



Developing an Optical Interferometric Detection Method based biosensor for detecting specific SARS-CoV-2 immunoglobulins in Serum and Saliva, and their corresponding ELISA correlation

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ABSTRACT

The standard rapid approach for the diagnosis of coronavirus disease 2019 (COVID-19) is the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA. The detection of specific anti-SARS-CoV-2 immunoglobulins is crucial for screening people who have been exposed to the virus, whether or not they presented symptoms. Recent publications report different methods for the detection of specific IgGs, IgMs, and IgAs against SARS-CoV-2; these methods mainly detect immunoglobulins in the serum using conventional techniques such as rapid lateral flow tests or enzyme-linked immunosorbent assay (ELISA). In this article, we report the production of recombinant SARS-CoV-2 spike protein and the development of a rapid, reliable, cost-effective test, capable of detecting immunoglobulins in serum and saliva samples. This method is based on interferometric optical detection. The results obtained using this method and those obtained using ELISA were compared. Owing to its low cost and simplicity, this test can be used periodically for the early detection, surveillance, detection of immunity, and control of the spread of COVID-19.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an RNA virus responsible for the pandemic that has infected over 120 million people and caused over 2.1 million deaths worldwide [1]. The infectivity of the virus, coupled with the extent of globalization has been responsible for its rapid spread, reaching most countries within a couple of months [2]. The infectivity and virulence also posed a problem for many countries where a large percentage of sanitary workers were infected, presenting an obstacle to the functioning of healthcare systems [3]. Therefore, countries need to be able to test the population in a fast,

cheap, convenient, and reliable manner. In fact, since January 2020, the World Health Organization has encouraged each country with three words: “test, test, test” [4]. Frequent testing is also important to gain an understanding of the transmission, infectivity, and morbidity of the virus, and the herd immunity in the population.

In Spain and other European countries, most of the tests conducted are serum-based. These are generally of two types: the first test, which is more commonly used, analyzes total immunoglobulin (Ig) levels; the second test analyzes the presence of anti-SARS-CoV-2 IgM and IgG antibodies. The third diagnostic test is the polymerase chain reaction (PCR)-based method used for the detection of viral RNA in a

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nasopharyngeal sample. Usually, the first type of test is used in patients and the second and/or third tests are conducted only if the first yields positive results [1]. Although this strategy has several advantages, it has been found to produce a large number of false positives, require repetitions (which increases the possibility of cross-contamination), and is more invasive and uncomfortable for the patient [2].

By September 2020, over 150 tests have been carried out per 1000 people in Spain [1]; these showed a prevalence of SARS-CoV-2 of 8.9%. However, many of these tests were repetitions or were conducted in conjunction with other tests [3].

Data from epidemiological studies conducted around the world have shown the necessity to develop new types of tests (as mentioned above), which use samples that are easier to obtain, such as saliva. Saliva has previously been used for the detection of infections [4]. IgA levels in saliva samples have previously been reported for patients testing positive for SARS-CoV-2 [5]; testing these levels would also facilitate the population-based mass screening for COVID-19 [6].

The spike protein is a glycosylated protein present on the outer surface of SARS-CoV-2; it plays a key role in viral entry into host cells [7, 8]. Cryogenic electron microscopy studies have shown that trimeric arrangement and structural changes are necessary for the fusion of host and viral membranes [9]. The S1 and S2 subunits of the spike protein are affected to different extents by these changes, which ultimately allow the receptor-binding domain to access the target. The nature of the function of the spike protein is the basis of many studies, which target the protein with the goal of neutralizing the virus. Antibodies detected in patients infected with SARS-CoV-2 have also been reported to target the spike protein [10–12].

While these characteristics of the spike protein drive its use in test systems, different complications arise. Each monomer of the spike protein is 180 kDa and is composed of two subdomains, which are folded in a complicated manner. The protein is heavily glycosylated and can undergo other post-translational modifications, including acylation and phosphorylation [13]. The safety, yield, and reproducibility, among other factors, dictate the use of the recombinant protein, as opposed to the spike protein isolated from the natural source. However, not all systems are adequate for the production of proteins with these characteristics. *Pichia pastoris* has previously been used for the expression of difficult proteins with high yields, at cheap prices, using scalable protocols (including industries) [14]. In fact, a domain of the SARS-CoV spike protein was successfully expressed in *P. pastoris* in 2009 [15].

Immunoassay technology for *in vitro* diagnostics is of relevance in healthcare, clinical, agro-food, environmental, and pharmaceutical research, as well as for drug control and other fields. The field of optical techniques has experienced significant developments in biochemical sensing, which can be divided into approaches based on interferometry and resonance, absorption, and fluorescence. The latter two are based on labeled technologies such as enzyme-linked immunosorbent assay (ELISA) or lateral flow-based systems [16]. However, the significant advantages of optical biosensing systems based on combined interferometry and resonance, used with or without optical waveguides, are worth mentioning [17,18]. The applications of surface plasmon resonance-based biosensors [19–21], diffraction grating coupled interferometry [22], photonic crystals [23,24], ring resonators [25–27], Mach–Zehnder [28,29], guided-mode resonance biosensors [30,31], Young interferometers [32], and resonant nanopillars [33], among others, have been widely reported.

