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Review

Ultrasensitive detection of micrococcal nuclease activity and *Staphylococcus aureus* contamination using optical biosensor technology-A review

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ABSTRACT

One of the most common and important pathogenic bacteria is *Staphylococcus aureus* (*S. aureus*) which is known as a foodborne illness all over the world. The detection of micrococcal nuclease (MNase) can act as a unique diagnostic biomarker for the identification of *S. aureus*. So far, various complex methods have been introduced for the evaluation of *S. aureus* bacterium. However, they have different limitations such as labor-intensive, inaccurate results and time-consuming procedures. Thus, it is of particular attention to develop fast, easy, simple and more approachable detection methods based on nanotechnology and MNase detection. In this review, recent advances and modern techniques of ultrasensitive biosensors based on quantum dots (QDs), noble metal and magnetic nanoparticles (NPs), graphene oxide (GO) nanosheets, and also transfer energy strategy have been discussed for the identification of MNase activity and *S. aureus* contamination. Besides, advantages and disadvantages of different types of fluorescent, phosphorescent and colorimetric biosensors have been discussed.

1. Introduction

There are a wide variety of pathogenic spherical gram-positive bacteria [1], that the *S. aureus* is one of the most pathogens and it is associated with food poisoning worldwide [2]. In 1880, for the first time, it has been discovered by Alexander Ogston [3–5] and the meaning of the words *Staphylococcus* and *aureus* are bunch of grapes and gold respectively, because *S. aureus* grows in large yellow grape-like clusters colonies with a size of about 1 μm [5–7]. It can generate energy and produce mainly lactic acid by fermentation and aerobic respiration and also for growth, it requires some different nutrients such as amino acids and vitamins [8]. This bacterium exists naturally not only in the water, sewage, air, environmental surfaces and foods [9], but also skin and mucous membranes of healthy human beings [10]. It is able to survive under different conditions [11] and can grow in temperature ranges (10–50 °C) and different pHs (4.0–10) [7]. Approximately, thirty percent of the world population is a carrier of *S. aureus* bacterium without showing any symptoms [12,13] and basically, this bacterium causes a wide range of infections in humans such as skin and prosthetic device infections [14,15], meningitis and pneumonia [16,17].

One of the most common and important foodborne illnesses is staphylococcal food poisoning (SFP) that caused by the massive growth of *S. aureus* strains and can generate staphylococcal enterotoxins [11]. There are two kinds of staphylococcal enterotoxin, classical and newly identified enterotoxins that the classical enterotoxins are the main reason of SFP (about 95%) [12]. Generally, vomiting, diarrhea and nausea are the most routine symptoms of SFP and they require hospitalization, and it can lead to death in some susceptible people [18]. In the USA, yearly, more than 240 thousand cases of foodborne disease have been caused by *S. aureus* bacterium, which shows a major public health problem [15].

Different enzymes like proteases, lipases, nucleases, collagenase and

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hyaluronidase are produced by *S. aureus* [19]. These exoenzymes digest biomolecules into the food for growth the bacteria and increase invasive disease potential [19,20]. Among them, MNase enzyme is the most routine ones that is used as a diagnostic biomarker in order to detect the content, growth and contamination of *S. aureus* [20–22]. In 1956, this enzyme was firstly discovered by Cunningham and co-workers from *S. aureus* bacterium [23]. The MNase or thermostable nuclease (TNase) normally secreted by more than 90% of *S. aureus* strains [24]. Indeed, it is a nonspecific endoexonuclease that is able to digest both single and double-stranded DNA (ssDNA and dsDNA) and RNA [25–27] and can cleave and hydrolyze the AT or AU-rich regions specifically [28,29]. Besides, this enzyme has considerable stability under either food processing or storage conditions and after 60 min incubation at 97 °C, it stays alive and active [20]. More importantly, there is a deep correlation between *S. aureus* count and the existence of heat-stable MNase [30,31]. Thus, interestingly, detection and identification of MNase enzyme could be used as a unique indicator of *S. aureus* contamination [29].

There are different methods available for the detection and identification of *S. aureus* [5,14]. Among these, traditional procedures such as culture single-based assays, radioactive labeling, gel electrophoresis and enzyme-linked immunosorbent are widely used for *S. aureus* detection. Although, the detection results of the cultural traditional methods are accurate and appropriate but suffer from several drawbacks including time-consuming, selective enrichment, selective plating, arduous, requiring sophisticated instrumentation or isotope labeling, biochemical screening, serological confirmation and bacterial colony isolation and also some of these methods need three to five days to obtain the proper results of bacterial cells [32–35]. Therefore, in order to overcome the shortcomings of traditional methods, some unique and sensitive methods based biosensing platforms have been presented. To this aim, it is of supreme importance to develop new detection methods according to the biosensors [36,37].

A biosensor is an analytical device, utilized to detect biomolecules and combines biology, physic and chemistry [38–40]. It includes a biological recognition segment for specific/selective binding with a target molecule (proteins, enzymes, antibodies, drugs, cell receptors, tissues, toxins, DNA, etc.) and a physiochemical detector which converts a biological response to an electrical and optical signal [41–44]. Nowadays, the development of biosensors has attracted widespread attention because they are rapid, easy, highly sensitive, and highly selective and biosensors technology may have the potential to overcome numerous of the above-mentioned restrictions [45–48] and also they can be alternative systems for traditional methods in accurate analysis and diagnosis [48–51]. Different biosensing approaches have been reported for the direct detection of *S. aureus* and other bacteria based on antibody-antigen and aptamer recognition including surface plasmon resonance [52], enzyme-linked immunosorbent assay [53], electrochemical biosensing [54], optical based immunoassay [55,56] and quartz crystal microbalance [57,58]. In these systems, specific bio-affinity molecules such as antibody and/or aptamers are used for specific binding to target molecules *via* physical and chemical interactions. However, these modern direct detection methods have various excellent advantages, but they have some limitations such as high cost of production, low stability, and difficulties associated with purification and modification of antibodies. Hence, recently, other biosensors have attracted scientific attentions that detect the bacteria like *S. aureus* in indirect process. These biosensors operates based on the amplification of specific nucleic-acid sequences [59,60] or detection of toxins and other biomolecules excreted by the pathogen under certain situations [61,62]. Thus, these biosensors do not require further oligonucleotides like aptamers that they are expensive and susceptible to digestion by nucleases.

In our previous review, the direct biosensors based aptamers have been reviewed for *S. aureus* determination [36]. But to the best of our knowledge, there is no published review for indirect detection of *S. aureus* based on MNase activity. Thus, it is of particular attention and pressing need to review and investigate indirect assays for *S. aureus* determination that they are fast, easy, simple and more approachable detection methods based on nanotechnology and MNase activity and we believe, in a short time, there will be an enormous progress in the design of biosensors for detection of MNase if their shortcomings like lack of high selectivity are addressed well. Basically, in the case of indirect detection of *S. aureus*, only the optical biosensors have been studied based on fluorescent, phosphorescent and colorimetric methods [63]. Thereby, in the following sections, the ultrasensitive optical biosensors based on aforementioned methods have been reviewed and discussed for the detection of MNase and *S. aureus* contamination.

