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Review Article

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Naked-Eye Sensing of SARS-CoV-2 Utilizing Nanozymatic Nanoassays

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Abstract

The first clinical diagnosis of the new infectious disease, COVID-19 was reported on December 31, 2019. The origin of this new infectious disease is a new generation of coronavirus, i.e., SARS-CoV-2. Since, the exploration of this concept, several methods have been developed for the diagnosis of COVID-19 via the detection of SARS-CoV-2. Among different methods, the nanozyme-based colorimetric sensors have attracted good attention due to the naked-eye response and simple procedure. Hence, the aim of this review is a quick overview of the nanozyme-based sensing and detection methods for early diagnosis of COVID-19. The main basis of these sensors is the detection of color variation of a nanozyme-mediated oxidation reaction in the presence and the absence of antigens of COVID-19. In this review article, we aimed to review the recent nanozyme-based colorimetric sensors for the detection of SARS-CoV-2 to provide a brief comprehensive insight into naked-eye sensing of SARS-CoV-2. The colorimetric nanozyme-based sensing of SARS-CoV-2 is based on the interaction of nanozymes with SARS-CoV-2 nucleocapsid protein, spike (S1) protein of SARS-CoV-2, SARS-CoV-2 phosphoprotein, SARS-CoV-2 RNA, or receptor of CD147 and then probing the color change of the nanozyme-mediated oxidation of peroxidase substrates. The progress or inhibition of color intensity of the reaction system is then assigned to the presence of SARS-CoV-2 and consequently positive result of the COVID-19 test.

Keywords: Nanozymes; SARS-CoV-2, Naked-eye sensing of SARS-CoV-2, COVID-19, Nanozyme-based colorimetric sensors

1. COVID-19 and its Origin

On December 31, 2019, the first case of a novel infectious disease with unknown origin (causative agent), features, duration of human transmission, and epidemiological parameters was confirmed in a designated hospital in Wuhan, a major city in China [1,2]. The studies on this new infectious disease revealed that a new generation of coronavirus, SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), is its causative agent [3-5]. Coronaviruses are a group of Coronaviridae families with a broad distribution in mammals which are known as the non-segmented positive-sense RNA viruses [6]. This novel disease caused by SARS-CoV-2 was called Coronavirus disease 2019 and termed COVID-19 by WHO on 11 Fed 2020 [7]. Although the human infections resulting from coronavirus are mild in most cases [6]. Shortly after the first report of COVID-19, the novel COVID-19 exhibited a high potential for outbreaks and becoming an epidemic disease and even a pandemic, as now we see in the world [8-11]. We know that common coronavirus infections such as SARS damage the cells through the binding of the SARS-CoV to the target cells via ACE2. In fact, the epidemiological findings in 2019-nCoV infections can be explained by the host's angiotensin-converting enzyme polymorphism. Additionally, some other researchers also believe that the novel pathogenic coronavirus, SARS-CoV-2, acts via binding to the ACE2 enzyme. More precisely, they believe that the novel coronavirus accesses host cells by affecting the ACE2 enzyme (angiotensin-converting enzyme 2) by connecting to the peplomer (a special surface glycoprotein) of the enzyme. The ACE2 is the most abundant enzyme in the type II alveolar cells of the lungs, hence, the 2019-nCoV has entered the host cells through the peplomer and destroyed the lungs. Although researchers noted that hypertension is a high risk for COVID-19 patients, however, the mechanism underlying this link is unknown. They emphasized that the high blood pressure in COVID-19 patients may damage the ACE2 receptor-expressing endothelial or alveolar epithelial cells in the lung [1-11].

2. Nanozymes And Their Potential Application

The fast development of nanoscience and material chemistry has increased interest in researching new and innovative synthesis methods to produce new nanomaterials with unique catalytic activity [12,13]. unique optical properties [14-16]. high active area antibacterial properties and high biocompatibility [17-19]. The new field of nanozyme-based catalysis, which has been introduced as an alternative to enzyme-based catalysis, is called nanozyme chemistry. On the other hand, nanozymes are known as nanomaterials with high enzyme-like activity and can be used to simulate enzymatic reactions in harsh environmental conditions (for example, higher temperature or wider pH range) [20-27]. Although, the traditional way for improving the enzyme

