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Detection of SARS-CoV-2 virus via dynamic light scattering using antibody-gold nanoparticle bioconjugates against viral spike protein

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Mass testing for the diagnosis of COVID-19 has been hampered in many countries owing to the high cost of genetic material detection. This study reports on a low-cost immunoassay for detecting SARS-CoV-2 within 30 min using dynamic light scattering (DLS). The immunoassay comprises 50-nm gold nanoparticles (AuNPs) functionalized with antibodies against SARS-CoV-2 spike glycoprotein, whose bioconjugation was confirmed using transmission electron microscopy (TEM), UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), and surface-enhanced Raman scattering spectroscopy (SERS). The specific binding of the bioconjugates to the spike protein led to an increase in bioconjugate size, with a limit of detection (LOD) 5.29 \times 10^3 \text{TCD}_{50}\text{mL}.
1. Introduction

Early diagnosis of COVID-19 caused by SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) [1] is essential to handle the pandemic, which is why the World Health Organization (WHO) [2] has recommended mass testing. Unfortunately, mass testing for COVID-19 has not been possible in many developing countries, including Brazil, because it is mostly performed using expensive methods that require specialized laboratories and personnel. COVID-19 diagnosis has been based on detecting the viral genome, or a specific part of its sequence [3], with reverse transcription-polymerase chain reactions (RT-PCR) as the gold standard test [4–9]. RT-PCR detects the genetic material of the virus (ribonucleic acid, RNA) using a swab, mainly collected in the nose and throat [10]. Less commonly used molecular techniques are reverse transcription loop-mediated isothermal amplification (RT-LAMP) [11], 12] and clustered regularly interspaced short palindromic repeats (CRISPR) [13, 14], but none of them are amenable to point-of-care diagnostics. Genosensors have been developed to detect the genetic material of SARS-CoV-2 using portable instruments and simple procedures [15], even though they are not commercially available.

Accurate diagnosis can also be achieved by exploiting antibody-antigen interactions in immunosensors, e.g., used to detect the spike protein [15–19]. The widespread serologic tests to detect IgM and IgG immunoglobulins are not suitable for early diagnosis of COVID-19 because noticeable immune responses take a few days after the infection [20–22]. The presence of IgM antibodies indicates the onset of the immune response whereas the IgG associates to the stage of immunization against SARS-CoV-2 [20]. Detection of virus proteins (S and N, e.g.), on the other hand, yields faster, cheaper tests [23,24], especially if one capitalizes upon extensive knowledge about nanobiosensors [25–27]. These immunosensors may contain gold nanoparticles (AuNPs) whose physicochemical properties are tuned to amplify optical, electrical, and electrochemical signals. For example, the plasmon resonance in AuNPs is varied by changing particle size and shape, and size distribution [28]. AuNPs are suitable for functionalization to anchor active layers as they are biocompatible upon binding of biomolecules [29,30]. Immunosensors based on AuNPs conjugated with antibodies are capable of recognizing antigens, cells, microorganisms, and proteins through varied principles of detection [31]. Bioconjugation affects both the color and size of the AuNPs, thus permitting colorimetric or size assays with dynamic light scattering (DLS) [32,33]. Driskell et al. [34], for instance, have reported the use of DLS to detect the H1N1 virus. To the best of our knowledge, DLS has not been used to detect SARS-CoV-2, using AuNPs functionalized with anti-SARS-CoV-2 spike glycoprotein antibodies, which is the aim of the present study.