2. Ultrasensitive detection of MNase

2.1. Optical sensors

Optical sensors have emerged as an appropriate platform for the detection of biologically active materials. They have been developed due to their high sensitivity, quick reply, and simple method [63–65]. As mentioned earlier, optical biosensors can be categorized into various subclasses like reflection, absorption, dispersion, colorimetric, surface plasmon resonance, fluorescence, phosphorescent and chemiluminescence methods. Among these methods, biosensors based on fluorescence and phosphorescent detection have been attracted a great attention due to their high selectivity and sensitivity and have been generally used in chemistry, physic, biology, and pharmacology for the selective detection of biologically important molecules for more than one decade [66–68].

2.2. Fluorescent-based MNase sensors

Fluorescence is one of the most prevalent optical techniques and has been broadly utilized to biosensors due to its unique characteristics such as high sensitivity, high efficiency, fast detection speed and simplicity [69–71]. Fluorescence occurs when an excited molecule or nanomaterial emits light in reverting to its ground state [71]. Fluorescence-based detection techniques often use the incorporation of the fluorescent biorecognition element linked with an optical transducer. In this respect, optical-based biosensors usually use fluorescence resonance energy transfer (FRET) to detect various molecules [72,73]. FRET is based on the non-radiative transfer of excess energy between an excited molecule (generally called the donor) and an acceptor substance [74,75]. Numerous fluorophore donors and acceptors such as autofluorescent proteins, some natural colors, and inorganic nanostructures would be exploited as a segment of FRET-based biosensors [76].

QDs are becoming popular fluorophores for the fluorescence based biosensors due to their amazing properties such as strong and spectrally wide absorption, large surfaces for the addition of different molecules, color adjustment and high brightness and photostability [77]. QDs are able to act as both great energy donors and acceptor for different types of organic and inorganic molecules and nanomaterials [78].

Huang et al. for the first time used the QD-FRET bioprobe as a rapid and simple method for the quantitative detection of MNase enzyme with high sensitivity and specificity. They monitored MNase activity with using this fluorescence bioprobe for content detection of *S. aureus* bacterium. For this purpose, their fluorescence bioprobe consisted of two main parts: the modified ssDNA and the streptavidin-conjugated QDs. The 3′-end of the ssDNA was modified with biotin and 5′-end was modified with 6-carboxy-X-rhodamine (ROX), that operated as an acceptor. The optimum molar ratio of streptavidin-conjugated QDs to modified ssDNA was selected 1:2.46 to develop the method. In the absence of MNase, a color change was happened in the system from green to orange-red due to the transfer energy between QDs section and ROX moiety. Conversely, in the presence of MNase, because the MNase decomposed the ssDNA into the small DNA sections, the FRET did not occur between two sections and the color came back to green. As well as,

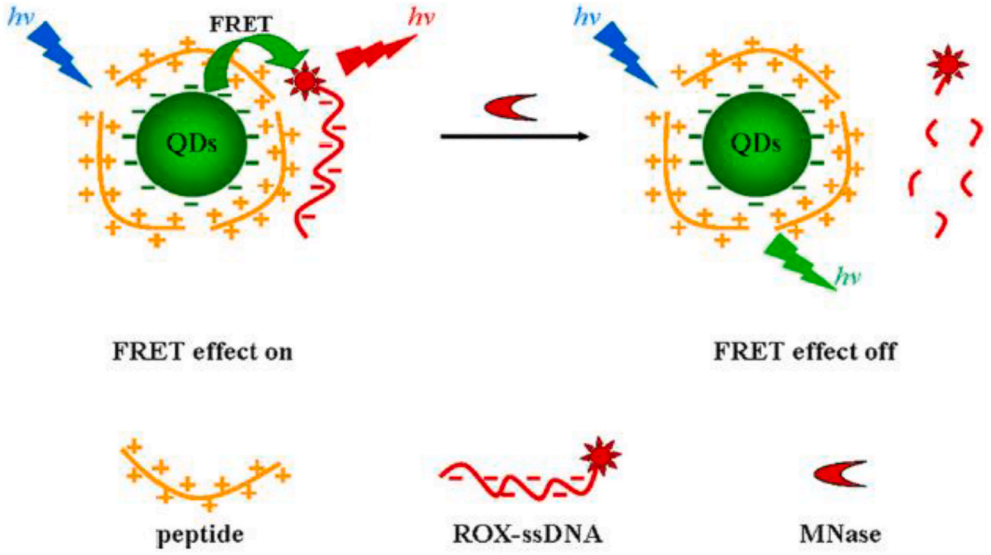

Fig. 1. Schematic description of the QDs peptide-based method for MNase detection. Reprinted with permission from Ref. [83].

this fluorescence QD based bioprobe was also examined for detection of another phosphodiesterase nuclease (ss-specific nuclease, S1) activity in different concentrations. The proposed fluorescence biosensor was not efficient for S1 nuclease and these observations confirmed the high specificity of biosensor for MNase detection. The limit of detection (LOD) of this method was determined up to 0.06 U mL^{-1} MNase [79].

Qiu et al. proposed a novel procedure based on electrostatic interactions and FRET between CdSe/ZnS core@shell QDs with positive charge and dye-labeled ssDNA with negative charge for quantitative detection of MNase. In that study, QDs supported lysozyme (lysozyme-QDs) with positive charges were considered as the energy donor and ssDNA with a sequence of 5-ROX-TAT ATG GAT GAT GTG GTA TT-3 and labeled by ROX (ROX-ssDNA) served as the energy acceptor. In order to change the negatively charged QDs to positively charged materials and better electrostatic interactions with negatively charged ROX-ssDNA, they employed lysozyme with a high isoelectric point (pI = 10.7–11) to coat the QDs and design lysozyme-QDs. The ROX-ssDNA was absorbed to the surface of lysozyme-QDs *via* electrostatic interaction, which led to the energy transfer from lysozyme-QDs to the dye. Before the MNase enzymetic reaction, due to the energy transfer from QDs to ROX-ssDNA, the fluorescence intensity of the QDs was reduced while the fluorescence intensity of the ROX-ssDNA increased. After the ROX-ssDNA was cleaved by MNase into the small DNA sections, the FRET efficiency was decreased due to weak electrostatic interactions between QDs and the shortened ssDNA. As a consequence, the fluorescence of the QDs was recovered. They claimed that, one of the advantages of this study compared to previous one was this method did not require streptavidin–biotin affinitive ligation and they used direct electrostatic interaction between negatively charged ROX-labeled ssDNA and modified positively charged QDs with assistance of lysozyme protein without any needs to chemical covalent bonding between Dye-labeled ssDNA and QDs. Besides, the specificity of their method was investigated for detection of two nucleases (S1 and EcoR I). These nucleases had no obvious effect on the fluorescence intensity of ROX-ssDNA and lysozyme-QDs [80]. Although, both S1 and MNase digested the AT-rich region of ssDNA, but S1 had the weaker activity compared to MNase and required certain pH and Zn(II) as an activator [81]. These results approved the high specificity of this biosensor for MNase detection. After optimum conditions, the LOD was reported 1.6×10^{-3} U mL^{-1} with a linear MNase concentration over the range of 8×10^{-3} to 9.0×10^{-2} [82].