properties, is the immobilization of the native enzyme on support or their cross-linking to each other using a two-side activated linker [27-30]. However, recently, the design and development of nanozymes were considered as an interesting way for this aim [21,31]. The first report on the enzyme-like nanomaterials was in 2007 by Gao et al. [32] which introduced the iron oxide nanoparticles as the peroxidase-mimicking materials with high catalytic efficiency and characteristic stability. After the first report of Prof. Gao as the pioneer and founder of the nanozyme field in sensing, detection, and catalysis, various nanomaterials such as noble metals, metal oxides, and carbon materials were introduced as enzyme mimics (nanozymes) [33-41] (Figure 1). For instance, up to date, different types of nanozyme-based sensors such as single nanozymatic sensors, enzyme-nanozyme hybrid sensors, etc. have been developed. Recently a new generation of nanozyme-based systems called the "multinanozyme system' was introduced by Hormozi Jangi et al. (2020) [35,36]. During the last years, a wide variety of nanozyme-based colorimetric sensors have been developed for the detection and quantification of a variety of analytes for instance, glutathione (GSH), dopamine, tetracycline, metal cations, glucose, H2O2, explosives, and cysteine. It is notable that after the first report of COVID-19 in 2019 [1,2]. The nanozymes were utilized for the detection of SARS-CoV-2.

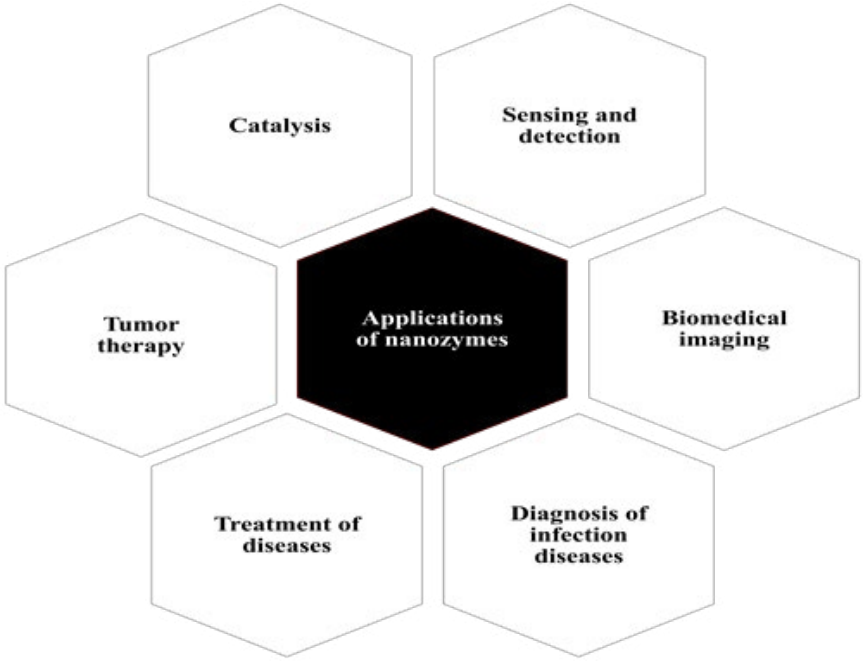

Figure 1: Different applications of nanozymes in the real world.

3. Current Methods For Detection Of Sars-Cov-2

Currently, there are several methods for diagnosis of COVID-19 via detection of SARs-CoV-2 or other ways (Figure 2) including real-time reverse transcription-polymerase chain reaction (rRT-PCR), hematology examination, polymerase chain reaction (PCR), diagnostic guidelines based on clinical features, Chest CT scans [1]. Here, it is challenging to develop effective diagnostics and therapeutics against SARS-CoV-2 [11]. However, yet actual testing methods to identify SARS-CoV-2 are limited. Besides, diagnosis of this new pandemic over SARS, MERS, and H1N1 is one of the most challenges due to their very similar clinical characteristics. To overcome these difficulties, recently, nanozmyes-based systems have been applied for fast, accurate, reliable, and cost-effective early diagnosis of COVID-19.