2. Experimental details

2.1. Materials and virus samples

Boric acid was purchased from Vetec™ (Duque de Caxias, RJ, Brazil). Gold nanoparticles (50 nm, ~3.5 × 10¹⁰ particles/mL), bovine serum albumin (BSA), and 3,3’-dithiobis (sulfosuccinimidyl propionate) (DTSSP) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Phosphate buffered saline (PBS) solution was purchased from Laborclin (Pinhais, PR, Brazil). Sodium tetraborate was obtained from Dinâmica (Indaiatuba, SP, Brazil). Polyclonal antibody against the N-terminal of the spike protein of SARS-CoV-2 from rabbits was kindly provided by the Virolology and Microscopy Laboratory, Universidade de Brasília, Brazil. Samples from influenza and SARS-CoV-2 viruses were kindly donated by Butantan Institute (São Paulo, Brazil). The samples used were A/Brisbane/2018-IVR-190 (H1N1), A/Washington/02/2019 (B), B/Phuket/3073/2013 (B), A/South Australia/34/2019-IVR-197 (H3N2), and SARS-CoV-2 inactivated by gamma irradiation. The concentrations of the virus samples were determined by TCID₅₀/mL (Tissue Culture Infectious Dose), which defines the dilution of virus required to infect 50% of the cells.

2.2. Antibody-modified AuNPs (pAbS1N@AuNPs)

An aliquot of 1.2 mL (2.6 × 10¹⁰ particles/mL) suspension of 50-nm AuNPs had the pH value adjusted to 8.9 with borate buffer (50 mM, pH 8.9). Next, 10 μL of 10 mM of 3,3’-dithiobis (sulfosuccinimidyl propionate) (DTSSP) were added to the AuNPs suspension and incubated at room temperature under light protection for 40 min. This suspension was transferred into a 2 mL Lobind® Eppendorf tube (Eppendorf, Hamburg, Germany) and centrifuged at 7000 g for 5 min at 5 °C. The supernatant containing the excess of DTSSP was removed, and the AuNPs were resuspended in 1100 μL of 2 mM borate buffer pH 8.9. The centrifugation and resuspension cycle of AuNPs was repeated two more times. Next, the polyclonal antibody (21.6 μg, 32.4 μg, or 43.2 μg) was added to the AuNPs suspension and incubated at room temperature for 90 min. After this 90-min reaction, the suspension was centrifuged at 7,000 g for 5 min at 5 °C. The supernatant containing the excess antibody was removed, and pAbS1N@AuNPs were resuspended in 1100 μL of borate buffer (2 mM, pH 8.9) containing 0.25% BSA. For complete removal of the antibody, the centrifugation/resuspension process was repeated twice again. The samples containing 21.6 μg, 32.4 μg, and 43.2 μg antibodies are referred to pAbS1N18@AuNPs, pAbS1N27@AuNPs, and pAbS1N36@AuNPs, respectively.

2.3. Incubation protocol of pAbS1N@AuNPs with viruses

Diluted virus stock was prepared using PBS and 20% sucrose as the diluent for influenza (1 × 10¹⁰.3 TCID₅₀/mL for A/Brisbane, 1 × 10¹⁰.6 TCID₅₀/mL for B/Washington, 1 × 10⁷ TCID₅₀/mL for B/Phuket, and 1 × 10¹⁰.7 TCID₅₀/mL for A/South Australia). Twelve serial dilutions of the virus stock (2.11 × 10⁶ TCID₅₀/mL) were prepared using PBS as the diluent for SARS-CoV-2. First, 90 μL of the respective dilution of virus and 10 μL of pAbS1N18@AuNPs or pAbS1N36@AuNPs were added into a cryotube, and the sample was incubated at room temperature for 30 min on a Kline orbital shaker (400 rpm; Nova Tecnica, Piracicaba, SP, Brazil). Then, 50 μL of pAbS1N@AuNPs and the virus mixture were transferred to a small volume cuvette (ZEN0118 model, Malvern Instruments Ltd, Worcestershire, UK) for dynamic light scattering (DLS) measurements.