A similar strategy was designed by Chen and co-workers for the simple and sensitive detection of *S. aureus* with adequate sensitivity of the MNase at 2.9×10^{-3} UmL^{-1}. On this platform, the energy donor was the mercaptoacetic acid immobilized QDs and the energy acceptor was the ROX-ssDNA. Also, in this project, they used positively charged peptide supported QDs that its role was the bridge between the QDs and negatively charged ROX-ssDNA which it can operate as an electrostatic linker to induce the close electrostatic interactions and better energy transfer as well (FRET). The isoelectric point of peptide was 10.76 with ten arginine units that indicated the appropriate positive charge. Similar to previous work, in the attendance of MNase, the ssDNA-ROX was hydrolyzed into the short DNA sections, and FRET efficiency reduced due to the feeble electrostatic interactions between peptide supported QDs and the short DNA sections (Fig. 1). Therefore, the fluorescence intensity of ROX-ssDNA acceptor was reduced and presented a sensing device for MNase detection and *S. aureus* contamination. In this study, the concentration of peptide and pH were the critical factors to increase the FRET efficiency. With the increase of concentration of positively charged peptide, due to the stronger interactions of modified QDs and ssDNA, the FRET efficiency was raised. The optimal peptide concentration was 2.0×10^{-6} $molL^{-1}$. Besides, the best pH was selected between 8.0 and 9.0 and with upper and lower these amounts, the fluorescence intensity of dye was reduced. The reason was, at the lower pHs, the less protonated carboxylic acid groups were formed on the surface of QDs and decreased the electrostatic interactions and at upper pHs, the formation of less charged peptide, reduced the interactions. Moreover, this sensing platform was studied on different ROX-ssDNA chains (5-mer, 10-mer, 20-mer) to investigate the effect of oligonucleotide length on the energy transfer. Interestingly, the fluorescence of dye gradually raised with increasing length of oligonucleotide (5–20 mer). On the other hand, they believed that, this method has less complexity compared to previous study and requires just one step conjugation reaction to obtain positively charged QDs, while the previous one about using the lysozyme is time-consuming and tedious to get positively charged QDs with two-step conjugation reaction and long modification processing. The MNase detection was successfully performed with a LOD of 2.9×10^{-3} U mL^{-1} [83].

Chandan et al. developed a new and rapid procedure for selective and sensitive detection of TNase which was the precise biomarker for quick identification of *S. aureus* based on monoclonal antibody and QD fluorescent sensing platform. In this research, with the use of streptavidin coupling strategy, the water-soluble CdTe QDs were conjugated with biotinylated monoclonal antibody (anti TNase). It is worth to mention that, after conjugation of biotinylated antibody with CdTe streptavidin-QDs, the bio-recognition specificity and bioactivity anti-

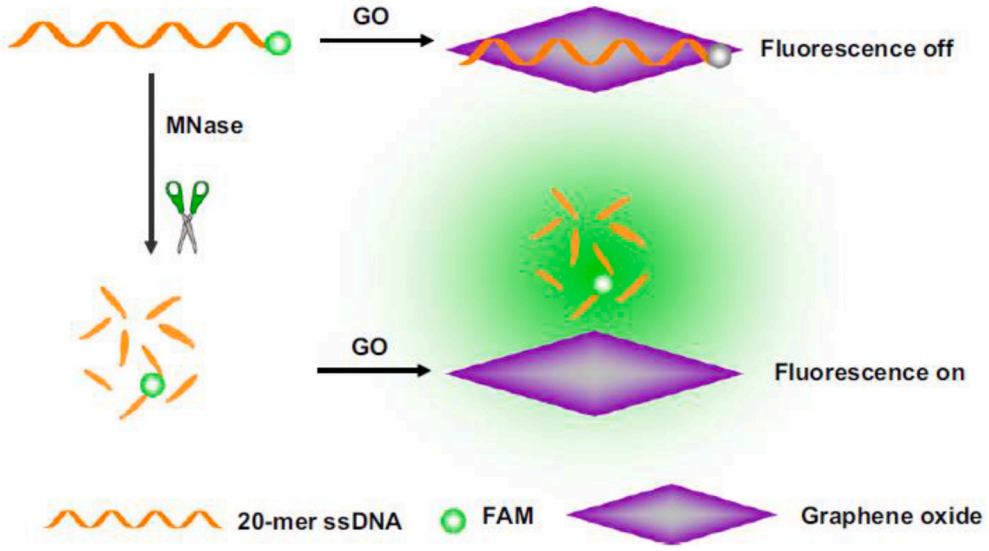

Fig. 2. Schematic mechanism of graphene oxide-based biosensor for the MNase detection. Reprinted with permission from Ref. [88].

TNase antibody were still retained. In this study, two assay methods were developed, FRET and strip based assay (nitrocellulose membrane). Briefly, In the case of FRET assay, the photoluminescence intensity of CdTe streptavidin-QDs was decreased proportionately with the increase of TNase (analyte) concentration. It was due to the increase in the number of acceptors and energy transfer from QDs-streptavidin to TNase. The LOD for this modified procedure was reported 0.5 ng mL^{-1} and a complete loss of photoluminescence intensity was obtained beyond the 50 ng mL^{-1} of TNase concentration. In the case of strip assay, with decrease the TNase concentration from 10 μg mL^{-1} to 3 ng mL^{-1}, the fluorescence was reduced proportionately and in the absence of TNase, no fluorescence emission was observed because of the lack of antigen antibody interactions. The LOD of strip assay was detected 3 ng mL^{-1}. Moreover, these assays were employed for *S. aureus* detection in several food and environmental samples such as bakery products (10 samples), chicken (6 samples), water (5 samples) and milk (5 samples). Interestingly, six samples of bakery products, two samples of chicken and three samples of water and milk were stayed positive for the target pathogen contamination, although, the strip assay was positive for only bakery products and water samples. Also, they believed that, with the use of this modified QD based method, immune assays like FRET assay and nitrocellulose paper-based QD-assay would be developed [84].

Graphene is a significant allotrope of carbon that recently due to the various features like electronic, mechanical, and thermal is become well-known between researchers [85,86]. In next researches, the biosensors have been developed based on the GO as an excellent energy acceptor [87,88]. It can decrease the fluorescence intensity of dye labeled ssDNA due to the strong electrostatic interactions [89]. In the first study, He and co-workers reported an ultrasensitive fluorescent sensing probes based on graphene and ssDNA for MNase detection. GO can adsorb nucleotide molecules like ssDNA *via* π-π stacking interactions, but cannot act as a good platform for adsorbing dsDNA and other biological molecules [90,91]. Thus, in this work, the 20-mer ssDNA (5′-FAM-TATATATATATATATA-3′) was modified with AFM dye as the nuclease target. In the absence of MNase, the high quenching of fluorescence intensity was observed due to the close proximity of FAM-labeled 20-mer ssDNA to the surface of GO nanosheets and energy transfers. Interestingly, with the use of MNase enzyme, it digested the FAM-labeled 20-mer ssDNA into the short oligonucleotide fragments and fluorescence intensity was gradually increased because of the low affinity of the short DNA fragments to be adsorbed on the surface of GO nanosheets (Fig. 2). More importantly, as the concentration of MNase increased, the fluorescence intensity of dye was promoted. Besides, with the increasing number of the bases of ssDNA from 5- to 20-mer, the fluorescence quenching of GO to oligonucleotide was raised. 99% of fluorescence quenching was obtained in presence of 20-mer ssDNA, while, it was just 46% for 5-mer ssDNA. These observations showed that the affinity of GO to adsorb the long ssDNA (20-mer) is remarkably higher than that of the short ssDNA (5-mer) [92]. The specificity of the method has been studied for other nucleases such as S1 and exonuclease I. Both of them could digest the ssDNA. The relative fluorescence F/F_0 (fluorescence intensity of the biosensor without and with nucleases, respectively) of the biosensor in the presence of MNase, S1 nuclease and exonuclease I was 22.43, 0.93 and 1.28, respectively which it was much bigger for MNase than others. The results showed that this platform was specific for MNase detection and was not efficient for other nucleases. In that study, MNase could be identified in a range of 8×10^{-5} to 1.6×10^{-3} U mL^{-1} with a LOD of 2.7×10^{-5} U mL^{-1} [88].

