

The Applications of Magnetic Nanoparticles in SARS-CoV-2 Virus Test

Lian Bu *

Beijing huijia private school, Beijing, China

* Corresponding author: 23bulian@huijia.edu.cn

Abstract. In the past few decades, significant progress has been made in the applications of magnetic nanoparticles. Due to the ultra-high surface area-to-volume ratio of magnetic nanoparticles, the magnetic nanoparticles have relatively high chemical reactivity and strong catalyzing ability, magnetic nanoparticles became increasingly important in the field of biochemistry and medical treatment. Especially in recent years, magnetic nanoparticles have played an important role in the detection of corona Virus. This article is going to introduce the application of magnetic nanoparticles in the identification corona virus. It will explain the working principle of magnetic nanoparticles when functioning independently, and analyze how will it cooperate with other corona virus tests.

Keywords: SARS-CoV-2 virus test, magnetic nanoparticles, COVID-19.

1. Introduction

Coronavirus disease (COVID-19), which is a severe acute respiratory syndrome, is caused by the infection of SARS-CoV-2 virus. It will spread from a patient's mouth or nose in small liquid particles while he/she is coughing, sneezing and speaking [1]. Therefore, people will easily catch this disease in their daily life, which resulted in an extremely fast spreading rate. Until now, almost 10% of people in the world have been infected, and the mortality is gigantic [2]. Hence, in order to prevent the disease from further spreading at a fast rate, effective diagnostic tools are urgently needed.

The magnetic nanoparticles are small particles (between 1~10nm) that contain several magnetic elements such as iron, nickel, cobalt, chromium and their chemical compounds. They are super-paramagnetic, which means that their magnetization can flip to another directions under different temperature levels. Because of their nano scaled size, there are countless possible future inventions that are related to either their bare forms or coated forms that are designed for specific uses. The magnetic nanoparticles have been applied in different fields such as cell separation, protein purification and metallurgy industry. Therefore, under this kind mature and well-developed technological background, the usage of magnetic nanoparticles in the test of COVID-19 is potential and promising [3].

In recent years, because of the massive background information provided by different researchers and the urgent need of an economic, efficient and sensitive test, the usage of magnetic nanoparticles in the detection of the corona virus become the center of attention. In other words, it can not only be used as a single test but also cooperate with many other existed SARS-CoV-2 virus tests, such as real-time polymerase chain reaction, enzyme-linked immunosorbent assay and gold nanoparticles test, and the pitfalls of these tests will be improved through the special properties of magnetic nanoparticles. Based on the information hitherto gathered, this article is going to evaluate these three tests that have been currently used widely and explain how can magnetic nanoparticles work as a tool of minimizing the inaccurate results, improving the efficiency for other tests and letting the test become more applicable. Therefore, the long-cherished goal of inhibiting the virus can be achieved [4-5].

2. The application of magnetic nanoparticles in detecting corona virus

2.1. Independent usage

The functionalized magnetic nanoparticles which have been coated by protein A (Figure 1a) are going to be used to detect simulative corona virus that is composed of a single streptavidin-coated polystyrene bead with one hundred SARS-CoV-2 spike proteins attached (Figure 1b). Then, the magnetic nanoparticles are going to be exposed under a strong magnetic field which will normally the magnetic nanoparticles can freely rotate. However, if the corona virus exists, the rotation is going to be restricted. Therefore, poor harmonic spectra are being produced, which will result in lower MPS signals (figure 1 c). Consequently, by recording the MPA signals, people can gather quantitative data when detecting SARS-CoV-2 virus particles[6].

