELISA) system is widely used in different research area to assay antibodies [18], and consist of an antigen as the target, an antigen-capturing antibody and a detection antibody able to produce signal when antigen is available [19]. In addition, ELISA assay is highly sensitive with specificity that depends on the quality of primary antibody [20], and can recognize antigens at ultralow concentrations [21]. Although, it has been widely applied in many fields like food safety, an antibody based immuno-essay has some challenges such as protein-based antibody reagents are not extremely stable in non-refrigerated applications whereas DNA aptamer are extremely stable in both hydrated and lyophilized form [22]. Second, unlike antibodies, which undergo irreversible denaturation at room temperature or higher, aptamers can reform [19]. In addition to batch-to-batch variations during the production of antibody, it is no easy and challenging to generate specific monoclonal antibodies especially against non-immunogenic molecules [19]. Besides, aptamers show more flexibility and can be produced for targets to which antibodies are difficult to generate. These problems highlight the need for an alternative replacement over antibodies with aptamer. More importantly, unlike antibody, aptamers can be directly amplified by PCR. Since their discovery in the late 1990s, aptamers have been widely used in many applications, including target detection, enzyme inhibition, receptor regulation, and drug delivery [3]. Hence, this review aims to cover comprehensively the use of aptamer as an alternative to antibody conjugated gold nanoparticles for detection of *S. aureus* in food stuffs for food safety.

II. ADVANTAGES OF APTAMERS OVER ANTIBODIES

Aptamers displayed more competitive advantages over antibodies (**Table I**) ; where, once the amino acid of aptamer's nucleotide sequence is known, it can be synthetized chemically in huge portions without using experimental animals during production, at low price,

and accurately, thus leading to amazing batch-to-batch fidelity (John and Alicia, 2016, Lv et al., 2016). Aptamer can be produced for targets to which antibodies are difficult to generate (Kim et al., 2010, Yi-Chung Chang et al., 2013). In addition, Aptamer possess a number of potential advantages over antibodies such as stability, variability, low cost, simply synthetized, chemically modified, minimally immunogenic, no or low toxicity and reusability (Yiyang Dong et al., 2014). Because no animals are used in aptamer selection, molecules that do not illicit an immune response are also good targets for selection purposes. Another standpoint advantage of aptamers over antibodies is that they are inherently stable, so that they can be subjected to numerous rounds of denaturation and renaturation, thus allowing for an easy regeneration of the sensor surface [23].

TABLE I: COMPARISON OF THE PROPERTIES OF ANTIBODIES OVER APTAMERS

Properties	Antibodies	Aptamers
Cost of production	Animal synthesis and expensive	Chemical synthesis with lower cost
Types of targets	Common protein or haptens	From ions to whole cells
Size	larger	smaller
In vitro application	Limited to physiologic conditions because of animal immunization	In vitro selection under a variety of conditions
Batch activity	varied	uniform
stability	Antibodies are susceptible to high temperatures and pH changes; Denatured antibodies cannot be repaired	Aptamers are fairly stable at ambient temperature and are easily refolded if denatured and can withstand repeated round of denaturation without loss of function
Detection range	Good	better
Shelf life	Shelf life is limited. Requiring a continuous cold chain	Long shelf life. Tolerant of transportation without any special cooling requirements
Reusability	Regeneration may result in a loss of activity Poor reusability due to irreversible conformation changes	Regeneration can be performed without affecting activity Good reusability through a reversible conformation
Transportation	On ice	Room temperature
Ability to conjugate	Difficult through protein modification	Easy through various modification
Immunogenic response	High immunogenicity and low bioavailability	Low immunogenicity and high bioavailability
Development process	Initial antibody generation requires an immune response in an animal model	Enrichment of an oligonucleotide library through SELEX process
Modification	Antibodies are typically conjugated with one type of signaling or binding molecule	Aptamers can be modified at both the 5’ and 3’ end
Affinity and specificity	High affinity and good specificity	High affinity and greater potential for specificity due to nature of the isolation process
Synthesis and manufacture	Produced in animals and then by recombinant methods Poor batch to batch reproducibility	Produced in vitro(no animal use) Low batch-to batch variability Chemically synthetized
In vivo complications	Can cause immune response if used as a drug	No intrinsic immune response
Handling and storage	Refrigeration and freezing Easily denatured	Stable at ambient temperature No freezing or refrigeration needed
Mass	High molecular weight (~150 kDa for IgG).	Low molecular weight (13–26 kDa).

