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BIOENGINEERING

Diagnosis of COVID-19 and innovative alternative methods based on optic fiber immunosensor

BIOINGENIERÍA

Diagnóstico de COVID-19 y métodos alternativos innovadores basados en inmunosensores de fibra óptica

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Abstract

This work shows a world overview of COVID-19 diagnostic methods, analyzing their effectiveness and sensitivity. With a special emphasis on biosensors, specifically, those that are based on fiber-optic technology, simply explaining their operation and their ability to detect virus as SARS-CoV-2. With these technological advances, the clinical diagnosis will be made faster, cheaper, and applied to patients in remote places where there are no hospitals or clinical laboratories, either due to poverty, geographic difficulties, or violence, factors found in Colombia.

Keywords: *Biosensor, COVID-19, Clinical Diagnostics, Medicine, Technology and Optical fiber.*

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Resumen

Este trabajo muestra una revisión mundial de los métodos diagnósticos de COVID-19, analizando sus efectividades y sensibilidades. Con un énfasis especial en los biosensores, particularmente, los que se basan en la tecnología de las fibras ópticas, explicando de manera simple su funcionamiento y su capacidad para detectar virus como el SARS-CoV-2. Con estos avances tecnológicos, el diagnóstico clínico será rápido, económico y podrá aplicarse en pacientes que vivan en lugares remotos donde no hay hospitales ni laboratorios clínicos, debido a la pobreza, las dificultades geográficas o violencia que son factores que se encuentran en Colombia.

Palabras clave: *Biosensor, COVID-19, Diagnóstico clínico, Medicina, Tecnología y Fibra óptica.*

1. Introduction

To limit the spread of the coronavirus infection and execute a correct treatment for the patients, worldwide experts have demonstrated the importance of developing fast tests to improve the diagnosis of COVID-19 (Table 1). To date, the World Health Organization (WHO) has recommended the use of an assay based on reverse transcription-polymerase chain reaction (RT-PCR) in respiratory samples as the Gold Standard for the diagnosis of COVID-19 [(1)]. Unfortunately, RT-PCR is affected by several practical limitations, including relatively invasive sampling, a time-consuming procedure to process and generate results, the need for specialized operators and certified laboratories. Therefore, the use of RT-PCR is particularly challenging in environments with limited resources. As well, increased global demand for diagnostic tests is limiting the availability of operating material for respiratory sample collection and molecular diagnostics. On the other hand, one of the most used techniques for the detection and diagnosis of diseases is enzyme-linked immunosorbent assay (ELISA). However, complex procedures, a long time, and expensive equipment are required for its use.

Reviewing the literature recently published in the National Center for Biotechnology Information (NCBI), searching the terms diagnosis and fiber optic (June 3, of 2020) 15769 documents were found. However, only one article implemented a fiber-optic biosensor that combines a sandwich immunoassay with the surface plasmons and fluorescence to detection recombinant SARS-CoV nucleocapsid protein N, and was shown an improvement of the detection limit (1 pg/mL) to be increased by 10^4 - fold using the same monoclonal antibodies in comparison with conventional antigen capture ELISA [(2)], showing that fiber optic technology can offer even greater sensitivity than the best conventional methods used.

There are tests to determine if an individual has been infected with SARS-CoV-2: 1) viral nucleic acid detection and viral antigen detection -acute infection-, and 2) detection of antibodies to the virus -prior infection-. Despite being sure that there is a worldwide discussion on the use and use of tests that detect antibodies [(3)], new tests, with high sensitivity, specificity, and using low-cost technology need to be created [(4)]. Therefore, postulating the creation of devices based on optical fibers is suitable and perhaps they would help control, with fewer adverse effects for society, this, and new pandemics.

Every biosensor is composed of three well-differentiated parts: the substrate, the biolayer, and the immobilization interface. The substrate in optical fiber biosensor is silica, where the optical transduction process occurs. The biolayer oversees detecting the target molecules based on the corresponding bioreactions. Finally, the immobilization interface provides the biolayer attachment to the substrate. In the last decades, a

wide variety of immobilization techniques have been developed and have been used for biosensing applications [5].

An Optical biosensor can be defined as a transducer through which biological measurands interact with the light that is either guided through an optical fiber, or that is guided to an interaction region by an optical fiber [6], to produce a modulated optical signal with information related to the parameter being measured, which means, that fiber interacts with an external parameter and carries the modulated light signal from the source to the detector. The input measurement information can be extracted from this modulated optical signal (Figure 1).