In the second study, Ravikumar and colleagues described the MNase detection method based on the surface energy transfer mechanism using an ultrasensitive and selective fluorescence sensor. In this project, the "ON-OFF-ON" GO based platform was designed for MNase detection using a monoclonal antibody conjugated GO ensemble. As mentioned before, the GO acts as a great acceptor and monoclonal antibody CdTe QDs operate as an excellent donor. Thereby, after a close contact to each other, the energy was transferred from monoclonal antibody QDs to GO which caused the quenching the fluorescence intensity of monoclonal antibody QDs. In addition, after sensing, due to the stronger affinity of monoclonal antibody QDs to MNase, the energy transfer did not occur from the QDs to GO and this strategy permitted the fluorescence emission. It is worth mentioning that, in FRET energy transfers, due to the dipole-dipole interactions, the acceptor (generally dye labeled DNA) and donor (normally QDs) come to a close contact and energy is transferred. But, in this research, monoclonal antibody had a bigger size compared to dye-ssDNA and the energy was transferred by nanometal-surface energy transfer and not FRET that could happen beyond 10 nm of distance between donor and acceptor. Notably, the "ON–OFF–ON" MNase detection probe was fabricated based on immobilized monoclonal antibody QDs on the solid support strips (nitrocellulose membrane). In first step, the monoclonal antibody QDs were absorbed on the surface of nitrocellulose membrane strips and there was not any nanometal-surface energy transfer and the state was "On" fluorescence emission. In the next step and in the absence of MNase, the GO was adsorbed on strips containing monoclonal antibody QDs *via* π-π stacking, thus causing the close proximity of monoclonal antibody QDs into the GO and consequently quenching of fluorescence of QDs by nanometal-surface energy transfer

(OFF). Conversely, in the presence of enzyme, the affinity of the monoclonal antibody to MNase was dominant due to the antigen antibody interactions and this inhibited the surface energy transfer from QDs to GO and led to fluorescence recovery (ON). Furthermore, different samples from local water (Harohalli and Kaggalipura lakes and Laksmanteertha river) and food sources (old and fresh milk bread and cow's milk) have been analyzed for *S. aureus* detection. Their results showed that the method was appropriate and effective for only water samples and both solution and strip assays showed the MNase presentation in local water samples. However, in the case of old milk bread (15 days old), the solution method was also effective and demonstrated the *S. aureus* contamination properly. The LOD for fluorescence-based assay and strips was reported 0.3 ng mL^{-1} and 0.5 ng mL^{-1}, respectively [87].

guanine (G)-quadruplexes are unique nucleic acid structures that are created by accumulated G-tetrads in G-rich DNA. G-quadruplexes have various biological functions and potential applications in diagnosis and therapy [93]. Due to their adjustable structures, they have hugely attracted researcher's interest for design ultrasensitive fluorescent biosensors [94–96]. He and Joao developed a simple and easy method for MNase detection based on G-quadruplex DNA and N-methyl mesoporphyrin IX (NMM) as a signal reporter. The fluorescence intensity of NMM is very low, but it can be enhanced when the NMM is interacted with G-quadruplex DNA [92]. Hence, in the absence of enzyme, after the addition of KCl, the G-rich ssDNA (d[$G_3(T_4G_3)_3$]) formed the G-quadruplex structure and then, the fluorescence intensity of NMM was extremely increased. In the attendance of MNase, the G-rich ssDNA was cleaved into small DNA sections and hindered the formation of G-quadruplex structure. Thus, the fluorescence of the NNM was reduced dramatically. Consequently, the emission intensity changes depend to the MNase concentration. It is worth mentioning that, K^+ is an important factor in order to form G-quadruplex structure and with increasing KCl concertation in the solution, the fluorescence intensity of mesoporphyrin increased proportionally. Interestingly, this like simple systems do not need complex and sophisticated design, materials and experiments, which decrease the complexity, overall assay time and experimental costs. On the other hand, they claimed that, this method can introduce the G-quadruplexes as new transducers for other nuclease sensing systems. This G-quadruplex-based biosensor system could be developed for other nucleases like S1 nuclease just by using the different reaction buffer. Under optimal conditions, the LOD was measured as 7.1 $\times 10^{-5}$ U mL^{-1} with a linear correlation to MNase concentration in a wide range of 1.2 $\times 10^{-4}$ to 2.4 $\times 10^{-3}$ U mL^{-1} [97].

A sensitive and label-free MNase detection was developed by Peng and co-workers using 5-mer ssDNA-templated Ag nanoclusters as a fluorescence indicator. In this study, the 5-mer acted not only as the substrate for MNase detection, but also as the scaffold for the silver nanoclusters (Ag NCLs) synthesis. Without the MNase, the ssDNA induced the synthesis of fluorescent Ag NCLs and showed the excitation/emission fluorescence peaks at 560 and 610 nm based on the undigested ssDNA scaffold. In the presence of enzyme, the oligonucleotide was cut by MNase, which prohibited the synthesis of Ag NCLs due to the lack of the ssDNA scaffold, resulting in decrease the fluorescence intensity. Moreover, the effects of pH and HNO_3 were investigated on the synthesis of Ag NCLs. The fluorescence intensity of the Ag NCLs enhanced gradually with an increment of pH value from 4.0 to 6.0 and HNO_3 concentration from 0.5 to 2.0 μL. With increasing pH beyond 6.0 and HNO_3 concentration beyond 2.0 μL, the fluorescence intensity reduced significantly. Compared to the previously studied fluorescence detections, this approach did not require any ssDNA labeled by dye and quencher and occurred without transfer energy. It is also a convenient, simple, and low cost method with high sensitivity and selectivity and can be developed to other nuclease assays. The fluorescence intensity showed a linear correlation to MNase concentration in the range of 0 U mL^{-1} to 2 $\times 10^{-4}$ U mL^{-1} with a LOD of 8 $\times 10^{-6}$ U mL^{-1} [98].

Recently, a new fluorescent method has been reported by Qing and co-workers based on copper nanoclusters (Cu NCLs) dsDNA template [99]. They developed a high selective and ultra-sensitive fluorometric strategy for the detection of MNase enzyme and evaluation of *S. aureus* contamination by dsDNA templated Cu NCLs. In that study, a dsDNA with AT-rich regions and protruding 3′-termini was selected as a template for Cu NCLs and MNase assay. Indeed, without the MNase enzyme, the lengthy AT-rich regions of dsDNA induced the synthesis of Cu NCLs with yellow excitation/emission fluorescence peaks at 340 and 570 nm. In contrast, with MNase, the dsDNA was digested into small and mono oligonucleotide sections and prevented the successful synthesis of fluorescent Cu NCLs and thus, quenching the fluorescence intensity was happened. They claimed that, one of the most advantages of the method was the high specificity and selectivity for MNase detection by employing the modified dsDNA which was not digested by other nuclease. To this aim, different types of ds and ssDNAs were exploited and examined for enzymatic digestion reaction. ssDNA and complete dsDNA were also excised in the presence of other nucleases, lacking absolute selectivity in MNase detection. While, dsDNA with AT-rich regions and protruding 3′-termini could be only digested by MNase, and showed the higher selectivity and specificity and negligible interference from other exonucleases. This method works in the 1.0 $\times 10^{-3}$ to 5.0 $\times 10^{-2}$ U mL^{-1} MNase activity range with the LOD value of 1.0 mU *S. aureus* mL^{-1} [99].