In order to detect specific anti-SARS-CoV-2 immunoglobulins in the serum or saliva samples of patients, immune responses must be emulated *in vitro* in a biosensor. To achieve this, we immobilized the recombinant SARS-CoV-2 spike protein in the sensing area of photonic biosensors. These photonic biosensors are based on biophotonic sensing cells (BICELLS), which have recently been reported [34–36]. Not only are the biosensors important, but also is the manner in which the transducing signals are readout in them. The interferometric optical detection method has been reported [37,38] to be a reliable system to

archive the demanding limit of detection required for most *in vitro* diagnostic systems. It also has capacity for high-throughput screening. Both of these features are essential for the detection of antibodies specific to SARS-CoV-2.

Therefore, the work described here intends to present an *in vitro* diagnostic system consisting of a truncated and recombinant SARS-CoV-2 spike protein immobilized in BICELL photonic transducers. This system aims to detect anti-SARS-CoV-2 IgG, IgM, and IgA antibodies in serum and saliva samples of patients testing PCR-positive, using the interferometric optical detection method (IODM); the results obtained were compared and correlated with those obtained by ELISA.

ELISA is a gold standard laboratory technique that allows for the specific detection of a large variety of specific analytes in various types of samples. Therefore, it has diverse applications in the fields of clinical diagnostics, food quality, and biotechnology, among others. However, it has several disadvantages: it is a laborious procedure, requires a large sample volume, has limited options for multiplexing, and has a detection limit just lower than the nanomolar range [39,40]. The success of the IODM has already been proven. Its ability to conduct high throughput screening and its cost-effectiveness could also be beneficial in health systems.

2. Materials and methods

2.1. Production of recombinant truncated spike protein using *Pichia pastoris*

SARS-CoV-2 complementary DNA was kindly donated by Isabel Solá (Consejo Superior de Investigaciones Científicas – Centro Nacional de Biotecnología, CSIC-CNB, Spain). The truncated region of the *spike* gene was first amplified by PCR using specific primers. After cloning into the pPICZalpha plasmid, which served the vector, *P. pastoris* Bg11 cells were electro-transformed, and the protein was produced as per the manufacturer's instructions. A fragment corresponding to the largest epitope was produced, as previously described [41].

Recombinant SARS-CoV-2 spike protein (rS1) was purified from the supernatant of the yeast culture and isolated by chromatography. Briefly, supernatants were dialyzed against 0.1 M ammonium acetate (pH 6.8) for 8 h at 4 °C (Spectrum Labs Spectra/6–8 kD MWCO RC Dry dialysis membrane, Fisher Scientific, Waltham, MA, USA). After freeze-drying, the dialyzed supernatant was fractionated by size exclusion chromatography on a Sephacryl S-200 High Resolution system (GE Healthcare, Chicago, IL, USA) in 0.1 M ammonium acetate (pH 6.8) (1 mL/min, 5 mL fractions). Fractions were quantified by bicinchoninic acid test (Thermo Scientific, Waltham, MA, USA) and analyzed by Coomassie staining and immunoassays using specific antibodies against the SARS-CoV-2 spike protein (Invitrogen, Thermo Fisher, Carlsbad, CA, USA).

The protein quality was assessed by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry, and its identity was verified by peptide-mass fingerprint analysis, using an Ultraflex workstation (Bruker Daltonics, Bremen, Germany) equipped with a 337 nm nitrogen laser by standard methods. FlexControl Software version 2.4. (Bruker Daltonics) was used for sample analysis and for control of the analytical method parameters.

2.2. Human samples

To evaluate the immune activity of rS1, ELISA was performed using saliva samples collected during the voluntary and experimental pilot trial for the early detection, surveillance, and control of COVID-19 of the Universidad Politécnica de Madrid carried out at the Center of Biomedical Technology (<http://www.ctb.upm.es/blog/>).

The presence of SARS-CoV-2 antigens in the serum samples collected from patients with and without SARS-CoV-2 infection was confirmed by PCR. These samples were supplied by the Health Research Institute

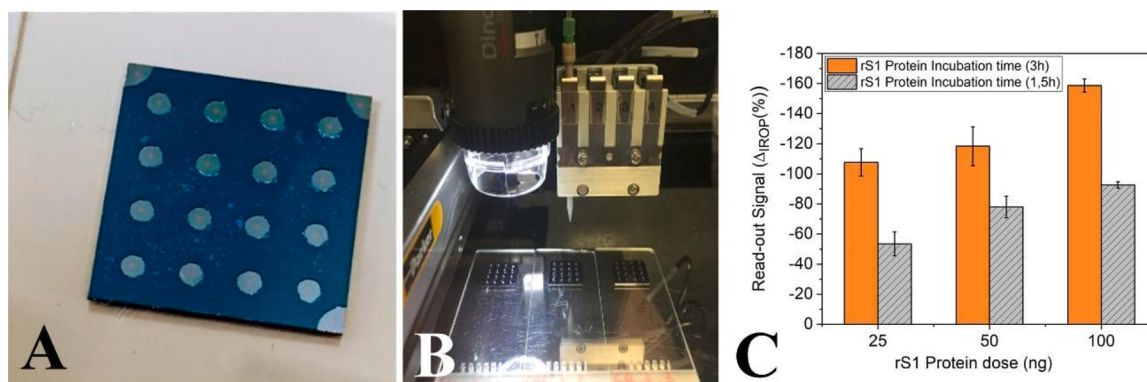


Fig. 1. Biofunctionalization of biophotonic sensing cells (BICELLS). (A) Sixteen 200 µm BICELLS integrated into one biokit. (B) BioDot dispensing 1 µL of reagent per BICELL in three biokits. (C) Sensing recognition curve for 200 µm BICELLS. A calibration curve of ΔIROP (%) vs concentration of rS1 protein (25–100 ng/cell) after incubation for 1.5 and 3 h. $n = 4$ BICELLS for each concentration. Abbreviations: ΔIROP , difference in the increased relative optical power signal between the reference and after recognition of the biological event; rS1, recombinant SARS-CoV-2 spike protein.

biobank, belonging to the Hospital Clínico San Carlos of Madrid (B.0000725; PT17/0015/0040; ISCIII-FEDER). Clinical studies were approved by the local ethics committee of the Hospital Clínico San Carlos (20/404-E_COVID). The samples were classified as per the clinical diagnostic criteria, as moderate, severe, or mild. Once the serum samples were received, they were thawed and treated at 56 °C to achieve complement deactivation.