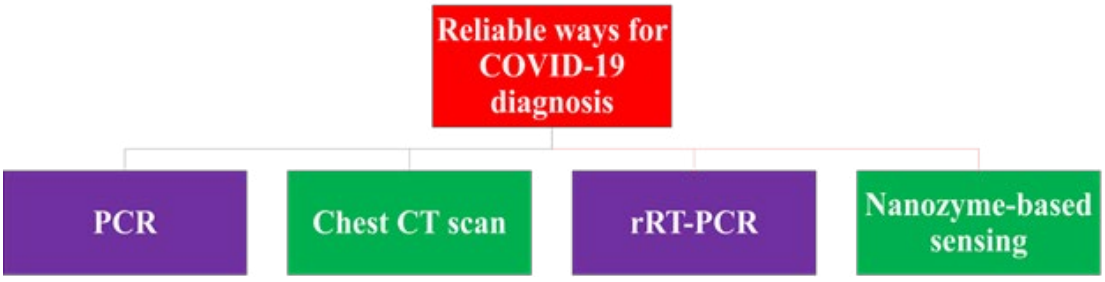

Figure 2: Current reliable methods for the clinical diagnosis of COVID-19.

4. Nanozymes Application For Diagnosis Of Covid-19

There are several reports in the literature regarding the application of nanozymes for the diagnosis of COVID-19 via the detection of SARS-CoV-2. In fact, the colorimetric nanozyme-based sensing of SARS-CoV-2 is based on the interaction of nanozymes with SARS-CoV-2 nucleocapsid protein, spike (S1)

protein of SARS-CoV-2, SARS-CoV-2 phosphoprotein, SARS-CoV-2 RNA, or receptor of CD147 and then probing the color change of the nanozyme-mediated oxidation of peroxidase substrates. The progress or inhibition of color intensity of the reaction system is then assigned to the presence of SARS-CoV-2 and consequently positive result of the COVID-19 test. For example, Liang et al. (2021) [42].Developed a nanozyme-linked nanosensor for the rapid and quantitative diagnosis of COVID-19 by detecting the SARS-CoV-2 nucleocapsid protein in human blood (Figure 3). The system is integrated with disposable immunochromatography assay and optical sensor devices. Immunoreaction and enzyme-catalyzed substrate color reaction were carried out on the chromatographic strip in a device, of which the light signal was read by a photometer through a biosensor channel, and the data was synchronously transmitted via the Bluetooth to the app in-stored smartphone for reporting the result. With a limit of detection (LOD) of 0.026 ng/mL NP, NLICS had a linear detection range (LDR) between 0.05 and 1.6 ng/mL NP, which was more sensitive than conventional ICA. NLICS took 25 min to report results. For the detection of NP antigen in clinical serum samples from 21 COVID-19 patients and 80 healthy blood donor controls, NLICS and commercial enzyme-linked immunosorbent assay had 76.2% or 47.6% positivity, and 100% specificity, respectively, while a good correlation coefficient (r = 0.99) for quantification of NP between two assays was obtained.

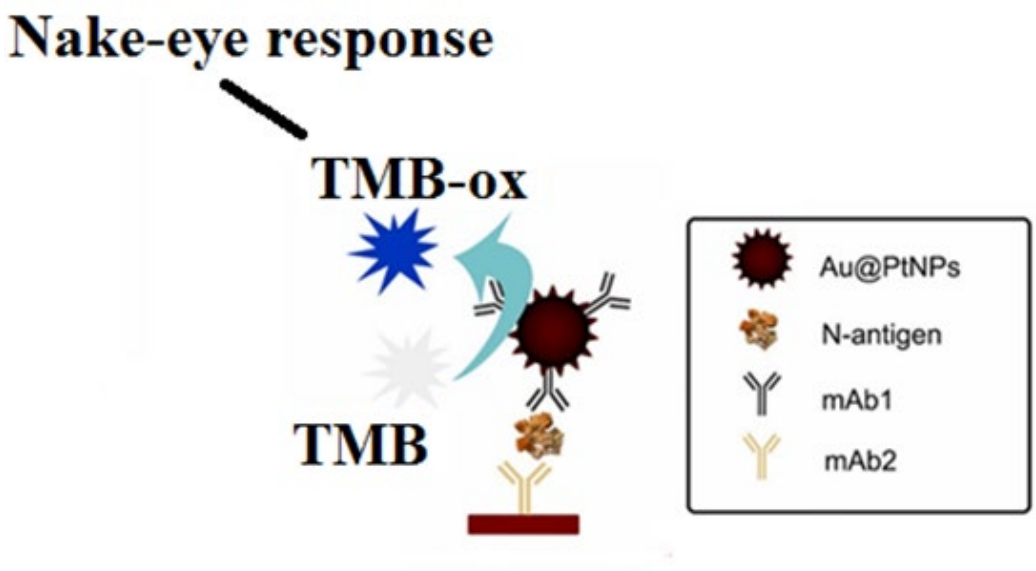

Figure 3. Au@ptNPs as nanozymes for naked-eye detection of SARS-CoV-2 via interaction of nanozymes with SARS-CoV-2 nucleocapsid protein (adopted from Liang et al. [42]).