Very recently, another ultra-selective fluorescent protocol based on dsDNA and MNase activity was developed by Wang and co-workers. In this research, the previous strategy was utilized by enzymatic digestion of specific dsDNA (3′ protruding terminus), because ssDNA could be hydrolyzed by other nucleases such as Exonuclease I and interfere the MNase detection. Moreover, the difference of this method compared to previous one was employing nucleic acid dye SYBR Green I as the indicator of fluorescence intensity. Similar to the previous study, in the first step and absence of the enzyme, the AT-rich regions of dsDNA boosted the fluorescence intensity of SYBR Green I [100]. Although, the fluorescence increment of SYBR Green I was sequence-dependent [101]. In the second step and presence of the enzyme, it hydrolyzed the AT-rich regions of dsDNA and produced short oligonucleotide fragments and as a consequence, failed to improve the fluorescence intensity of SYBR Green I. On the other hand, the fluorescence signal of SYBR Green I was studied in the presence of denatured MNase. The solution contained inactive enzyme demonstrated the high fluorescence signal that was almost equivalent with the condition without MNase. It showed that, this ultra-selective fluorescent protocol can evaluate MNase activity either in biological or buffer samples with high selectivity. The advantages of this selective substrate-based fluorescent protocol were saving the testing costs of probes and reducing the probe labeling time compared to other procedures like nanomaterial based methods. The LOD of this method was reported 0.013 U mL^{-1} [102].

Lee et al. demonstrated a nuclease-responsive DNA probe for rapid and simple bacterial detection. They designed the probe including of a quencher (BHQ-1) labeled on the 3′-end and a FAM dye fluorophore labeled on the 5′-end in dsDNA structure that could be cleaved by nucleases, including DNase, exonucleases and endonucleases. These nucleases were released from both *Escherichia coli* and *S. aureus* bacteria after lysis buffer optimization. Briefly, before nuclease release, there are a close proximity between quencher and FAM dye fluorophore and resulted in minimize the fluorescence intensity due to the transfer energy. In the presence of these enzymes, the DNA probe digested by them, and the fluorophore and quencher were separated from each other and led to increase the fluorescence intensity. Besides, the fluorescence intensity of the dsDNA was studied in presence of different nucleases including DNase I, exonuclease and endonuclease secreted by *Escherichia coli* and *S. aureus* bacteria. Basically, the fluorescence intensity of the probe enhanced after release of these nucleases. Meanwhile, a high fluorescence signal was observed in presence of DNase I compared to other nucleases. The most advantage of this protocol was its rapid bacterial detection with only 1-min time-consuming and they believed that this protocol can be used for on-site bacterial contamination assay

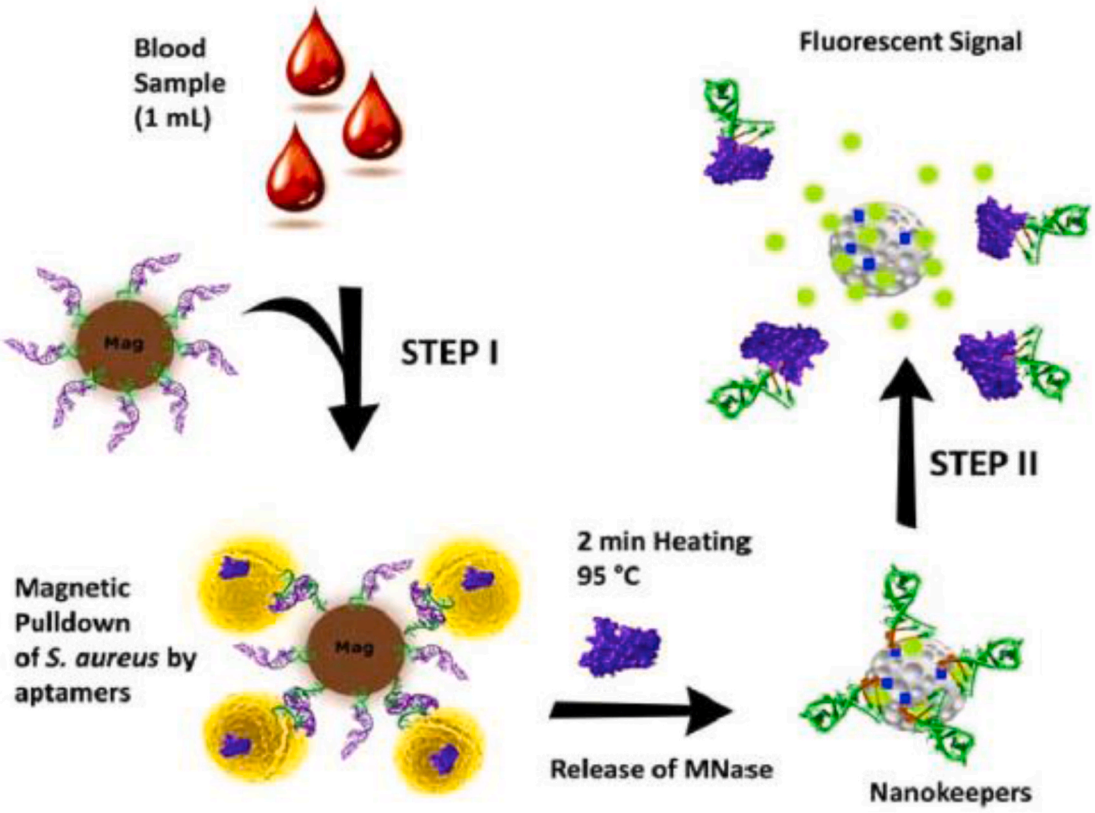

Fig. 3. Schematic representation of the stepwise determination of *S. aureus* by the specific opening of the pores of fluorescein loaded Nanokeepers. Reprinted with permission from Ref. [106].

in future. Moreover, this procedure could apply for both dead and live bacteria, preventing the false-positive signals unlike the adenosine triphosphate-based assay [103]. Therefore, two conditions consisting of heating or 70% EtOH treatment were selected to prepare dead bacterial samples. Attractively, the fluorescence intensity in heat-treated *Escherichia coli* and *S. aureus* bacteria was higher than 70% ETOH-treated condition. It indicated that the heat-treated method could not kill the bacteria completely and some of them were intact and hydrolyzed the DNA probe [104]. The LOD of this method was reported 10^3 CFU for *Escherichia coli* and 10^4 CFU for *S. aureus* [105].

Another study was conducted by Borsa et al., in which they developed the fast, sensitive and specific assay for MNase enzyme based on silica and magnetic NPs and triggered release of FAM dye fluorophore. This project had two steps. The first step was the magnetic preconcentration procedure from blood samples in which they prepared aptamer functionalized magnetic NPs and it was used for isolation of *S. aureus* bacterium from blood samples with pull-down procedure and after short heating of the sample, the MNase was released into the solution. In next step, the nanokeeper consist of MCM-41 type silica NPs immobilized with oligonucleotide probe and FAM dye fluorophore and quencher (called as TT probe) was added to the previous solution. It was observed that, in presence of *S. aureus* cells and secreted MNase enzyme, the fluorescence intensity was increased significantly due to the release of FAM (Fig. 3). The most advantages of this method were the effective detection of *S. aureus* infections directly in the human blood samples in only 10 min and the next one was that magnetic field separation and fluorescent reading of this procedure which could be exploited into in portable devices and automated systems. Moreover, the method was used as a sensing probe for the efficient detection of *S. aureus* bacteria as low as 682 CFU in whole blood samples [106].