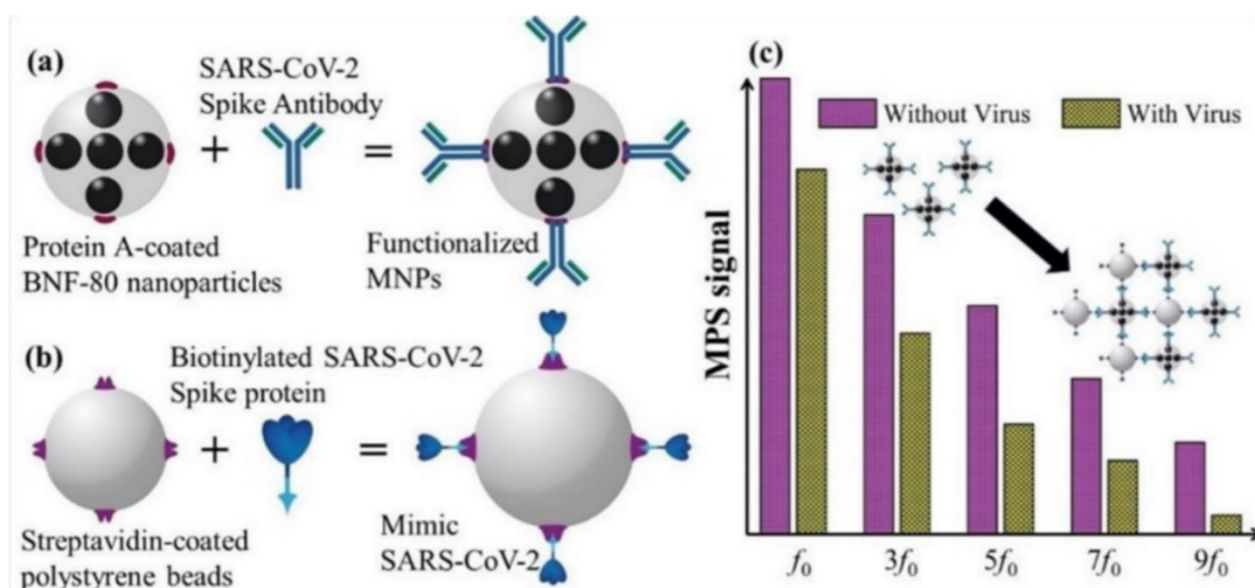


Figure 1. Working principle of magnetic nanoparticles (a) The production of functionalized magnetic nanoparticles (b) The production of mimic corona virus. (c) The MPS signal recorded with and without the presence of simulated corona virus.

2.2. Combination between magnetic nanoparticles test and RT-PCR

Currently, the RT-PCR is regarded as the most widely accepted diagnostic test. It works by matching the SARS-CoV-2 primers with the DNA in the sample and amplify the sequence to create many copies that can maximize the viral property. This test is the only specific diagnostic test with a specificity of up to 95%, which allows it to be widely used in most places [7].

However, the RT-PCR test is still waiting to be improved in certain aspects. Firstly, it can not identify the true positive and true negative results. According to previous researches, in a group of 10,000 individuals, there are 1000 positive cases and 9000 negative cases. In the group of 9000 uninfected people, 8820 of them are true-negative cases and 180 of them are false-negative cases. The false-negative results can be affected by certain factors [8]. The first one is the place where the samples are derived from. In other words, different specimens that have been taken on the same person can be different just because of the variance in viral load on the tested site. According to researches, the RT-PCR tests that are taken in nasal and oropharyngeal are more accurate [9]. The second factor is related to the denaturation and degradation of the viral RNA samples that's being caused by improper dealing processes or storage methods. Hence, the quantity of intact RNA that can be used for the test will decrease and the false-negative results will be generated. The situation will even become more serious at places where the technology hasn't been well developed or the operators haven't been professionally trained, since the probability of the contaminated samples is going to increase. The third factor is related to the duration of the sample. Since a usable viral load is limited

within a limited period. During this specific period, viruses can replicate themselves rapidly and escape from the attack of cells. However, once the period exceeds this particular span, the results will become inaccurate, because the viral samples become incomplete. For the false-positive identification, according to diagnostic examples, in 1000 infected people, 950 of them are true negative and 50 of them are false negatives [8]. The false-positive result occurs accompanied with the non-professional manipulation. Then, the contaminants from other viral sample will lead to wrong diagnostics[10]. Since bio-samples from the human body, which in this case is RNA, are unique; once the sample has been contaminated, the consequence is irreversible and the data will be wasted[11]. In addition, Since the RT-PCR is a RNA-based test, it simply detect the existence of viral DNA, so ever after the recovery of patients, it can still detect some left viral RNAs in the sample and provide a false positive result[12]. Therefore, the patient may suffer great mental pressure and there's a high risk of accidentally being infected under the condition when this person has more chance to contact a disease-carrying object or person.