Counting sensitivity and selectivity, one of the fundamental characteristics that make most biosensors, so the potential is the possibility of performing the analysis of the substance to be determined in real-time and directly (without the need for the intervention of a marker). These characteristics give biosensors the possibility of carrying out not only a qualitative and quantitative analysis, but also the possibility of evaluating the kinetics of the interaction and, therefore, clear up the fundamental mechanisms of said interaction.

Indubitably, implementing new techniques that in real-time and in a dynamic way make the diagnosis of a disease without the use of long procedures and highly qualified personnel would mean an important technological advance, chiefly for the early detection of some diseases whose diagnosis It can be very costly and tedious, and it can affect populations in inaccessible locations where contact to a clinical laboratory is naught.

Several structures optical fiber-based have been shown as feasible to be used as biosensors: Fiber Bragg Gratings (FBG) [7], Long-Period Fiber Grating (LPFG) [8], Surface Plasmon Resonance (SPR) [9], Lossy Mode Resonance (LMR) [10], and a wide variety of interferometers [11] which stands out tapered fibers [12], and multimode fibers (SMS) [11].

The physical principle in all of them is based on the change in the biolayer produces a change in the light that propagates inside the fiber, making it possible to detect if an antibody or antigen has bound to the biolayer.

Immobilization of bioreceptors onto the biosensor surface can be accomplished by different mechanisms as adsorption, covalent bonds, entrapment, cross-linking, or affinity. Figure 2 depicts an immunoassay used in some papers when a direct immunoassay is carried out on optical fiber [11,13]. First, the fiber optic surfaces should be functionalized by immersion in a solution of copolymer using a solvent. The polymeric deposition provides carboxylic

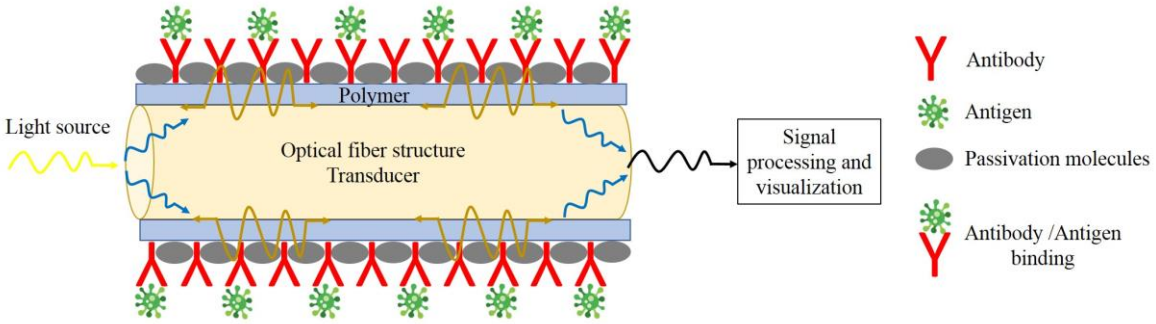

Figure 1. *Optical fiber immunosensor scheme for antigen, viral nucleic acid detection and viral antigen detection*