2.4. Characterization methods

Size and surface morphology for the AuNPs and bioconjugates were characterized by transmission electron microscopy (TEM) using a transmission JEOL 1011, Tokyo, Japan, operated at an accelerating voltage of 100 kV. AuNPs and pAbS1N18@AuNPs were prepared by placing a drop of the sample (5 μL) on a 400-mesh copper grid coated with formvar film. After that, the sample was air-dried, and to enhance the contrast, osmium tetroxide vapor was used. Grids were kept at room temperature until further analysis. Four hundred AuNPs were counted in different images for each sample using Imagej - Plugin Analyse (set scale) software. The binding affinity of SARS-CoV-2 viruses and pAbS1N36@AuNPs conjugates was confirmed via TEM using a modified negative staining technique [35]. A formvar-carbon coated 400-mesh copper grid was floated on a 40-μL drop of pAb1SN36@AuNPs reagent/sample mixture for 30 min at room temperature. Next, the excess sample was removed with filter paper. The grid was then floated on a 40-μL drop of 0.7% glutaraldehyde for 5 min to fix the sample. After removing excess fixative, the sample was negatively stained by floating the grid on 2% aqueous phosphotungstic acid at pH 7.0 for 60 s. The stain was removed from the grid, and the sample was allowed to dry overnight before obtaining the TEM images. DLS was performed with a Zetasizer Nano ZS90 Model (ZEN6900 (Malvern Instruments Ltd, Worcestershire, UK) equipped with a 4.0 mW 632.8 nm (red) laser and at a scattering detector angle of 90° to measure particle size by using a
The electronic spectra for the nanoparticles in suspensions were recorded on a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan) using quartz cells with a 10.00 mm optical length without dilution for AuNPs, pAbS1N18@AuNPs, pAbS1N27@AuNPs, and pAbS1N36@AuNPs samples. The FTIR experiments were performed using a Bruker spectrometer, Vertex 70 model (Billerica, EUA), with an attenuated total reflectance module (ATR). The measurements were averaged over 96 scans, taken at a resolution of 4 cm\(^{-1}\). The measurements were averaged after 96 scans, taken at a resolution of 4 cm\(^{-1}\) from 400 to 4000 cm\(^{-1}\).

Antibody conjugation was assessed onto the surface of the AuNPs by surface-enhanced Raman scattering (SERS). Silver films were used as a substrate for SERS measurements, and they were prepared using the electrolytic method, with AgNO\(_3\) electrolyte solution at 0.1 mg/mL. For SERS studies, a solution of 10 \(\mu\)L BSA, pAbS1IN antibody, DTSSP@AuNPs, and pAbS1N36@AuNPs samples were diluted in 10 \(\mu\)L distilled water, dropped onto the Ag film, then dried with dry nitrogen. Raman and SERS analyses were performed using LabRAM HR Evolution (HORIBA Scientific, Palaiseau, France) equipped with an 1800 lines/mm grating and CCD detector. The measurements were conducted with a 532 nm laser excitation source (10 mW) using a DuoScan Raman imaging system (Horiba Scientific) with a 50X objective. DuoScan was used to distribute the laser beam on the sample surface by obtaining average spectral information of an area of 20 \(\times\) 20 \(\mu\)m\(^2\).