Secondly, the RT-PCR requires thermocycler apparatus that can provide the accurate cycle of temperature changes, meanwhile this equipment is very expensive. In addition, it is often confined to a laboratory and can only be manipulated by specific workers. Besides, the spectrofluorimetric apparatus coupled with computers that are required for giving out quantitative data of the RNA amount in the samples is also very expensive and uneasy to operate [13]. Therefore, some poor countries and remote areas that have no ability to access to or manipulate those machines can't use RT-PCR as their test, which means that a wide propagation of this testing method can't be achieved.

Thirdly, the RT-PCR might require 4 hours to interpret the data and perform the PCR reaction [14]. Therefore, the low efficiency will add workloads to analyzers, especially when a great amount of diagnostics such as community-based tests are required.

However, after the combination with magnetic nanoparticles, these three problems can be solved. Firstly, the magnetic nanoparticles test is an approach to detect living viruses which can provide the final test results according to the amount of living viruses in the sample. Therefore, false-positive diagnose can be partially eliminated. In other words, the magnetic nanoparticles allow the purification of RNA be improved, which can increase the number of clinical tests for corona virus. [12]Second, all required materials for magnetic nanoparticles are affordable and harmless for the users even in remote area and poor countries. Therefore, the combination of magnetic nanoparticles and RT-PCR test can reduce the expensive and complex equipment that was originally required by RT-PCR test [6]. Thirdly, the time of analyzing will be significantly shortened by using the magnetic difference to quickly attract the target particles. Since the magnetic separation does not need to replicate the viral RNA and it can balance and select the most suitable condition, such as the optimum growing condition for the expression of protein, it can provide high-throughput analysis[15]. Therefore, the efficiency of RT-PCR will be greatly improved.

2.3. Combination between magnetic nanoparticles test and ELISA

The ELISA can be used for the detection of both qualitative and quantitative data about the presence of corona virus. The blood sample is inserted in the tiny wells of an ELISA plate that has been coated with the antigen or the virus's inactivated form. If the blood includes antibodies, they will bind to antigens, requiring the addition of a substrate solution. When the reaction is finished, a color change occurs, indicating the presence of antibodies [16].

The combination of magnetic nanoparticles and ELISA test is achieved by producing and purifying His-tagged form of the SARS-CoV-2 nucleocapsid N protein, which then will be immobilized on Ni²⁺ magnetic beads. Then, these beads were challenged for 2 minutes with infected human serum. The beads were then rinsed twice for 30 seconds each time. Then, they are going to be submerged in a solution that contains anti-human IgG HPR before being washed twice more. Finally, the beads are going to be dipped into the HPR chromogenic substrate and experience incubation. At the end of the experiment, all the beads are going to be withdrawn so that the results could be examined visually.

Positive COVID-19 samples will show a prominent blue hue, indicating that the IgG has interacted with the protein, while the negative results will be completely blank [17].

The traditional ELISA exhibits several advantages compared with RT-PCR. Comparing with the testing period of RT-PCR (48hours), the ELISA only requires 2~5hours, because the procedure doesn't require pre-treatment, such as replicating the RNA sequence, which made the whole process more simple. In addition, as simultaneous analysis can be performed, it's suitable for testing a whole community or a bunch of people[14]. In addition, the ELISA is generally safe and environmental friendly since it does not require radioactive substances and large quantities of organic solvents. This characteristic is critical for its sustainable development, which can provide a better developing place for other technology in the future. Furthermore, since the whole test only requires some basic and cheap materials, the cost of production is lower than the cost of production of RT-PCR, which will result in cheaper price and higher degree of propagation [16].