III. APTAMERS AS MOLECULAR RECOGNITION ELEMENTS

Studies have proven that, Nucleic acids are those molecules that allow organisms to transfer genetic coding information from one generation to the next and have long been thought to be less complex than proteins[24]. The two main types of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) [13]. Aptamers are nucleic acid ligands that are synthetically isolated from libraries of oligonucleotides by an in vitro selection process called systematic evolution of ligands by exponential enrichments

(SELEX) [25]. However, like proteins, nucleic acids are able to fold into intricate tertiary structures that have the potential to perform a variety of functions including gene-regulation, catalytic activity and ligand-binding[26]. Several researchers have been made to know the ability of molecular recognition properties through developing synthetic DNA/RNA motifs that bound specifically to molecular targets [19]. In other words, these DNA/RNA structures, called aptamers, and were selected using an in vitro selection procedure called systematic evolution of ligands by exponential enrichment (SELEX)[26]. The application of SELEX to synthesize aptamers for a large number of targets has facilitated a wide range of applications that use aptamers [27]. Like antibodies, these synthetically derived molecular recognition probes were found to be selective and able to bind to their targets with high affinity[28]. Similar to antibodies, aptamers can fold into specific three-dimensional conformations structures to bind with target molecules containing stems, internal loops, purine-rich bulges, hairpin structures, hairpins, pseudoknots, kissing complexes, and G-quadruplex structures[29].

Today, there is a growing need for rapid, sensitive and specific and inexpensive methods for sensing and detecting purposes. While antibodies have long been considered to be the standard in molecular recognition and the use of antibodies as recognition probes, the relatively new technology of aptamers offers several advantages [30]. The so-called aptamers offer great flexibility in terms of structure variants to bind to a variety of targets with high affinity and specificity, and they are also promising candidates for bioanalytical applications [31]. Firstly, the in vitro aptamer selection process allows a greater control over aptamer binding conditions. No physiological salt concentrations, temperatures and pH can be used in successful selections [32]. Aptamers can show an improved stability over their protein-based antibody counterparts. In particular, aptamers can be reversibly denatured by changing the surrounding conditions[33]. For example, a variation in pH, temperature, ionic strength, or use of denaturants irreversibly denatures antibodies, while aptamers simply unfold; thus the aptamer structure can then regain its functionality when return to its original binding conditions[34]. However, due to the nucleic acid nature of aptamers, they bind to complementary nucleic acids as well as their targets. Aptamer generation has been achieved for a wide variety of targets molecules with high specificity and strong binding affinity from small molecules to protein, virus and even whole cells [25, 29, 35]. Generally, the SELEX process consists of several rounds of selection of sequences that bind to a target molecule (**Fig.1**). Each round includes three main stages:

- The oligonucleotides random DNA or RNA library is incubated with the target of interests;
- The bound oligonucleotides complexes with the target are washed from non-bound oligonucleotides;
- The bound sequences are amplified by PCR. As a result, Enrichment of the oligonucleotides library with sequences exhibiting the increased affinity to the target molecule occurs [36]. The winner sequence is named Aptamers that come out and the in vitro process is called SELEX.