3. Results and discussion

3.1. Conjugation of antibodies onto AuNPs

Successful conjugation of polyclonal antibodies onto the AuNPs was experimentally confirmed. The UV–Vis spectra of 50-nm AuNPs (Fig. 1A) indicate that the conjugation leads to a redshift from 534 nm to 537 nm owing to a change in the refractive index of the nanoparticles. This redshift does not depend upon the specific number of nanoparticles, but its band intensity is proportional to the amount of antibodies used in the bioconjugation process, as expected from the results by Driskel et al. [34]. A second absorbance band is observed within 650–750 nm if larger particles or aggregates are formed [36], turning the dispersion dark blue. Since this effect has not been observed for the bioconjugates (Fig. 1A), one assumes there is no formation of aggregates. DLS data shown in Fig. 1B indicate that the bioconjugates are ca 13% larger than AuNPs. This increased size was also observed in the TEM images of pAbS1N18@AuNPs (Fig. 1D and E), where spherical and hexagonal structures can be noticed with average sizes from 55 nm to 60 nm. These values are smaller than those obtained from DLS (−80 nm in diameter) because TEM allows obtaining core sizes for the AuNPs; meanwhile, DLS can be related to the hydrodynamic diameter [37,38]. The zeta potentials plotted in Fig. 1C indicate a reduction seen in the number of negative charges after immobilization of pAbS1IN antibody from ca −60 mV to −40 mV. A similar decrease in charge has been reported for protein-coated AuNPs after surface modification with BSA (bovine serum albumin) (or streptavidin) [37]. Our stability studies indicated that AuNPs (before and after bioconjugation) had their sizes kept unchanged for 210 days, with larger hydrodynamic diameter seen for AuNPs containing the antibody pAbS1IN (pAbS1IN18@AuNPs) as compared to unmodified AuNPs (Figures S-1 and S-2, Supporting Information). The presence of BSA and antibody over the AuNPs surface reduces self-aggregation for AuNPs in aqueous media or buffer solutions [39].

Fig. 2 shows the FTIR spectra (A) for the anti-SARS-CoV-2 spike glycoprotein antibody before (pAbS1IN) (B), after coupling to AuNPs (pAbS1IN36@AuNPs) and AuNPs (C). The spectrum (Fig. 2C) provides information on AuNPs covered by citrates. The adsorption of citrates on AuNPs is confirmed by the vibration modes of carboxylates, with absorption bands at 1400 cm\(^{-1}\) (\(\nu_s\), (COO\(^{-}\))) and 1630 cm\(^{-1}\) (\(\nu_a\), (COO\(^{-}\))). The spectrum for the free antibody (pAbS1IN) in Fig. 2A displays the typical bands - amide I, amide II, and amide II bands, respectively. The band at 1080 cm\(^{-1}\) can be assigned to C = O symmetric stretching of COO\(^{-}\) group and CH\(_2\) scissoring vibration, respectively. The band at 1080 cm\(^{-1}\) can be predominantly attributed to C–O–C and C–O ring vibration. The fingerprint region between 500 and 900 cm\(^{-1}\) can be assigned to vibration in amino acids, e. g., phenylalanine, tyrosine, tryptophan, etc. [40]. Fig. 2B shows the FTIR spectrum of antibody conjugated with AuNPs, with bands at 1654, 1543, and 1243 cm\(^{-1}\) assigned to amide I, amide II and amide II bands, respectively. Note that the bands are shifted to higher wavenumbers compared to the spectrum of AuNPs alone. The intensity ratios of the amide I and amide II bands for pAbS1IN and pAbS1N36@AuNPs are ca 1.95 and 1.0, respectively, suggesting that conjugation causes
conformational changes in antibodies after Au functionalization. From the FTIR data one infers that the electron-donating effects from free NH$_2$ groups and carboxylate ions are responsible for antibody coupling to terminal succinimidyl ester, thus forming an amide bond by the nucleophilic attack of the amine to the ester carbonyl carbon [41].