However, although the ELISA test has a cheaper and more efficient process, several problems still exist. The most significant shortcoming is this method is not suitable to apply to the point-of-care analysis. However, once it combines with magnetic nanoparticles, the result can be interpreted visually without affecting the accuracy. In fact, the ELISA test combined with magnetic nanoparticles can achieve the sensibility of 96% and the specificity of it can be up to 99% by simple visual interpretation. This is because the magnetic bead ELISA in the negative results group showed abnormally low cross-reaction background, and the mean raw OD equals to 0.08. This allows tester to identify positive samples in a better sensitivity than normal ELISA with simple low antibody titer. The sensitivity in this case is crucial for ensuring that the cases will be identified, and specificity in this case is crucial for ensuring the false-positive or false-negative results are being minimized [17]. What's more, the magnetic bead ELISA requires only a small amount of instruments and lesser time to provide results. To be specific, when all the apparatus have been set up, the whole process takes less than 12 minutes, while the traditional ELISA takes about 2~5 hours. In addition, by using a simple magnetic extractor/mixer device that can be built inside the house, the government can reduce the cost to 2 US dollars. Therefore, this kind of test can be validly used at a point-of-care analysis. Moreover, the possibility of deriving inaccurate results is still really high. In this case, all false results can be caused by an insufficient covering of the surface of the testing plate by using antigen. Nevertheless, the magnetic nanoparticles can address this problem by replacing the original ELISA plate with the nanoparticles, which have a high surface-area-to-volume ratio. Hence, the contacting area between antigen and antibody will increase, and the reactivity will also increase, which will promote the reaction to go more completely [12]. Consequently, the result will be more accurate. According to the receiver operating characteristic (ROC) of 165 samples, the combination of magnetic nanoparticles and ELISA provides better result with an area under the ROC curve of 0.97 than normal ELISA which has area of 0.96 that's under the ROC curve. In addition, after the corporation of magnetic nanoparticles, the specificity of 97% can be reached and the sensitivity of 97% could be achieved for the magnetic bead ELISA, while for the classic ELISA, 100% of specificity can be reached, but the sensitivity is only 90%[17]. Therefore, the magnetic bead ELISA is obviously more applicable and accurate than the traditional ELISA.

2.4. The combination between magnetic nanoparticles and gold nanoparticles

The gold nanoparticles test works by using a highly specific molecule coupled to the gold nanoparticles to detect a specific protein that is part of the specific gene sequence of corona virus. If the corona virus is present, its antigen will form a complex with the monoclonal antibodies, which will then bound to the gold nanoparticles. Subsequently, the whole complex will be captured by c antigen-specific antibodies which are being fixed on the nitrocellulose membrane during the migrating way that's caused by capillary action. Since the antigen-antibody complexes are immobilized and kept accumulating at one specific line, a visible blue line will appear.[18]

Gold nanoparticles in this case have several advantages, including eminent stability and extraordinarily high absorption coefficients. Because of these characteristics, gold nanoparticles

forms stable and highly active biological compounds with common biological targets like DNA and protein, allowing for very sensitive and specific identification [19]. Thus, one of the outstanding advantage of gold nanoparticles is its sensitivity.

Also, since the gold nanoparticles will produce bright blue color after detecting SARS-CoV-2 virus, it's easy to spot changes which allows the visual identification becoming possible.

The production of gold nanoparticles, on the other hand, is usually reliant on processes that unavoidably require wet chemical reduction techniques. Although these techniques can support fast production on a large scale, the synthesis of gold nanoparticles that all have a uniform with size and shape is especially challenging [20]. However, scientists have already established economic and stable way to coat magnetic nanoparticles by using gold nanoparticles.

The manufacture of nano DNA probes can be substantially simplified by using the trisodium citrate-auric acid reduction approach to prepare the gold coat of magnetic nanoparticles. Furthermore, citrate works as a reducing agent and a surface stabilizer in the process of producing gold nanoparticles by the trisodium citrate-auric acid reduction method, because citrate immobilization on the surface of heterogeneously grown gold nanoparticles. Therefore, the quality and usability of particles can be ensured.

In addition, by using gold nanoparticles to coat the magnetic nanoparticles, the magnetic, light and electric properties of magnetic nanoparticles will be improved and magnified. Therefore, the Au-coated magnetic particles can test the mixed DNA sample [21].

3. Conclusions

Recent studies reported several quantitative detection methods of COVID-19, meanwhile their shortcomings were exposed as well. Magnetic nanoparticles were reported to be helpful to overcome these limitations when combined with other detection methods. Meanwhile, magnetic nanoparticles could also detect the corona virus function independently. Therefore, it was believed that through the aid of magnetic nanoparticles, a cheap, high-effective and accurate corona virus test could be achieved. In some developing and undeveloped countries without professional testing equipment, it is of great importance to develop such a method. In this work, magnetic nanoparticles combination with RT-PCR, ELISA and gold nanoparticles were discussed. The improvements before and after the modification were combed in detail. Magnetic nanoparticles show excellent modification effect. Through the summary of this article, the detection methods modified by magnetic nanoparticles will offer more promising ways to improve virus detection means.

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