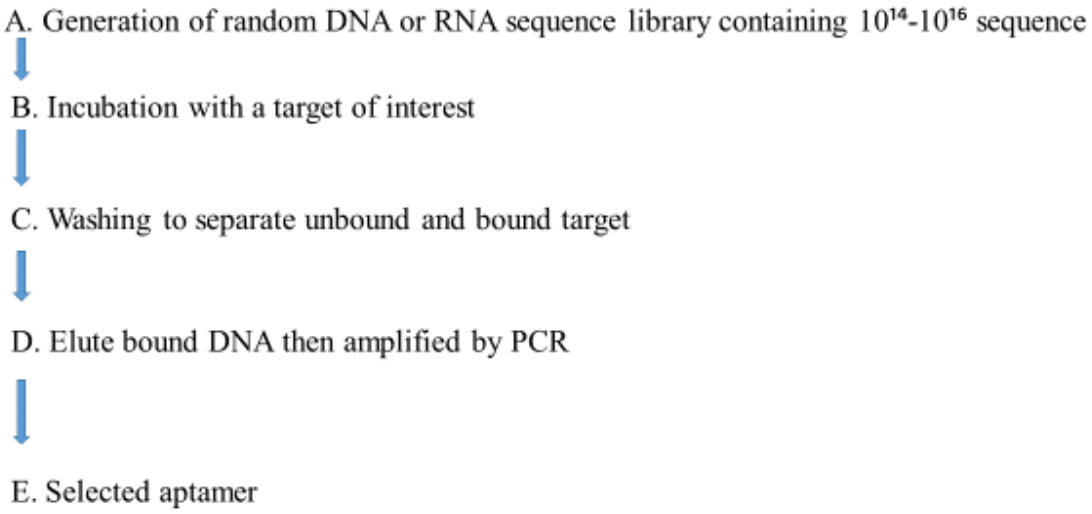

Fig.1.a

typical aptamer selection (SELEX) process

IV. APTAMERS AS ANALYTICAL REAGENTS

Nucleic acids were seen merely as informative molecules in opposition to proteins that carry the conformational information[16]. Aptamers are able to fold into a specific 3D structure including secondary structure elements of loops, hairpins, pseudoknots, triplexes, and quadruplexes with high affinity toward the specific target, from small chemical molecules to proteins and even whole cells [34]. Their ability to fold into numerous tertiary conformations to interact with almost any target render them to become increasingly important molecular tools for detection [37], Upon binding of the target, the aptamer undergoes structural changes and the target is bound by an induced-fit mechanism [38]. The binding process is based purely on the complementarity of molecular recognition of aptamer and specific target via the formation of intermolecular hydrogen bonds, electrostatic interactions, stacking interactions, and hydrophobic interactions bonds [15]. These affinities are similar or even superior to the affinities of monoclonal antibodies thus, their alternative is of much possible [32]. In addition to the high affinity and specificity, in other words, aptamers exhibit high selectivity. Moreover, the specificity of aptamers can be controlled during SELEX process [13].

V. APTAMER BIOSENSORS AS A NEW STRATEGY FOR DETECTION

A biosensor is a device that detects, transmits and records the information on a biological analyte [24]. Biosensors based on DNA or RNA aptamers (aptasensors) represent new type of the sensor that utilize unique properties of artificial receptor-aptamers [23]. Moreover, aptasensors are of considerable of great of interest due to their application in detection practically in unlimited kind of compounds of various sizes [33]. However, aptamers can be easily chemically modified by various chemical groups including fluorescence probes[39], colorimetric[40], quenchers [15], electrochemical indicators[41], nanoparticles or enzymes[42], which provides an extraordinary flexibility in different assays[43]. Furthermore, DNA aptamers are modified to improve their properties, and, more interestingly, they are reversibly denatured, converting them into ideal capture molecules for sandwich assays [44]. Therefore, aptamers modified to various solid

supports, provides their high stability and utilize various methods of detection in different research area for different purposes [15]. Thus, aptamers can be advantageously used as an alternative to antibody in the development of affinity biosensors [32]. On the other hand with antibodies, which at certain conditions can be irreversibly denatured, aptamers-based biosensors can be regenerated without loss of integrity and selectivity [23].

VI. GOLD NANOPARTICLES AS A NEW RECOGNITION ELEMENT

The typical structure of gold nanoparticals is spherical nano-sized gold particles [45], but they can also be composed of a thin gold shell surrounding a dielectric core, such as silica (gold nanoshells) [46]. In the last year, gold nanoparticles (GNPs) also named as colloidal gold, have a rich history in chemistry where they were used to stain glasses, cotton fibers for decorative purposes and employed as anti-aging components for skin protection [47] but nowadays, GNPs have attracted increasing attention in many research field for detection purposes [48]. Evidently, gold nanoparticles (ranging from 1 to 100 nm) are the type of nanomaterials and becoming emerging concept in nanotechnology for their food aspects and detecting platforms[49]. Additionally, GNPs have been synthesized by an array of methods which mainly are based on the reduction of chloroauric acid in the presence of a stabilizing agent [47]. The most commonly used method, the citrate synthesis method, includes reduction of chloroauric acid using trisodium citrate resulting into the formation of GNPs [50, 51]. Citrate capped GNPs are negatively charged, which can be exploited for electrostatic interactions with some positively charged biomolecules like antibody, aptamer and other biomolecules [47, 52]. Owing to their intrinsic characteristics, Gold nanoparticles (GNPs) have large specific surface area, good stability and interesting electrical, optical properties, and biocompatibility and are easy to prepare [15, 53]. Synthetic and biological compounds can be anchored onto the surface of GNPs via the strong metal–ligand interaction between sulfur and gold (the S–Au binding) [54]. More importantly, GNPs provided a good pathway of electron transfer and enhanced the immobilized amount of biomolecules [55]. In addition, Gold nanoparticles (GNPs) have attracted tremendous attention due to their unique optical properties, robust nature, stability, and their large surface areas in different research areas. Benefiting from these advantages, GNPs can consequently be applied to biosensors as molecular recognition element, disease detection, and clinical therapy[56]. By combining gold nanoparticle properties with aptamer specific ability and affinity, The use of aptamer-conjugated GNPs in different research area including food stuffs offers excellent possibility[17]. This is due to the fact that aptamers with their low cost, facile, pH and heat tolerance, minimum batch to batch differences, greater specificity and binding affinity to the cognate targets [48]. Thus, GNPs serve as excellent candidates for protein and aptamer bio-conjugation, because they readily react with the amino and cysteine thiol groups of proteins or modified aptamer[57]. Gold nanoparticles can be conjugated with DNAs, antibodies, enzymes and other bio-molecules, which can afford them promising applications in signal enhancement of bio-chemical detection [58]. Gold nanoparticles (GNPs) coupled with biomolecules such as aptamers have become increasingly important biological nanoprobes [20].