2.3. Activity assays

Briefly, polystyrene 96-well microtiter plates (Costar 3590, Corning) were coated with 50 µL purified rS1 (5 µg/mL) and incubated for 2 h at 37 °C. After blocking, the plates were washed and incubated with saliva samples at 1:10 dilution (ON, 4 °C). The presence of specific IgA antibodies was detected by incubation with polyclonal horseradish peroxidase (HRP)-labelled anti-IgA antibodies (Thermo Fisher) for 1 h at 25 °C. The plates were washed again and then developed by treatment with 50 µL peroxidase substrate buffer (Ultra-TMB, Thermo Scientific). After 30 min, the reaction was stopped by treatment with 50 µL 2 N HCl, and the optical density was measured at 450 nm. Phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) was used as a negative control. Assays were performed in triplicate.

When the recombinant protein activity was studied, 98 % of patients who tested PCR-positive for SARS-CoV-2 ($n = 54$; March–June 2020) showed positive titers for IgA, IgG, and IgM antibodies. The test results of patients who had not been in contact with the virus ($n = 14$) were negative. Therefore, even though the percentage similarity between the activity of the natural protein and the recombinant fragment is not known, we can confirm that it is a good bioreceptor for use in the detection of anti-SARS-CoV-2 antibodies.

In ELISA, the negative control was coated with casein blocking buffer; signals greater than 0.1 absorbance units were not obtained in any of the cases. The final data shown were obtained by subtracting the value of the negative control from the values obtained for the recombinant protein.

2.4. Use of multiplexed biosensors on a kit

In this study, 16 independent BICELLS, transduced on chips, packaged on a kit, were used, since they were easy to handle. Each BICELL is based on two Fabry-Perot interferometers: one layer of SiO_2 and a thin SU-8 polymeric film which exhibits reliable optical biosensing [42,43].

For the fabrication of the BICELLS, SU-8 2000.5 (MicroChem Corp., Newton, MA, USA) was spin-coated on a silicon substrate with a thin layer of SiO_2 . It was then soft-baked at 115 °C for 30 s. An ultraviolet light-exposure process was then conducted, followed by a post-baking

step at 115 °C for 4 min and a developing step for 2 min (Laguna et al., 2015). Finally, the BICELL surface was activated by means of an O_2 plasma process [44], to immobilize the recombinant SARS-CoV-2 spike protein onto the sensing surface. We tested and verified that the biofunctionalized biosensors can maintain their stability and activity for at least 3 months.

2.5. Biosensor readout

All immunoassay measurements were carried out using the IODM to read the 16 multiplexed BICELLS on a single kit (Fig. 1A). The read-out used to evaluate biosensing was based on the increased relative optical power (IROP) signal; this has been reported previously [38]. In brief, IROP is the unit of measurement used for the quantification of changes in the optical readout for the two interferometric signals. When one of the interferometers changes its response due to biological recognition, the IROP signal changes. We define ΔIROP (%) as the difference between the reference IROP and the IROP after recognition of the biological event. Therefore, this methodology allows us to detect the concentration of the target biomolecules in an analyzed sample after specific binding to the biosensing response, as previously reported [37, 43].

2.6. Biofunctionalization

The whole batch of immunoassays was performed using rS1, produced in *P. pastoris* and purified as described in Section 2.1. The rS1 protein was automatically dropped over each BICELL (1 µL, as a 300 µg/mL solution in PBS [pH 7.5]) (Sigma-Aldrich, St. Louis, MO, USA) using an automated liquid dispensing platform, the BioDot AD1520TM (Fig. 1B). The BICELLS were then incubated for 3 h in a humid environment at 37 °C. After incubation, the biokits were washed by directing a stream of ultrapure water onto the surface of each BICELL and dried using filtered air. The ΔIROP (%) signal of the rS1 biofilm was then measured and the values were confirmed to be within our admissible limits of tolerance. A dose-response curve (concentration- ΔIROP [%]) was used to determine the concentration of protein to be biofunctionalized and to establish the tolerance limits (Fig. 1C). All kits were blocked with casein hydrolysate 1x (Sigma-Aldrich) for 1 h with agitation. Finally, the BICELLS were washed with 20 mL ultrapure water, shaken with ultrapure water for 45 s, and dried using clean air. The BioDot dispensing system can accurately dispense small volumes of reagent (in the order of 100 nL), allowing excellent reproducibility in the biofunctionalization stage.