Fu et al. (2021) [43]. Used porous metallic gold@platinum nanozymes for the diagnosis of COVID-19 via colorimetric detection of spike (S1) protein of SARS-CoV-2, obtaining a wide linear working range over 10–100 ng mL^{-1} along with a low limit of detection (LOD) of 11 ng mL^{-1}. The schematic representation of this colorimetric sensor is presented in Figure 4, as seen in this figure, the basis of the system is the detection of SARS-CoV-2 via the interaction of antibody-linked nanozymes with the spike (S1) protein of SARS-CoV-2 and probing the color change of peroxidase substrate, TMB. In fact, in the presence of SARS-CoV-2, the antibody-linked nanozymes interact with the spike (S1) protein of SARS-CoV-2 and then the oxidation of colorless TMB by hydrogen peroxide can catalyze by the conjugated system to produce the corresponding blue-colored oxidation product which can be used for naked-eye sensing of SARS-CoV-2.

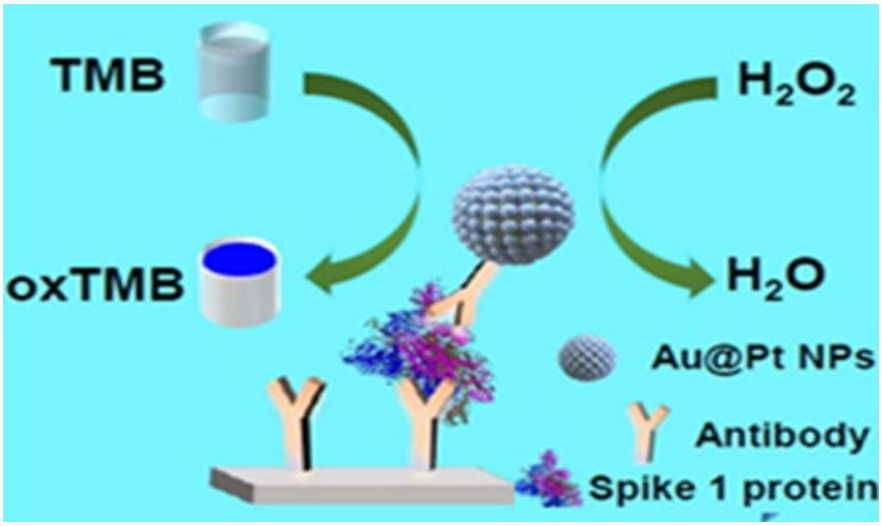

Figure 4. Colorimetric nanozyme-mediated sensor for detection of SARS-CoV-2 via interaction of antibody-linked nanozymes with the spike (S1) protein of SARS-CoV-2 (adopted from Fu et al. [43]).

Liu et al. (2021) [44] introduced a smartphone-based nanozyme-linked immunosorbent assay for quantitative sensing of SARS-CoV-2 nucleocapsid phosphoprotein in 37 serum samples from 20 patients infected with COVID-19. Moreover, Zhao et al. (2022) [45] employed MIL-101(CuFe) nanozymes for accurate visual naked-eye diagnosis of COVID-19 via detecting the universal receptor of CD147, providing a very low detection limit of 3 PFU/mL and a detection time as short as 30 min. Besides, Wu et al. (2022) [46] developed a MnO2 nanozyme-mediated CRISPR-Cas12a system for naked-eye diagnosis of COVID-19. In this system, the MnO2 nanorods were initially linked to magnetic beads using a single-stranded DNA (ssDNA). Also, in 2023, He et al. [47] performed a nanozyme-based colorimetric method for naked-eye diagnosis of COVID-19 by iron manganese silicate nanozymes as peroxidase-like nanozymes (Figure 5). They constructed a cut-price colorimetric assay for the detection of SARS-CoV-2 RNA by utilizing iron manganese silicate nanoparticles as peroxidase-like nanozymes. The iron-manganese silicate nanoparticles catalyze the oxidation of chromogenic substrates to produce the colored products. However, the color intensity was effectively inhibited by pyrophosphate ions. Hence, thanks to the large number of pyrophosphate ions generated by amplification processes, SARS-CoV-2 RNA can be detected by a colorimetric readout visible to the naked eye, as reported by He et al. [47]. Overall, as can be seen in Figure 4, if the blue color of the oxidation product of peroxidase substrate, TMB, can be observed after interacting the nanozyme with the test sample, the test result is negative while upon inhibition of color intensity, the result of COVID-19 test is positive.