2.2.1. *Phosphorescent-based MNase sensor*

Phosphorescence is a type of photoluminescence related to fluorescence. In contrast of fluorescence, phosphorescent materials do not promptly emit the light. Since, transitions were occurred very slowly, absorbed radiation is re-emitted at a lower intensity for up to several hours after the original excitation [107,108]. Phosphorescence is a less usual phenomenon than the fluorescence for biosensing platforms, but it represents several outstanding advantages over fluorescence emission, such as the longer emission lifetime, the wider gap between the excitation and emission spectra, and the lowest interference of the short-lived autofluorescence [109]. It also solves interference from autofluorescence and scattering light of matrix [110]. Recently, the room temperature phosphorescence has attracted extensive interest in optical biosensors due to the longer lifetime and appropriate delay over the autofluorescence of complex matrix and scattering light [111,112]. It works based on the phosphorescent resonance energy transfer (PRET).

In this respect, Li and colleagues developed a room-temperature phosphorescence sensor for detection of MNase and *S. aureus* contamination. The sensor was based on the PRET between poly-(diallyldimethylammonium chloride)-3-mercaptopropionic acid immobilized manganese-doped zinc sulfide QDs (modified Mn–ZnS QDs) and ROX-labeled 20-mer ssDNA with a sequence of 5-ROX-TATATGGATGATGTGGTATT-3 (20-mer ssDNA-ROX). Specifically, the 20-mer ssDNA-ROX was selected as the energy acceptor and the modified Mn–ZnS QDs was chosen as the energy donor. At first, without the MNase, the positively-charged modified Mn–ZnS QDs adsorbed the negatively charged 20-mer ssDNA-ROX *via* electrostatic interaction and the energy can be significantly transferred from the modified Mn–ZnS QDs donor to the 20-mer ssDNA-ROX acceptor, which strongly reduced the room temperature phosphorescence intensity of modified Mn–ZnS QDs. Then, after presence of MNase enzyme, 20-mer ssDNA-ROX was decomposed into small fragments, which led to decrease of electrostatic

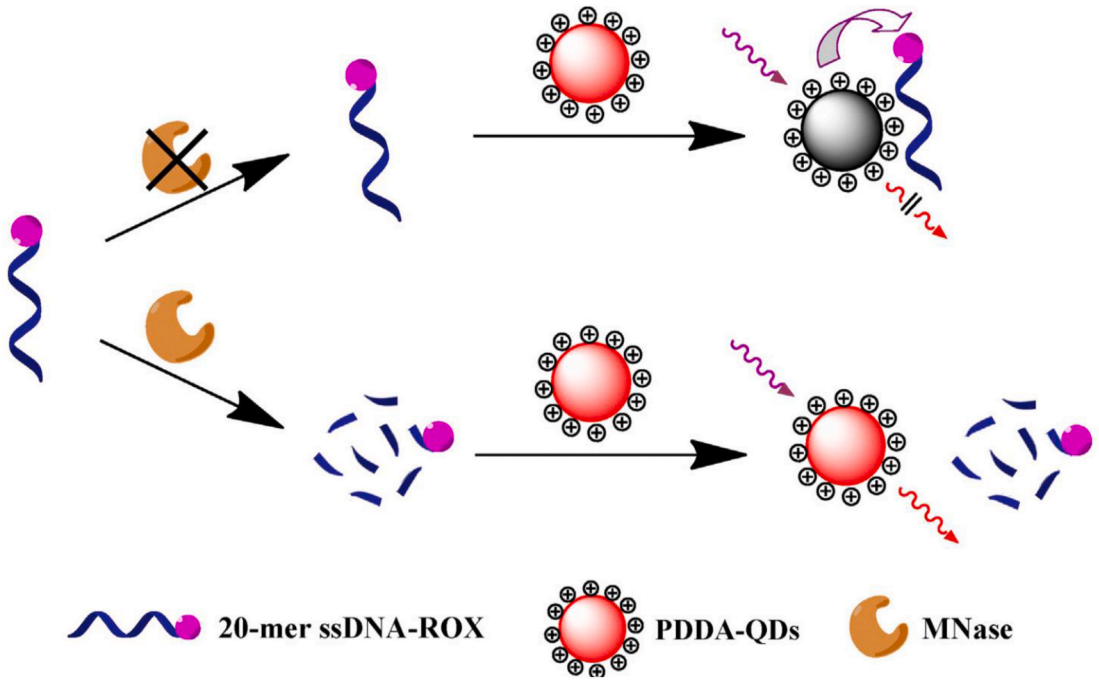

Fig. 4. Schematic illustration of fabricating of Mn–ZnS QDs for MNase detection. Reprinted with permission from Ref. [113].

interactions with Mn–ZnS QDs, and thus reduced the energy transfers and finally, gradual increase of room temperature phosphorescence intensity (Fig. 4). More importantly, with the concentration increment of 20-mer ssDNA-ROX, the phosphorescence intensity of modified Mn–ZnS QDs was slowly decreased, and maximum emission wavelength of modified Mn–ZnS QDs changed from 595 nm to 610 nm. Moreover, with the increment of MNase enzyme concentration, the room temperature phosphorescence intensity of the sample was gradually raised. Under the optimal conditions, the LOD of the method was reported 6×10^{-4} U mL^{-1}. This suggested room temperature phosphorescence sensor can solve interferences from autofluorescence and scattering light of matrix. Moreover, by changing the substrate and buffer conditions, this sensor would be an efficient method for detection of other nucleases and relevant biomolecules [113].

2.2.2. *Colorimetric-based MNase sensors*

Colorimetry is a solution-based assay that is used to ascertain the concentration of colored compounds [114]. Colorimetric identification