VII. APPLICATIONS

Recently attention has been paid by scientists towards Aptamer conjugated with the nanoparticles such as gold nanoparticles play vital role in many research field such as nanomedicine as the diagnostic and therapeutic tool and even in food safety delivery system [59]. However, Aptamers can potentially be applied to the detection of different pathogenic bacteria because they have advantages of high affinity and specificity, rapid and reproducible synthesis, and controllable modification [60]. Despite many previous

published citations, the characterization of aptamer−gold conjugates becomes easy due to advanced analytical techniques when compared with other sensing techniques (**Table 2**). The detection with aptamer conjugated gold nanoparticles indicated low detection limit compared to the other sensing methods.

TABLE 2: COMPARISON OF DIFFERENT SENSING TECHNIQUES FOR *S. AUREUS* AND APTAMER-CONJUGATED GOLD FOR OTHER TARGETS

Techniques	Application	Detection limit	Analysis time	References
Immunochromatographic assay	Chicken meat	10 cfu g^{-1}	25h	[52]
Electrochemical immunosensor	-	10 cfu mL^{-1}	-	[61]
Aptamer recognition coupled to tyramine signal amplification	water	8 cfu mL^{-1}	<4h	[62]
Colorimetric aptasensor	milk	9 cfu mL^{-1}	<4h	[14]
Electrochemical sensor	Beef meat	1.23 ng mL^{-1}	-	[63]
Rapid single cell detection by Aptamer conjugated gold	-	-	1.5 h	[3]
A colorimetric detection with aptamer–gold nanoparticle conjugates	Riboflavin	-	-	[64]
Gold nanoparticles conjugates-amplified aptamer Immunosensing screen-printed carbon electrode strips	Thrombin	25nM	-	[65]
A novel colorimetric aptasensor using gold nanoparticle	oxytetracycline	25nM	-	[40]

CONCLUSIONS

Aptamers are single-stranded synthetic oligonucleotides that are able to capture their target molecule with high affinity and specificity and interested the aptamer stability is of great importance for bacteria detection including *S. aureus* in food samples. Aptamers were discussed comprehensively to be attractive as an alternative to antibody-based biosensors for *S. aureus* monitoring and other bacteria or any other specific targets. Those aptamers are effective molecular recognition probes of high priority because they can detect different targets molecules with its ability to fold into numerous structure in different research area like food and drug quality, safety testing, disease diagnosis. etc. Therefore, aptamers can be thought of as nucleic acid-based alternative to antibodies, as they have a number of advantages over their amino acid-based antibodies. But arguably, one of these advantages is the high stability of nucleic acid of aptamers when subjected to a wide variety of conditions leading to a long shelf life and the possibility to regenerate the aptamer upon modification. Another standpoint of aptamer over antibody is the production of aptamer against small molecules where during antibody production, it fails. Consequently, aptamers are more flexible and can be more easily conjugated to a detecting agent (horseradish peroxidase, fluorophore molecule, or others) without perturbing the binding sites to which antibodies are difficult to produce. In addition, aptamers are selected in vitro selection process that can be settled out under desired conditions closely matched to those in which the resultant aptamer is required to work, thus ensures that the aptamers are

fit for purpose and optimized to work in the downstream assay conditions while for antibody, they don't work under desired conditions and they require extensive re-optimization. However, aptamer-conjugated gold nanoparticles have also high probability given the role of gold nanoparticles in stabilization and additional affinity of aptamers during detection.

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