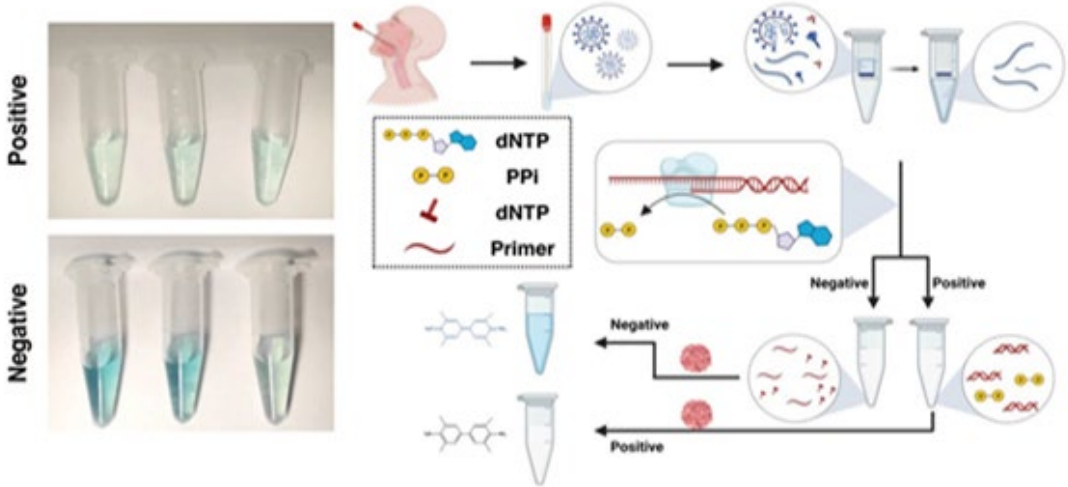

Figure 5. Nanozyme-based colorimetric method for naked-eye diagnosis of COVID-19 by iron manganese silicate nanozymes as peroxidase-like nanozymes via detection of SARS-CoV-2 RNA (adopted from He et al. [47]).

5. A Perspective on The Future of Nanozyme-Based Detection of Sars-Cov-2

The nanozyme-based systems and nanozyme chemistry are still growing and every year, several reports on the biochemical characterization of nanozymes and their application in different fields have been published by several researchers [48-53]. However, despite the considerable progress in this field in recent years, there is still space for progress in this field. since, the aim of this review is to focus on reviewing the nanozyme-based detection systems for diagnosis of COVID-19 via detecting SARS-CoV-2, in this section some key directions were proposed as a perspective for future studies on the nanozyme-based detection of SARS-CoV-2 to enhance the figures of merit of these systems; study of nanozymes;

1) Developing multinanozyme systems for the detection of SARS-CoV-2 to enhance the selectivity and sensitivity of the detection system and consequently reducing the false positive results of such sensors.

2) Developing commercial kits for accurate and fast naked-eye detection of SARS-CoV-2 with comparable accuracy to the standard real-time-PCR method.

3) Synthesis of new nanozymes with high specificity (specific active nodes) toward interaction with SARS-CoV-2 (specific not only selective) to reduce the false-positive or false-negative clinical results.

6. Conclusions

The first clinical diagnosis of the new infectious disease, COVID-19 was reported on December 31, 2019. The origin of this new infectious disease is a new generation of coronavirus, i.e., SARS-CoV-2. Since, the exploration of this concept, several methods have been developed for the diagnosis of COVID-19 via the detection of SARS-CoV-2. Among different methods, the nanozyme-based colorimetric sensors have attracted good attention due to the naked-eye response and simple procedure. Hence, the aim of this review is a quick overview of the nanozyme-based sensing and detection methods for early diagnosis of COVID-19. The main basis of these sensors is the detection of color variation of a nanozyme-mediated oxidation reaction in the presence and the absence of antigens of COVID-19. In this review article, the recent nanozyme-based colorimetric sensors for the detection of SARS-CoV-2 were reviewed to provide a brief comprehensive insight into the naked-eye sensing of SARS-CoV-2.

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