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Anti-SARS-CoV-2 IgG and IgM detection with a GMR based LFIA system

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A B S T R A C T

Since December 2019, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has caused millions of deaths and seriously threatened the safety of human life; indeed, this situation is worsening and many people are infected with the new coronavirus every day. Therefore, it is very important to understand patients’ degree of infection and infection history through antibody testing. Such information is useful also for the government and hospitals to formulate reasonable prevention policies and treatment plans. In this paper, we develop a lateral flow immunoassay (LFIA) method based on superparamagnetic nanoparticles (SMNPs) and a giant magnetoresistance (GMR) sensing system for the simultaneously quantitative detection of anti-SARS-CoV-2 immunoglobulin M (IgM) and G (IgG). A simple and time-effective co-precipitation method was utilized to prepare the SMNPs, which have good dispersibility and magnetic property, with an average diameter of 68 nm. The Internet of Medical Things-supported GMR could transmit medical data to a smartphone through the Bluetooth protocol, making patient information available for medical staff. The proposed GMR system, based on SMNP-supported LFIA, has an outstanding advantage in cost-effectiveness and time-efficiency, and is easy to operate. We believe that the suggested GMR based LFIA system will be very useful for medical staff to analyze and to preserve as a record of infection in COVID-19 patients.

1. Introduction

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that emerged in December 2019 has had a negative impact on human health and international economic development [1]. The disease is spreading rapidly; at the time of writing (October 22, 2020), the cumulative number of confirmed SARS-CoV-2 cases worldwide was 31,450,801 and the cumulative number of deaths was 967,495, affecting countries around the world. The new coronavirus is a positive-stranded single-stranded RNA virus with an envelope and no segments [2]. The particles are round or elliptical with a diameter of about 80–120 nm. Virus particles are wrapped in lipid bilayers provided by host cells and contain nucleic acid and nucleocapsid protein (NP) [3]. NP is the most abundant protein in coronaviruses. In the process of virion assembly, NP binds to viral RNA, leading to the formation of a spiral nucleocapsid. Due to its sequence conservation and strong immunogenicity, NP is often used as a diagnostic test tool for coronavirus. SARS-CoV-2 infection can be found in the patient’s respiratory epithelial cells within about 96 h of exposure. The common symptoms of respiratory tract infection after the patient is infected with the new coronavirus include fever, cough, shortness of breath, and difficulty breathing. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure, and even death [4,5].

After infecting the human body, the SARS-CoV-2 virus reproduces in the respiratory system. After a period of infection (usually 7–10 days), the body produces specific antibodies against the virus. IgM antibodies appeared first followed by IgG antibodies [6]. Immunological testing methods can be used to detect these specific antibodies and determine whether the human body has been infected with the virus. A positive IgM antibody indicates that the patient is in the early stages of infection, while a positive IgG antibody indicates that the patient has been infected with the new coronavirus. Recovered people usually react positively to IgG antibodies. If the nucleic acid test result is negative twice in 24 h, then the patient is not infectious. Antibody detection can be used to assist in the diagnosis of negative nucleic acid detection but it cannot replace nucleic acid detection methods and can be used in...
combination with nucleic acid detection.

At present, many medical centers use antibody testing to identify a patient’s infection history. Accurate and efficient test results improve clinical decision-making; therefore, understanding the assay’s performance is essential for medical staff to analyze the patient’s condition. The enzyme-linked immunosorbent assay (ELISA) is a detection method generally accepted by laboratories and hospitals, and its sensitivity is high [7]. However, ELISA requires well-equipped facilities with professional personnel and a long time to obtain the results. Huang et al. developed a lateral flow assay based on colloidal gold nanoparticles (GNPs) to achieve rapid diagnosis and on-site detection of IgM antibodies to SARS-CoV-2 virus [8]. However, conventional GNP can only achieve qualitative or semi-quantitative results, and its sensitivity and detection range are not good. Moreover, some LFIA use fluorescent dyes [9], surface-enhanced Raman spectroscopy (SERS) [10] materials, and quantum dots [11] as probes to analyze the optical signals to achieve quantitative results. In addition, the signal processing method based on a two-dimensional plane and background interference also limits the sensitivity and detection range of the optical biosensors [12]. Among many sensing probes, MNP has been developed as a promising point-of-care testing (POCT) bioensor probe candidate due to its inherent characteristics, i.e., enrichment, signal amplification, and signal readout [13-15]. Besides, of all three-dimensional analysis methods, the MNP-based LFIA can detect most of the magnetic signals captured by the test strip [16].