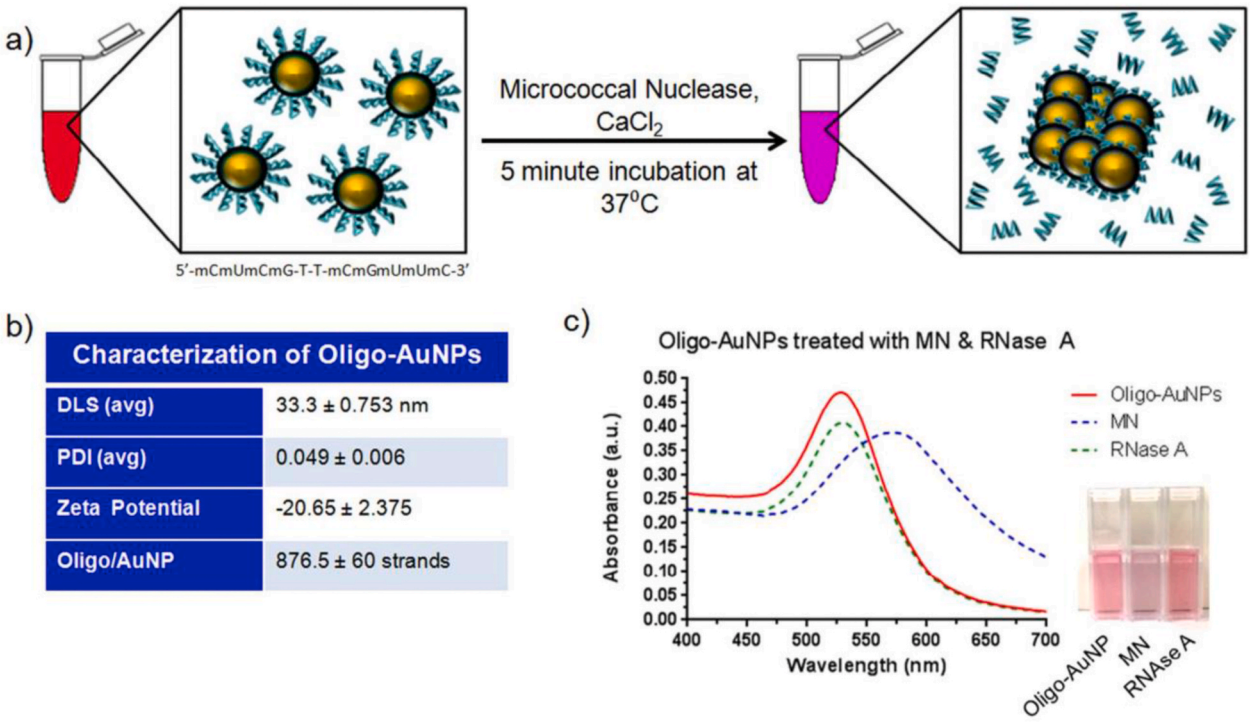

Fig. 5. a) Schematic description of MNase detection by AuNPs functionalized oligonucleotides, b) Characterization of Oligo-AuNPs by DLS, Zeta Potential, and DTT Displacement, c) Oligo-AuNPs were treated with 0.05 μM MNase or RNase A. Reprinted with permission from Ref. [124].

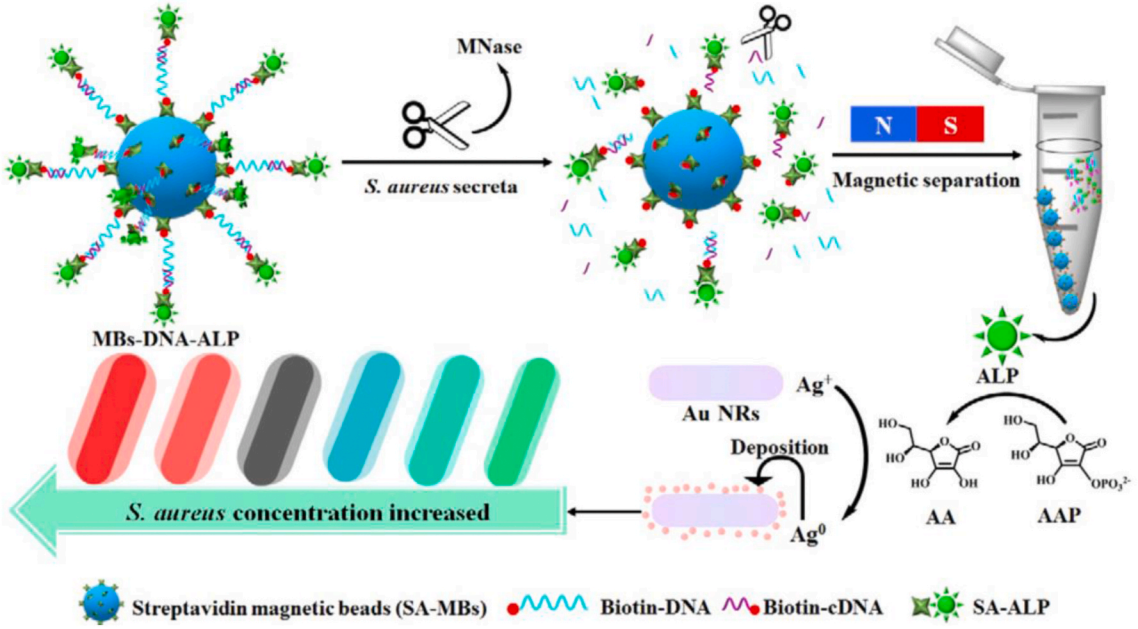

Fig. 6. The mechanism of dual enzyme-induced Au–Ag alloy nanoroads as colorful chromogenic substrates for the determination of *S. aureus*. Reprinted with permission from Ref. [125].

of biological compounds with the unassisted vision has attracted considerable attention due to its simplicity, low cost and practicality and these methods also do not require instrumentation [115]. These colorimetric procedures are usually carried out through the aggregation of metal NPs and changes in solution color [115]. One of the NPs that commonly and extensively used in the development of biosensors are gold (Au) NPs [116]. These NPs are perfect materials, owing to their unparalleled characteristics, such as facile water dispersal, biological non-reactivity, compliancy with surface functionalization, and capability to be tailored with uniform and various nano-sizes [117–119]. Au NPs have a surface plasmon resonance that is impressed by the geometry, size, ligand and proximity to other nanomaterials. Aggregation of Au NPs resulted in a shift in surface plasmon resonance and a colorimetric change from red to other colors [120] and these aggregations can be also induced by DNA sequences, proteins, and also metal ions [121–123]. Basically, this phenomenon is the fundamental principle behind many color-based biosensors. Au NPs functionalized with oligonucleotide can be employed as a selective and rapid method to detect *S. aureus* through color changes. The first oligonucleotide-functionalized Au NPs method has been reported by Tiet and co-workers. They immobilized the chemically modified 11-mer oligonucleotide with a sequence of 5-FAM-mC--mU-mC-mG-T-T-mC-mG-mU-mU-mC-ZEN-RQ-3 on the surface of Au NPs to detect MNase activity. Briefly, in the absence of MNase and *S. aureus*, the oligonucleotide-functionalized Au NPs were well dispersed in solution and showed a color of red. In contrast, in the presence of MNase enzyme, the 11-mer oligonucleotides was cleaved by MNase and led to the aggregation of Au NPs and finally, the λ_{max} shifted from 530 nm to 570 nm as well as a color changed from red to purple in solution (Fig. 5). Meanwhile, they found that the MNase required a salt to function as a co-factor. They examined the solution under three conditions, without salt, background levels of salt, and high levels of salt with $CaCl_2$. They observed that under condition in high levels of salt, the MNase acted selectively and it cut the 11-mer oligonucleotide at multiple sites. It suggested that by modifying the coating of Au NPs, the probe would be able to detect enzymes which secreted by other bacteria. Also, this oligonucleotide-functionalized Au NP method has been used in a more complex media such as ocean and creek waters with different concentrations of MNase. The LOD for creek and ocean water were 0.04 and 4.958 μM MNase, respectively. They claimed that, a huge decrease in sensitivity and a higher LOD of ocean water compared to creek water is due to the salinity of the ocean water or other organic contaminants that to overcome this problem, a purification step can also be performed before experiment. Thus, this approach held a big potential to detect of *S. aureus* bacterium in various water and food sources and presented fast and convenient responses with minimal sample preparation and required no extraneous instrumentation, but, this colorimetric approach was not a useful method for semi-quantitative visual detection. Moreover, the immobilization of biomolecules on the surface of Au NPs is often time-consuming and tedious. The LOD of this method was reported between 0.04 μM and 0.05 μM MNase [124].