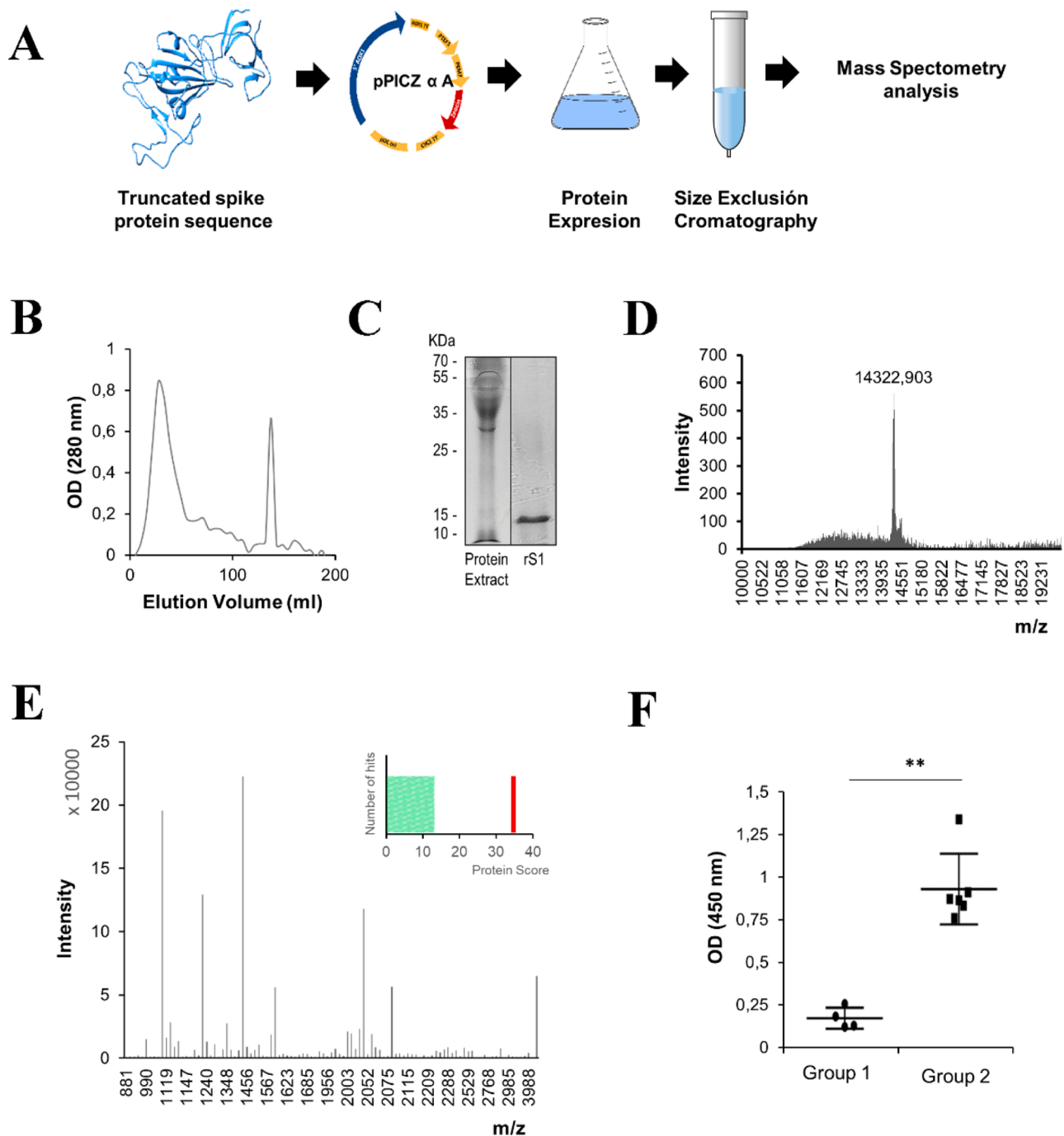


Fig. 2. (A) Scheme of the production of truncated recombinant SARS-CoV-2 spike protein (rS1). (B) The protein extract was fractionated by size exclusion chromatography on a Sephacryl S-200 High Resolution system. The elution profile of the extract was detected at 280 nm (shown above). (C) The protein extract and purified rS1 (5 μ g) were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis and Coomassie staining. (D) Molecular weight determination by matrix-assisted laser desorption/ionization-time of flight mass spectrometry of purified rS1. Measurements were performed in the linear positive mode, operating in the range of m/z = 4000–60000. (E) Peptide mass fingerprinting spectrum and Mascot scores ($p < 0.05$) of the purified rS1. (F) Activity assay to analyze the binding of rS1 with specific IgA in the saliva samples obtained from the volunteers. Group 1: saliva from volunteers who tested negative for SARS-CoV-2 by PCR and serological tests. Group 2: saliva samples from volunteers who tested positive for SARS-CoV-2 by PCR and serological tests. Statistical significance (** $p < 0.01$) determined by Mann-Whitney test for unpaired samples ($n = 6$). Abbreviations: OD, optical density.

2.7. In vitro detection of total Ig, IgG, IgM, and IgA in serum

Once the kits with the rS1 virus protein were biofunctionalized, serum samples were diluted 1:10 and 1.5 μ L of the sample was added per cell. This was then incubated for 3 h at 37 °C in a humid chamber to measure the total Ig levels. Washing was performed using 20 mL ultrapure water, followed by shaking for 10 min with PBS (Sigma-Aldrich),

and two syringes of 20 mL ultrapure water, before drying with filtered air. The IgG, IgM, and IgA titers were measured simultaneously, allowing the total Ig to be determined directly. To separate the titers from the antibodies, the kits were incubated with secondary antibodies (α IgG, α IgM, α IgA). The kits from the washing step were incubated with 1.5 μ L of α IgG (1:250, Sigma-Aldrich), α IgM (1:20, Sigma-Aldrich), and α IgA (1:10, Sigma-Aldrich) for 3 h at 37 °C in a humid environment.