The Internet of Medical Things (IoMT) holds great potential and can help medical staff realize intelligent perception and treatment of patients [17]. On the one hand, the IoMT-based LFIA detection method can reduce the doctor’s contact with potentially infectious patients, thereby reducing the doctor’s risk of infection. On the other hand, the integration of massive individual patient data is useful for the government and hospitals in preventing and predicting pandemic events. In addition, the use of IoMT-based sensors allows patients to monitor their health status. Through remote consultations with specialist doctors, patients are able to understand important health information [18].

In this paper, we present an MNP-based LFIA method to detect anti-SARS-CoV-2 IgM and IgG simultaneously while a supporting GMR device was used to block the unlinked carboxyl group of MNP. The proposed SMNPs have small particle size and strong magnetic properties, making them an ideal labeling material for LFIA. The experimental results showed that biosensor could obtain reliable test results in 10 min and the LODs of IgM and IgG were 10 ng/mL and 5 ng/mL, respectively. Combined with IoMT technology, our proposed SMNP-based biosensor would be very meaningful for doctors to understand patients’ infection history and provide treatments.

2. Experimental

2.1. Instrumentation and materials

A GeminiSEM 300 scanning electron microscope (SEM) (Carl Zeiss) was used to characterize the morphology of the MNPs. An X’Pert Platform Dispenser (HM3035) was used to fix the antibody to the NC membrane or conjugate pad. A test strip cutting machine (WM-100) was used to cut the test strip (4 mm) by a cutting machine.

Ferric chloride hexahydrate (FeCl₃·6H₂O), ferrous sulfate heptahydrate (FeSO₄·7H₂O), and ammonium hydroxide were supplied by Shanghai Macklin Biochemical Technology Co., Ltd. (China). 3-Aminopropytriethoxysiliane (APTES), triethylamine (TEA), 1-ethyl-3-(3-dime-}

pure grade, 99.0%), bovine serum albumin (BSA), and N, N-dimethylformamide (DMF) were purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd. The glass fiber, absorbent pad, and backing card were purchased from Shanghai Kinbio Biotech. Co., Ltd. The nitrocellulose (NC) membrane was purchased from Shanghai Jieyi Biotech Co., Ltd. Anti-SARS-CoV-2 IgM (2020T25P) and anti-SARS-CoV-2 IgG (2020T14) were bought from Beijing Bioc, Co., Ltd. Anti-human IgM (ncov-PS-GonAbM3) and anti-human IgG (ncov-PS-GonAbG3) antibodies were obtained from Shenzhen Fapon Biotech Inc. Human SARS-CoV-2 NP was purchased from Chengdu ABlink Biotech Co., Ltd. Two species-specific anti-immunoglobulin G antibodies (rabbit IgG and goat anti-rabbit IgG) were purchased from Hangzhou Clongene Biotech Co., Ltd. Deionized water was used for all experiments.

2.2. Preparation of carboxyl-modified SMNPs

We used the traditional co-precipitation method to prepare the SMNPs. First, FeCl₃·6H₂O (5.835 g, 0.0216 mol) and FeSO₄·7H₂O (3.001 g, 0.0108 mol) were dissolved in water (100 mL). Under vigorous mechanical stirring, the mixture was heated to 85 °C in the presence of nitrogen. After 30 min, ammonium hydroxide (7.5 mL) was quickly injected into the reaction system in one portion. The color of the solution immediately changed from orange to black, where the black substances were SMNPs; this reaction continued for 30 min. Finally, the resulting mixture was cooled to room temperature and the magnetic precipitate was collected and enriched by a magnet, and then washed three times with ethanol. After the SMNP-NH₂ was re-dispersed in DMF (15 mL), SAA (105 mg) and TEA (105 μL) were added to the solution and stirred at room temperature for another 24 h. Additionally, the schematic of the reaction is shown in Fig. 2. Then, continued to separate the product with a magnet, and washed three times with ethanol and water. Finally, the SMNP-COOH was re-dispersed in water for further use.

2.3. Preparation of LFIA

To link SMNP with NP or rabbit IgG to form SMNP-NP or SMNP-IgG conjugates, respectively, EDC (1.5 mg) and NHS (1.2 mg) were reacted with the prepared SMNPs (3.0 mg) in 1 mL MES (50 mM, pH = 6.0) for 1 h to activate the carboxyl-modified SMNPs. Then, the product was separated using a magnet and the buffer was changed to 1 mL PBS (pH = 7.2). After the solution was dispersed by ultrasonication (180 mW, 2 s), re-combinant NP (0.25 mg) or rabbit IgG (0.25 mg) was added and allowed to react with the activated SMNPs at room temperature for 4 h to label the SMNPs. After the protein coupling step is completed, a magnet was used to separate the product and disperse it in PBS (1 mL); then, α1% BSA solution (100 μL) was added to block the unlinked carboxyl group for 1 h. After completing the above process, the X’Y three-dimensional disperser was used to evenly disperse the NP- and rabbit IgG-labeled SMNPs solution onto the glass fiber (pre-treated with treatment liquid composed of 1.21% Tris, 1% PVP, 1% S9, 5% T-casein and water) in a concentration of 2.5 mg/mL. Then, using the same platform, the anti-human IgG (0.6 mg/mL), anti-human IgM (0.8 mg/mL), and goat anti-rabbit IgG (1 mg/mL) were dispersed onto the NC membrane, which was pre-fixed on the backing card, to form test line 1 (T1), T2, and the control line (C), respectively. Subsequently, the glass fiber and backing card were baked in a thermostat dryer at 37 °C for 10 h. In the end, the glass fiber and backing card were assembled together and then cut into individual test strips (4 mm) by a cutting machine.
2.4. LFIA procedure