The binding efficiency of antibodies to AuNPs was assessed with Raman spectroscopy and SERS by tracking bioconjugation from the first precoating procedure up to the attachment of the antibody moiety. The Raman spectrum of free DTSSP powder (Fig. 3A(i)) shows bands primarily due to the presence of 2,5-pyrrolidinedione ring and sulfur-containing groups, as better detailed in Table S-1. The SERS spectrum of DTSSP@AuNPs differs strongly from that of DTSSP powder, with various additional bands appearing due to the SERS effect. These additional bands are also listed in Table S-1. The most striking feature is the redshift of ν(C–S) modes and the quenching of disulfide vibrational modes (490-520 cm$^{-1}$ region), indicating that disulfide bonds were broken and DTSSP was adsorbed on AuNPs. Fig. 3B shows the SERS spectra of the pAbS1N antibody conjugated with AuNPs (pAbS1-N36@AuNPs) compared to those from the pAbS1N antibody and BSA. As expected, no bands from DTSSP are seen in the spectrum of pAbS1-N36@AuNPs, which is similar to the spectra of pAbS1N antibody and BSA, with bands arising from amino acids in pAbS1N and BSA, both bioconjugated to AuNPs. The amide bonds in the spectrum of pAbS1-N36@AuNPs coincide with those for the pAbS1N antibody (see Fig. 3B(i and ii) and Table S-1). The vibrational energy of these bands is typical of β-sheets which confirm that antibodies are structured [42]. These bands are seen at higher wavenumbers in the spectrum of BSA (see Table S-1), typically from an α-helix-rich structure [43]. One thus infers that the intensity of the dominant Raman signal in the spectrum of pAbS1-N36@AuNPs mainly comes from the antibody. Also relevant is the band at 1640 cm$^{-1}$ (Fig. 3C) for the pAbS1N36@AuNPs sample, absent for both free antibody and BSA. This band can be assigned to the C–O stretching of a secondary amide, probably due to the conjugation of AuNPs with antibodies [44].
3.2. Detection of SARS-CoV-2 virus by using pAbs1N@AuNPs bioconjugates

The incubation of pAbs1N18 or pAbs1N36@AuNPs bioconjugates with SARS-CoV-2 at 2.11 × 10^5 TCID_{50}/mL induced a shift at larger sizes in the DLS data (Fig. 4A). The shift is larger for pAbs1N18@AuNPs, which was used to estimate the limit of detection (LOD) for the SARS-CoV-2 virus. Fig. 4B indicates that the hydrodynamic diameter increases with increasing the concentration of the virus, SARS-CoV-2, from 1.65 × 10^5 to 2.11 × 10^5 TCID_{50}/mL. The LOD was obtained to be 5.29 × 10^3 TCID_{50}/mL by using the values of scattered light intensity and standard deviation for the bioconjugate without the virus (σ+3σ) (Figure S-3, Supporting information) [32].

The binding of SARS-CoV-2 to pAbs1N36@AuNPs bioconjugates was also suggested using TEM, with representative images in Fig. 5A-D. Fig. 5C shows the SARS-CoV-2 viral particles with spike proteins on the virus surface (marked in the image). The binding of pAbs1N36@AuNPs with SARS-CoV-2 can be observed in Fig. 5D, and the interaction of the virus surface spike protein with the bioconjugate is suggested with the magnified image in Fig. 5E.

In addition to a suitable sensitivity in detecting SARS-CoV-2, the interaction between antibodies and viruses must be sufficiently specific to avoid giving false-positive results. We have confirmed the selectivity of SARS-CoV-2 detection using DLS by testing pAbs1N18@AuNPs bioconjugate incubated with Influenza Type A Brisbane-H1N1 (H1N1@pAbs1N18@AuNPs), South Australia-H3N2 (H3N2@pAbs1N18@AuNPs), Influenza Type B Washington (Washington@pAbs1N18@AuNPs), and Phuket (Phuket@pAbs1N18@AuNPs) strains. Fig. 6A shows minor changes in nanoparticle peak size distributions, with the maximum difference being 10 nm for the Phuket strain – compared with almost hundreds of nm after SARS-CoV-2 incubation. The differences in size were confirmed by the hydrodynamic diameter (z-average) values in Fig. 6B, where the SARS-CoV-2 spikes protein led to a considerable increase in the hydrodynamic diameter for the bioconjugates. Furthermore, selectivity toward SARS-CoV-2 was confirmed in experiments in which no change in the size of the nanoparticles bioconjugates was noted upon interaction with influenza viruses. The main advantages of the method employed here include operational simplicity in the detection mode and offering lower prices for components and each test. The protocols and strategies used for the bioconjugates described here can be extended to colorimetric sensing, and one may envisage its use for low-cost mass testing.

Author contributions

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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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2022.123355.

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