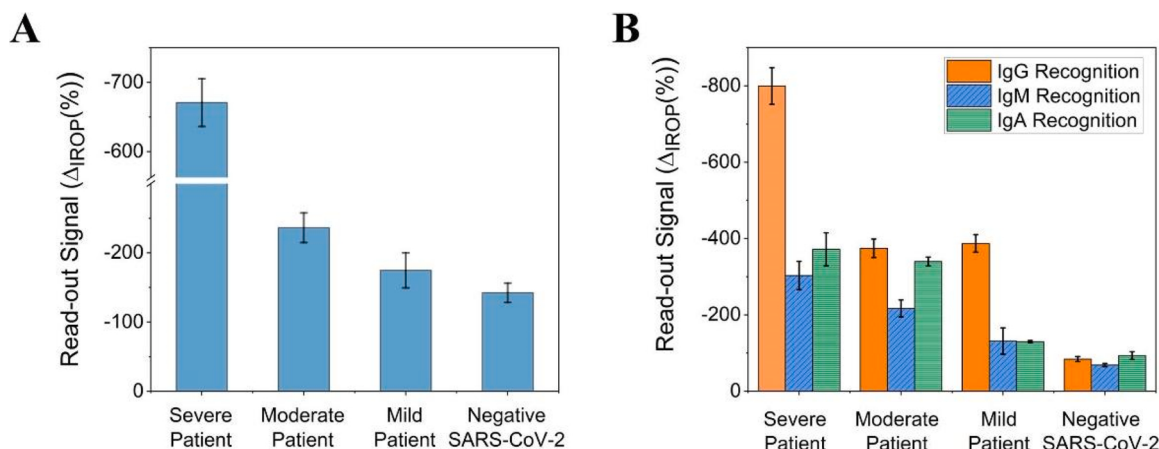


Fig. 3. *In vitro* detection of Ig total, IgG, IgM, and IgA in serum. (A) The vertical axis represents the $\Delta IROP(\%)$ corresponding to the total Ig levels detected in the sera of patients with severe, moderate, mild, and no SARS-CoV-2 infection. (B) The vertical axis represents the $\Delta IROP(\%)$ corresponding to the levels of specific IgG, IgM, and IgA antibodies in the same group of patients. Abbreviations: $\Delta IROP$, difference in the increased relative optical power signal between the reference and after recognition of the biological event; Ig, immunoglobulin.

Then, the washing step was performed using 20 mL ultrapure water and shaking for 2 min in Milli-Q water. The kit was then dried using clean and dry air. IROP (%) were measured after each stage of incubation.

2.8. *In vitro* detection of IgA in saliva

First, saliva samples were centrifuged for 10 min at 15,000 rpm and the supernatant was collected. The sensing surface was bio-functionalized with the rS1 viral protein, as described in Section 2.6. To measure the total Ig levels directly, 2 μ L of the saliva samples were incubated in each well overnight at 4 °C in a humid chamber. The washing step was performed with 20 mL ultrapure water and shaking for 45 s in Milli-Q water, before drying with clean air. To quantify the concentration of IgA in saliva and corroborate the results previously obtained, the kits were incubated with specific antibodies (α IgA). They were treated with 2 μ L α IgA (1:10, Sigma-Aldrich) and incubated for 3 h at 37 °C in a humid environment. Next, the washing step was performed using 20 mL ultrapure water and shaking for 2 min in Milli-Q water. The chip was dried with clean and dry air. IROP (%) values were measured after each stage of incubation.

To increase the speed of the measurements, the assay was conducted using the same protocol, changing only the saliva incubation time. In this manner, the samples were incubated for 1, 3, and 6 h, and overnight as a control; the duration of incubation with α IgA (1:10, Sigma-Aldrich) was not changed.

2.9. Correlation assays

To determine the correlation between the two techniques (BICELL-based ELISA and IODM), ELISA plates were coated with 50 μ L of purified rS1 (5 μ g/mL) and incubated for 2 h at 37 °C. After blocking, the plates were washed and incubated with serum samples (1:40 dilution) and saliva samples (1:10 dilution) in blocking buffer:PBS (1:4 dilution) overnight at 4 °C. The presence of specific IgG, IgM, and IgA antibodies was detected by incubation with HRP-labelled polyclonal antibodies against IgG (1:25000), IgM (1:20000), and IgA (1:10000) (Thermo Fisher) for 1 h at 25 °C. Plates were washed again with 0.05 % PBS-Tween and then developed with 50 μ L peroxidase substrate buffer (Ultra-TMB, Thermo Scientific). After 30 min, the reaction was stopped with 50 μ L of 2 N HCl, and the optical density was measured at 450 nm. PBS containing 1% BSA was used as a negative control. Assays were performed in triplicate.

B

3. Results and discussion

The domain of the SARS-CoV-2 spike protein (rS1) was expressed as a recombinant protein and purified from the media culture by size exclusion chromatography. It was then analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (Fig. 2A–C). To assess the purity and identity of the protein, two different mass spectrometry analyses were carried out. Molecular weight analysis of the protein by MALDI-TOF revealed the presence of a unique peak representing a mass of 14322 Da (Fig. 2D), while peptide-mass fingerprinting verified the identity of the protein (Fig. 2E). After confirming the purity and nature of the protein, the activity was evaluated using saliva samples obtained from volunteers who had tested PCR-positive (Fig. 2F). The specificity of recognition was evaluated by ELISA. The results showed that the IgA antibodies present only in the patients who tested PCR-positive for SARS-CoV-2 (Group 2) were able to recognize rS1.