The prepared test strips were placed into the cassette. A pretreated 90 μL sample (the mixture of anti-SARS-CoV-2 IgG and anti-SARS-CoV-2 IgM) was dropped into the small hole of the cassette. By capillary action, the SMNPs migrated along the test strip. After 10 min, the results were visible with the naked eye. The cassette was inserted into the GMR sensor and the stepper motor moved the test strip forward. The sample concentrations used in these experiments were 250, 200, 150, 100, 50, 25, 10, 5, and 0 ng/mL; all samples were diluted with whole blood buffer (pH = 7.4, Na₂HPO₄, NaCl, Casein-Na, and NaN₃).

3. Results and discussion

3.1. Principle of LFIA test strip

Fig. 1 illustrates the structure of the SARS-CoV-2 virus and the principle of SMNP-based LFIA to test antibodies of the virus, i.e., the “sandwich” format. The capture antibodies were immobilized at the TL1 (anti-human IgG), TL2 (anti-human IgM), and CL (goat anti-rabbit IgG) of the NC membrane; detected antibodies (NPs) were linked on the surface of the SMNPs. The protein-labeled SMNPs were evenly distributed on the glass fiber. When the SARS-CoV-2 antibody sample was applied to the test strip, IgG or IgM would combine with the SMNP–NP conjugate to form SMNP-NP-IgG or SMNP-NP-IgM, which was then transferred to the absorbent pad through capillary force. The complex is specifically bound to the capture antibody on the TL to form a sandwich complex (SMNP-NP-IgG-anti-human IgG or SMNP-NP-IgM), and its color intensity and magnetic signal intensity the concentration of antigen in the sample. When the negative sample is tested on the strip, no sandwich compound is formed on the TL. However, because the SMNP-rabbit IgG conjugate independently binds goat anti-rabbit IgG, CL will always appear in the case of successful experiments, thus indicating the normal function of LFIA.

3.2. Characterization of SMNPs

Dispersion, nanoparticle size, and morphology have a significant impact on the reaction time, sensitivity, and specificity of SMNP-based LFIA. SEM proves that our prepared SMNPs are uniform in size (as shown in Fig. 3A). Meanwhile, the average particle size of SMNP, SMNP-COOH, and SMNP-NP is, respectively, 68 nm, 237 nm, and 338 nm, with a 0.108, 0.199 and 0.244 PDI value by DLS, respectively (as shown in Fig. 3B), indicating the stable performance of the SMNPs. The zeta potential of SMNP-Bare is −26.1 mV. After modification of the amino and carboxyl groups, the zeta potential values are +27.6 mV and −18.6 mV, respectively (as shown in Fig. 3C). The changes in particle size and zeta potential indicate that each step has been successfully achieved. As shown in Fig. 3D, it is the hysteresis loop of the prepared bare SMNPs. Fig. 3D shows that the saturation magnetization value of the SMNP is about 62 emu/g; there is no hysteresis in the hysteresis loop and coercivity and remanence are zero, indicating that SMNPs are super-paramagnetic. Besides, it has a strong magnetic response: even if the magnet distance is 3 cm the product can be quickly manipulated. The feature of superparamagnetism can prevent the accumulation of SMNPs after withdrawal of the magnetic field. Therefore, a good magnetic response ability and superparamagnetism make this material an ideal labeling probe for LFIA.