Very recently, another Au NPs colorimetric biosensor was described by Zhou and co-workers. They introduced the colorful, rapid and sensitive colorimetric approach based on gold-silver alloy nanorods and magnetic NPs functionalized with alkaline phosphatase-oligonucleotide for semi-quantitative visual detection of *S. aureus*. Magnetic NPs functionalized with alkaline phosphatase-oligonucleotide were designed as a probe to detect the *S. aureus* bacterium. This procedure combined the enzymatic reaction with the excellent optical properties of Au NPs. Briefly and similar to previous studies, in the present of *S. aureus*, the MNase digested the AT-rich of oligonucleotide of probe into small fragments and the alkaline phosphatase was released into the solution. The released alkaline phosphatase induced the formation of Ag(0) NPs by ascorbic acid reduction reaction and eventually, generation of gold-silver alloy nanorods. Converting of L-ascorbic acid 2-phosphotrisodium salt into ascorbic acid was performed by the released alkaline phosphatase and the formed ascorbic acid reduced Ag + to Ag(0) and Ag(0) coated the Au nanorods. Consequently, the gold-silver alloy nanorods caused a notable shift of the surface plasmon resonance peak and an obvious rainbow-like color change from green to red (Fig. 6). With this colorful, rapid and sensitive method, a low concentration of *S. aureus* (25 CFU mL^{-1}) can be identified by naked eye observation. As such, this sensitive colorimetric approach has been analyzed for different complex biological fluids like urine, milk and river water. As they showed, urine and milk samples with a ten-fold dilution had the insignificant influence when compared with the MNase buffer sample. The water samples had no significant influence on the formation of gold-silver alloy nanorods. The most advantages of this study was the satisfactory recovery rates

Table 1
Comparison of different strategies of optical biosensors for indirect detection of MNase activity and *S. aureus* contamination.

Entry	Detection Method	Strategy	Type of materials used	LOD	Ref.
1	FL[a]	FRET between QDs and DNA-ROX and then FRET interruption due to the cleavage of DNA by MNase	CdSe/ZnS QDs ssDNA-ROX	0.06 U mL^{-1}	[79]
2	FL	FRET between positively charged lysozyme-QDs and negatively charged DNA-ROX and then FRET interruption due to the cleavage of DNA by MNase	CdSe/ZnS QDs ssDNA-ROX	1.6×10^{-3} U mL^{-1}	[82]
3	FL	FRET between positively charged peptide-QDs and negatively charged DNA-ROX and then FRET interruption due to the cleavage of DNA by MNase	CdSe/ZnS QDs ssDNA-ROX	2.9×10^{-3} U mL^{-1}	[83]
4	FL	FL quenching due to the antigen antibody interactions and FRET between QDs conjugated monoclonal antibody and TNase	CdTe QDs Antibody	0.5 ng mL^{-1}	[84]
5	FL	FL quenching due to the close proximity of DNA-FAM to the surface of GO and then FL recovery of FAM due to the cleavage of DNA by MNase and dissociation of DNA	GO nanosheets ssDNA-FAM	2.7×10^{-5} U mL^{-1}	[88]
6	FL	FL quenching due to the close proximity of monoclonal antibody QDs to the surface of GO and then FL recovery of QDs due to the antigen antibody interactions	GO, CdTe QDs Antibody	0.3 ng mL^{-1}	[87]
7	FL	FL quenching due to the cleavage of G-rich DNA and interruption in the formation of G-quadruplex structure	Mesoporphyrin G-rich ssDNA	7.1×10^{-5} U mL^{-1}	[97]
8	FL	FL quenching due to the cleavage of DNA and interruption in the formation of Ag NCLs	Ag NCLs ssDNA	8×10^{-6} U mL^{-1}	[98]
9	FL	FL quenching due to the cleavage of DNA and interruption in the formation of Cu NCLs	Cu NCLs dsDNA	1.0 mU mL^{-1}	[99]
10	FL	FL quenching due to the cleavage of DNA and interruption in the formation of dsDNA-SGI complex	dsDNA-SGI[d] complex	0.013 U mL^{-1}	[102]
11	FL	FL quenching due to the close proximity of DNA-FAM and BHQ-1 and then FL recovery of FAM due to the cleavage of DNA and dissociation of FAM from BHQ-1	BHQ-1[e] ssDNA-FAM	10^4 CFU	[105]
12	FL	FL recovery of FAM due to the cleavage of dsDNA by MNase and dissociation of FAM dye from quencher (TT)	Magnetic NPs TT-dsDNA-FAM	682 CFU	[106]
13	PS[b]	PRET between modified QDs and ROX-DNA and then PRET interruption due to the cleavage of DNA by MNase	Mn–ZnS QDs ROX-ssDNA	6×10^{-4} U mL^{-1}	[113]
14	CL[c]	Aggregation of AuNPs-conjugated-DNA due to the cleavage of DNA by MNase and color change from red to purple	AuNPs-ssDNA	0.045 μM	[124]
15	CL	Generation of Au–Ag NRs due to the cleavage of DNA by MNase and color change from green to red	Magnetic NPs Au–Ag alloy NRs	25 CFU mL^{-1}	[125]

[a] Fluorescence.
[b] Phosphorescence.
[c] Colorimetry.
[d] SYBR Green I.
[e] Black Hole Quencher.

and inexpensiveness, simplicity, and fast procedure (1 h), compared to microorganism culture approach which requires several days to count the colonies and also the good determination of *S. aureus* contamination in low-resource circumstances and it would be a convenient method for using in laboratory conditions and in environments. Besides, this method had better results in the case of different color changes compared to previous study [125]. Moreover, for better understanding and comparison of the above-mentioned optical methods, different strategies based on FRET (entries 1–3), fluorescence quenching (4–12), PRET (13) and color changes and colorimetry (14, 15) have been summarized in Table 1. In this review article, various inorganic materials like QDs, GO nansheets, noble metal NPs (Au, Cu and Ag) and magnetic and silica NPs and also different dyes and quenchers such as ROX, FAM, mesoporphyrin IX, SYBR green I and BHQ-1 have been reviewed and discussed.

3. Conclusion and future perspective

This review described an overview on recent advances in the using of biosensors for determination of MNase and *S. aureus* contamination. Due to the fantastic features of biosensors such as rapidity, simplicity, low-cost, high selectivity and sensitivity, they are becoming one of the most important methods for detection of biomolecules like proteins, enzymes, oligonucleotide and etc. and diagnostic applications compared to traditional methods. Generally, three types of routine approaches for the fabrication of MNase biosensors are fluorescent, phosphorescent and colorimetric methods. The colorimetric method is a sensing method that does not require extraneous instrumentation and it can be monitored by naked eye and also smartphone cameras. While, the fluorescent and phosphorescent methods are excellent analytical methods which are

developed based on signal transduction approaches. During the last decade, these detection systems are among the best and common ways to detect the MNase enzyme secreted by *S. aureus*. Thus, in this review, recent advances and modern techniques of ultrasensitive biosensors based on colorimetric, transfer energy, phosphorescence and fluorescence quenching strategies have been discussed. Besides, different nanomaterials and QDs as luminescent sources and various ss and dsDNA labeled by several dyes and quenchers have been reviewed for MNase detection and *S. aureus* contamination. We believe that in a short time, there will be a huge progress in the introduction of biosensors for detection of MNase if their shortcomings like lack of high selectivity are addressed well.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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