In this work, we report the detection of specific anti-SARS-CoV-2 immunoglobulins in the serum and saliva of volunteers, using 16 BICELL-multiplexed kits. We demonstrated the *in vitro* detection of specific total Ig in patient samples, while avoiding the nonspecific adsorption of the serum and saliva complex matrix.

In addition, this is a method for direct detection and does not require any chemical developers or signal amplification (Figs. 3 and 5AA). The use of O₂ plasma etching of SU8 epoxy resist to immobilize rS1 improved the specific detection of immunoglobulins, since the activation of the open epoxy rings is sustained for the first hour, during which the immobilization of rS1 takes place. We observed that the dose of the protein required to properly cover the sensing area of BICELLs was 100 ng, and the required incubation time was 3 h (Fig. 1C). This result was obtained by dropping 1 μ L of 100 μ g mL⁻¹ protein and comparing two incubation times (1.5 h and 3 h); saturation of the surface was observed at 3 h. Moreover, to prevent the non-specific adsorption of serum and saliva, we blocked the surface with casein hydrolysate.

To determine the type of immunoglobulins — IgG, IgM, and IgA in the serum and IgA in saliva — the corresponding secondary antibodies were used in the second step of recognition.

First, total specific immunoglobulins to SARS-CoV-2 and IgG, IgM, and IgA titers in serum samples were measured. Stronger read-out signals of total Ig levels were observed in patients with severe disease; the signals decreased in patients with moderate or mild disease (Fig. 3A). The titers differed based on the clinical classification (severe, moderate, and mild). Upon comparison of the individual immunoglobulin titers, we found that the level of IgGs was significantly elevated in patients with severe disease, compared to those with moderate disease (Fig. 3B).