3.3. Performance of the SMNP-based LFIA test strip

The proposed SMNP-based GMR sensor can realize the quantitative detection of IgG and IgM against SARS-CoV-2. A series of different anti-SARS-CoV-2 IgG samples (250, 200, 150, 100, 50, 25, 10, 5, and 0 ng/mL) were mixed with a constant anti-SARS-CoV-2 IgM concentration (100 ng/mL) before the LFIA process in order to verify the quantitative
performance of the GMR system. Besides, the other group is anti-SARS-CoV-2 IgG (100 ng/mL) mixed with different concentrations of IgM (250, 200, 150, 100, 50, 25, 10, 5, and 0 ng/mL) were tested. When the IgM concentration was constant, the color intensity and magnetic intensity on the TL1 gradually weakened while the value of TL2 remained stable (as shown in Fig. 4 A and B). A similar result was obtained for IgM samples (as shown in Fig. 4 C and D). There was no endogenous cross-reactivity between IgM and IgG, and a well-established linear relationship existed between the concentrations of target samples and the magnetic intensity. It should be noted that because the mouse antibody on the CL can always bind to goat anti-mouse IgG (a separate immune system), the magnetic signal on the CL tends to be roughly stable. Therefore, we use the T/C value to reduce the error of the test results. In addition, in order to verify whether the SMNP-based LFIA test strip can work reliably and stably in serum samples, we also used anti-SARS-CoV-2 IgG (100 ng/mL) and IgM samples (100 ng/mL) in fetal bovine serum solution for color intensity and magnetic intensity analysis. The results showed that TL1 and TL2 can still have stable visible bands and magnetic intensity value, so the fetal bovine serum solution has the same efficacy as the configured whole blood buffer, and the SMNP-based LFIA also has good stability for serum samples. Therefore, the proposed SMNP-based LFIA has good feasibility in the simultaneous quantitative analysis of anti-SARS-CoV-2 IgM and IgG.

We then evaluated the dynamic range and LODs of SMNP-based LFIA in the GMR system. As shown in Fig. 5, for both the IgM and IgG samples, the magnetic intensity on TLs increases with the increase of IgM and IgG concentration. For IgM, we reached a quantitative range of 10–250 ng/mL and for IgG, 5–250 ng/mL. We obtained the above LODs by setting the threshold above background noise. The signal is considered valid only when the calculated T/C value is 0.3 values higher than the noise baseline. We tested with a precise target concentration gradient and found that when the target concentration was lower than the specified LOD, the magnetic intensity on the TL became so weak that it was overwhelmed by background noise (the residual SMNPs on the test strip), resulting in an extremely low T/C value, so it cannot be regarded as a valid value. Besides, IgG and IgM have linear relationships of \( y = 0.49239 + 0.0224x \) and \( y = 0.0933 + 0.01153x \), respectively, and their \( R^2 \) are 0.9672 and 0.9893, respectively. Furthermore, as shown in Table 1, we made a comparison table between this study and other work.

### 3.4. SMNP-based IoMT application

SMNPs have no magnetic properties in the absence of a magnetic field so they will not attract each other. Use of a constant magnet provides an external magnetic field with the direction of the magnetic field perpendicular to the LFIA strip. The whole test process is controlled by the microcontroller unit (MCU). As shown in Fig. 1, the stepper motor carries the test strip through the external magnetic field at a constant speed and direction, and the SMNPs of TL and CL are magnetized. Then, the internal MR sensors will be affected by the response magnetic field,
resulting in an increase in resistance.

Our proposed GMR platform could also be adapted for use on an intelligent phone-based IoMT platform. Using the Bluetooth protocol, the patient’s test results were displayed on a mobile phone. Based on the app developed on the intelligent phone shown in Fig. 6A and B, the test results were sent to the medical center via the network in real time. Therefore, doctors can learn about the patient’s infection history and status online, thus reducing the workload of medical staff and the chance of infection. Besides, by combining the patient’s digital information recorded by the medical center, the government and doctors can also implement preventive policies for different regions.

4. Conclusion

In this work, we manufactured an SMNP-based LFIA test strip for the rapid on-site detection of the IgM antibodies and IgG antibodies of SARS-
CoV-2 virus simultaneously. SMNPs were synthesized by the co-precipitation method and used as a labeling tool to analyze antibody proteins. The proposed GMR system is capable of quantitatively detecting IgM and IgG in 10 min with LODs of 10 ng/mL and 5 ng/mL, respectively. Our LFIA test strip can provide doctors with preliminary test results as well as nucleic acid detection tools to make a correct analysis of a patient’s history of infection and formulate an appropriate treatment plan. Meanwhile, connection of the sensor to a smartphone allowed the test results to be uploaded on IoMT, which can provide critical guidance and records for users and doctors. This method is faster, more convenient, and cheaper than other methods. We believe that the proposed SMNP-based LFIA test strip has strong potential in point-of-care testing for COVID-19.

Credit author statement

Qiaoge Bayin: Experiment conducting, Data curation and original article writing, Lei Huang: Data curation, verification of antigen and antibody materials Chunhui Ren: System software development Yusheng Fu: System software development Xing Ma: Guiding material synthesis and material characterization, Jinhong Guo: Guiding system integration and testing.

Declaration of competing interest

There are no conflicts of any interests.

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Fig. 6. (A) IoMT application of the proposed GMR platform based on an app. (B) Detection result displayed on the interface.

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