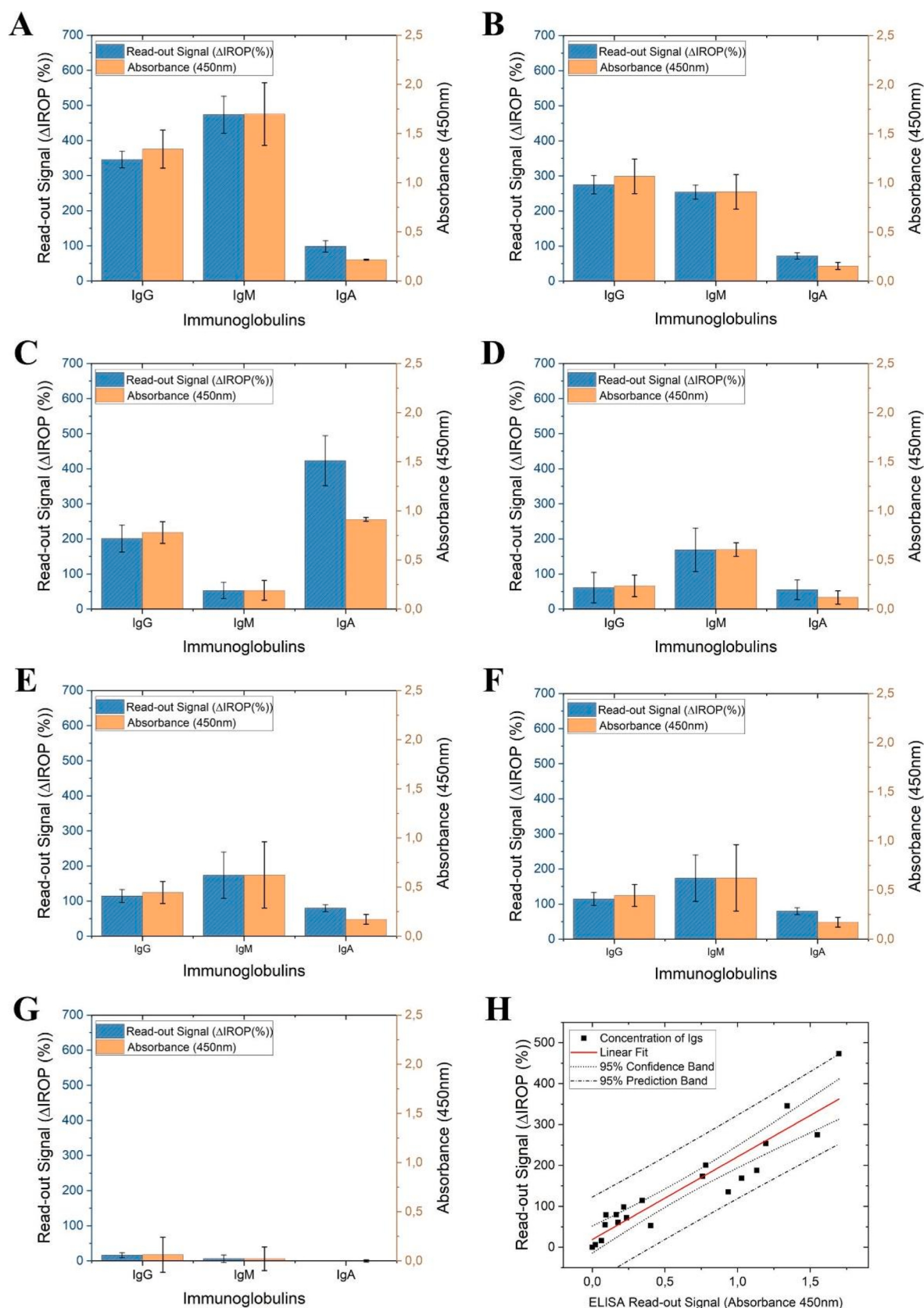


Fig. 4. Detection of IgG, IgM, and IgA in serum samples by measurement of Δ IROP and correlation of the results with those determined by ELISA. (A–C) Immunoglobulins levels in the sera of three patients with severe disease, measured by IODM and ELISA. (D–F) IgG, IgM, and IgA levels in the sera of three patients with moderate disease. (G) Samples of sera from healthy volunteers, who tested negative for SARS-CoV-2 infection, were used as a negative control. (H) Linear fitting between the results obtained by ELISA and the IODM, with a 95 % confidence interval and a 95 % prediction band. Abbreviations: Δ IROP, difference in the increased relative optical power signal between the reference and after recognition of the biological event; ELISA, enzyme-linked immunosorbent assay; IODM, interferometric optical detection method; Ig, immunoglobulin.

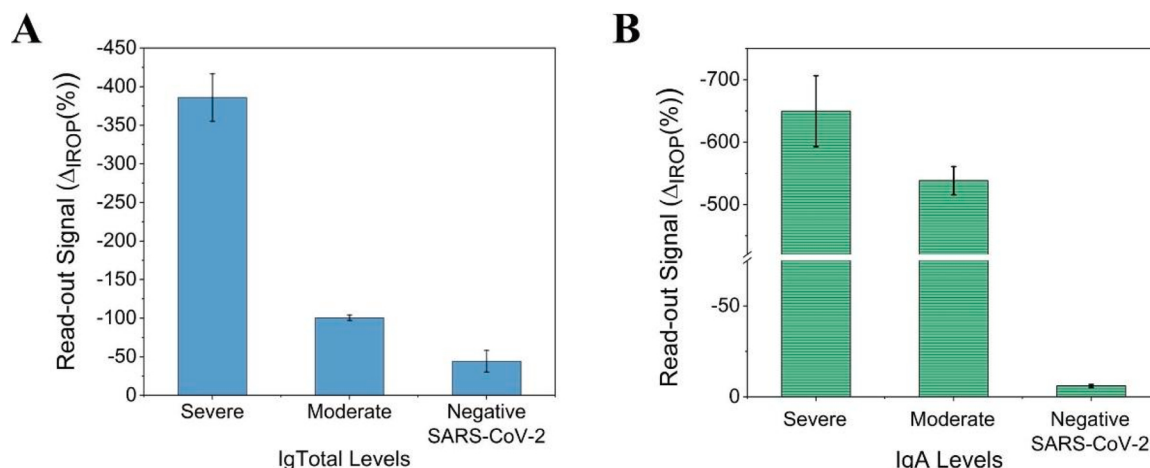


Fig. 5. *In vitro* detection of IgA in saliva. (A) Ig total levels in saliva determined by a direct detection method. The antibody titers in patients with severe, moderate, and no disease are represented. (B) The $\Delta IROP$ (%) corresponding to the specific anti-rS1 IgA antibody levels. Abbreviations: $\Delta IROP$, difference in the increased relative optical power signal between the reference and after recognition of the biological event; rS1, recombinant SARS-CoV-2 spike protein; Ig, immunoglobulin.

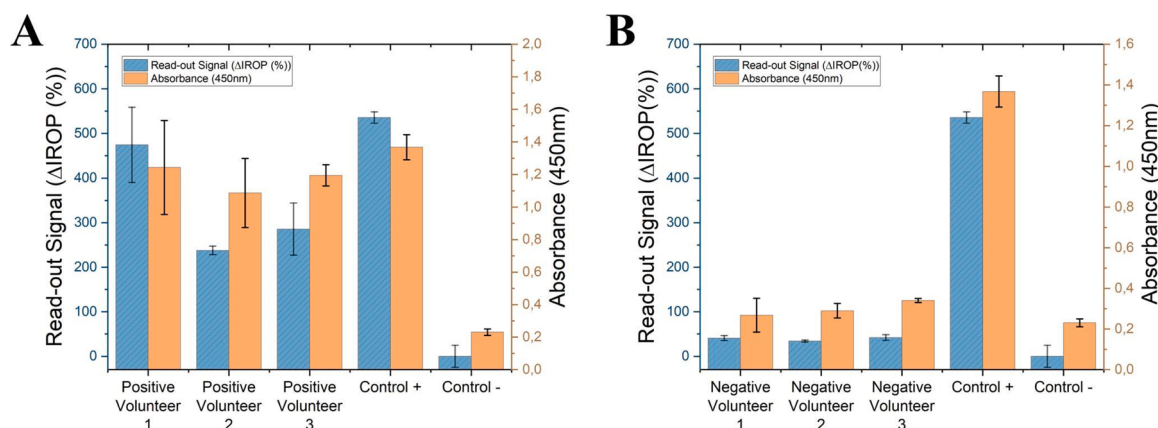


Fig. 6. Detection IgA in saliva by IODM and ELISA. (A) $\Delta IROP$ (%) and absorbance values for IgA in the saliva samples of volunteers who tested positive for SARS-CoV-2 infection. (B) IgA values measured by IODM and ELISA of volunteers who tested negative for SARS-CoV-2 infection. In all cases, the same PCR-confirmed positive and negative controls were used. Abbreviations: $\Delta IROP$, difference in the increased relative optical power signal between the reference and after recognition of the biological event; ELISA, enzyme-linked immunosorbent assay; IODM, interferometric optical detection method; Ig, immunoglobulin.

The levels of IgMs and IgAs were also found to be higher in patients who were classified as severe and moderate, in comparison with those who had mild symptoms (Fig. 3B). Finally, patients who tested negative for SARS-CoV-2 infection showed a low signal, representative of the background signal of the *in vitro* detection system.

To validate this method, these results obtained using the BICELL-based IODM were correlated with those obtained by ELISA, for serum samples of patients with severe, moderate, mild, and no disease. The levels of individual specific antibodies (IgG, IgM, and IgA) against SARS-CoV-2 were measured. The antibody titers of three patients with severe disease (Fig. 4A–C) and three with moderate disease (Fig. 4D–F) were measured, and similar results were observed using both techniques for all the patients. For both protocols, samples from a volunteer who tested negative for SARS-CoV-2 by PCR and tested by ELISA was used as a negative control (Fig. 4G).

Finally, as previously reported for the detection of interferon gamma [45], linear fitting, correlating the results from the BICELL-based IODM and ELISA was prepared; good correlation between both techniques was observed (see Fig. 4H).

After analyzing the serum samples, we tested whether this system could produce accurate results for other types of samples, such as saliva, since it is less invasive and simpler to obtain. Therefore, we measured the levels of anti-rS1 IgA antibodies (an early biomarker of COVID-19) in

the saliva of patients who tested positive for SARS-CoV-2 infections and had different antibody titers (severe and moderate) (see Fig. 5).

A correlation was observed between the total Ig levels in serum and saliva samples (Figs. 3A and 5A). A higher concentration of specific antibodies was observed in patients with a higher severity of disease, while those with moderate disease showed lower levels of these antibodies. It was observed that the specific IgA titer of both samples increased as the severity of the disease increased. The IgA titers in saliva were found to be higher than those in the serum samples, since it is a characteristic antibody of the mucosa (Figs. 3B and 5B). The background signals for both total Ig and IgA titers (antibody titers for volunteers who tested negative for SARS-CoV-2 infection) were very low Fig. 5.

Finally, to verify these results, saliva samples from three volunteers who tested positive and three who tested negative for SARS-CoV-2 infection were measured by ELISA and IODM. IgA recognition values measured by the two techniques showed good correlation. The same positive and negative controls (confirmed by PCR) were used for both techniques (see Fig. 6).

To shorten the waiting time to obtain the results, the saliva of a patient with a high antibody titer was incubated for 1, 3, 6, and 24 h. After 3 h of incubation, the sensing response was not significantly improved. It was also observed that the response at this incubation time

was similar to that for the direct detection of Ig total and the specific recognition of IgA. Therefore, for further experiments, saliva samples were incubated for only 3 h.

4. Conclusions

We report, for the first time, the specific detection of immunoglobulins in serum and saliva samples, using BICELLS biofunctionalized with a truncated SARS-CoV-2 spike protein with an IODM readout, without the need for any chemical developer. Moreover, we measured and compared the titers for different types of anti-rS1 immunoglobulins (IgGs, IgMs, and IgAs) to evaluate the *in vitro* detection system for the diagnosis of SARS-CoV-2 infection using serum and saliva samples. This technique was found to be suitable for use with both types of samples and the presence of specific antibodies against SARS-CoV-2 could be detected. Furthermore, the results showed a good correlation with those obtained by ELISA; the method produced results in the linear range of the correlation curve. It is worth mentioning that, here, we report only the features of this *in vitro* detection system for detecting specific immunoglobulins for SARS-CoV-2; the clinical implications of these results are beyond the scope of this article. Based on our findings, we can conclude that the *in vitro* detection system and the assays described in this paper present promising alternatives for the detection of SARS-CoV-2 infection, and open up the possibility of a new, cost-effectiveness, high throughput technique for patient screening. Additionally, this method allows the use of saliva samples, the collection of which is less invasive and traumatic than for other types of samples. This could facilitate the periodical use of this technique for early detection, surveillance, and control of the spread of SARS-CoV-2 infections.

Author contributions

A.M.M. Murillo: writing-original draft, investigation, methodology of the measurement system and immunoassay procedure, formal analysis and data curation, visualization; **J. Tomé-Amat:** production of the SARS-CoV-2 rS1 protein; **Y. Ramírez:** investigation, methodology of the measurement system, immunoassay procedure, formal analysis; **Maria Garrido-Arandia:** investigation and methodology of production of the SARS-CoV-2 protein; **L.G. Valle:** design of the dropping procedure for the biofunctionalization, biofunctionalization of the diagnostic kits; **G. Hernández-Ramírez:** production of the SARS-CoV-2 rS1 protein; **L. Tramarin and B. Santamaría:** design and micron-nano fabrication of the bio-detection kits, including protein immobilization; **P. Herreros:** diagnostic kit packaging, biofunctionalization of diagnostic kits, design of the protocol for the micro-dropping system; **Araceli Diaz-Perales:** conceptualization and supervision, methodology, writing-review and editing **Miguel Holgado:** conceptualization and supervision, methodology, writing- review and editing, project administration, funding acquisition.

Declaration of Competing Interest

None.

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