Table 1. *Diagnostic techniques to detect SARS-CoV-2*

Diagnostic Techniques	Description	Efficiency	Advantages	Disadvantages
rRT-PCR - Reverse Transcription-Polymerase Chain Reaction	The testing procedure includes: (i) specimen collection; (ii) packaging (storage) and shipment of clinical specimens (iii) (good) communication with the laboratory and providing the needed information; (iv) laboratory testing; (v) report the results [19].	The time required to obtain the results can be up to 2 or 3 days [19]. It is only 66 to 80% sensitive [20].	rRT-PCR technique requires sophisticated laboratory equipment that is often located at a central laboratory (biosafety level 2 or above) [19]. Therefore, its result is very reliable.	Commercial PCR-based methods are expensive and depend upon technical expertise, and the presence of viral RNA or DNA does not always reflect acute disease [19].
(Loop-mediated isothermal amplification) LAMP assay	This method uses a set of four specially designed primers and a DNA polymerase with strand displacement activity. LAMP uses strand-displacement polymerase instead of heat denaturation to generate a single-stranded template [19].	LAMP can synthesize target DNA up to 10^9 copies in less than an hour at a constant temperature of 65 °C. Diagnostic sensitivity> 95% [19].	LAMP has high specificity and sensitivity and is simple to perform but also has the advantage of running at a constant temperature, simultaneously reducing the cumbersomeness of a thermocycler as well as the energy required [19].	The clinical applicability of the LAMP technique has not been studied for SARS-CoV-2 yet [21].
IgM / IgG Rapid Test	Immunoassays are tests that identify specific antibodies in the patient's blood. A lateral flow immunoassay has been developed that can detect IgM and IgG in human blood just in 15 minutes [21].	COVID-19 IgM / IgG Rapid Test Sensitivity is 88.66% [21].	Using synthetic peptide as an antigen helps to enhance the stability and repeatability of the immunoassay, and theoretically would be more specific than using the virus as antigen [22].	Usually, the immunoassay only provides qualitative results [23].
A diagnostic model based on radiological semantic and clinical features	Based on CT imaging and clinical manifestations alone, the pneumonia patients with and without COVID-19 can be distinguished. These models will play an essential role in early and easy-to-access diagnosis, especially when there are not enough RT-PCT kits or experimental platforms to test for the COVID-19 infection [24].	Area under the curve value of 0.986 (95% confidence interval 0.966 ~ 1.000) and 0.936 (95% confidence interval 0.866 ~ 1.000) in primary and cohort validation, respectively [24].	The clinical and radiological semantic models provided better diagnostic performance and more considerable net benefits [24].	Were found 18 radiological features and 17 clinical features relevant to form the predictors of COVID-19 infection based on the study [24].

CRISPR-Based Assays	Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) represents a family of nucleic acid sequences found in prokaryotic organisms, such as bacteria. These tests have great potential for point-of-care diagnosis [(23)].	The CRISPR-Based Assays are low-cost and can be performed just in 1 h. Also, the essay follows the isothermal amplification of the target, resulting in a visual readout with a fluorophore [(23)].	For the COVID-19 virus detection, the CRISPR-based methods do not require complex procedures, just reading a paper strip it's possible to know the diagnosis without loss of sensitivity or specificity [(23)].	The methodology has been approved in the United States currently [(23)].
Nucleic Acid Hybridization Using Microarray	Microarray assays have been used for rapid high-throughput detection of SARS-CoV nucleic acids. The microarray assay has proven useful in identifying mutations associated with SARS-CoV and has been used to detect up to 24 single nucleotide polymorphisms (SNP) associated with mutations in the spike (S) gene of SARS-CoV with 100% accuracy [(23)].	A nonfluorescent, low-cost, low-density oligonucleotide array test has been developed to detect multiple coronavirus strains with sensitivity equal to that of individual real-time RT-PCR [(23)].	A portable diagnostic platform based on the microarray chip has been used to identify nucleic acids specific to the MERS coronavirus as well as to influenza and respiratory syncytial viruses [(23)].	One of the drawbacks of microarrays testing has been the high cost generally associated with it [(23)].
Amplicon-Based Metagenomic Sequencing	This diagnostic technique for identification of SARS-CoV-2 relies on a dual approach involving the use of amplicon-based sequencing in addition to metagenomic sequencing [(23)].	Amplicon and metagenomics MinION based sequencing was used to rapidly (within 8 h) sequence the genome of SARS-CoV-2 and the other microbiome in nasopharyngeal swabs obtained from patients with COVID-19 by the ISARIC 4C consortium [(23)].	This dual technique is particularly relevant to SARS-CoV-2 in the assessment of its rate of mutation and to detect its possible recombination with other human coronaviruses, both of which have implications for vaccine development and antiviral efficacy [(23)].	Even when the method is appropriate, it is not widely applied in the labs.
Enzyme-Linked Immunosorbent Assay (ELISA).	ELISA is a microwell, plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies, and hormones [(23)].	The test can be qualitative or quantitative, and the time to results is typically 1−5 h [(23)]	ELISA is speedy, can test multiple samples, and is adaptable to automation for increased throughput but can be variable in sensitivity and is suitable for point-of-care determinations [(23)].	This method often fails to detect the viral infection if the collection procedure is not optimal, or if the patient has low viral load due to an early stage of the disease or suppression by host immunity, or if the samples were obtained at a late stage in the course of infection [(25)].

Neutralization Assay	Neutralization assays determine the ability of an antibody to inhibit virus infection of cultured cells and the resulting cytopathic effects of viral replication [(23)].	The time to results for neutralization assays is typically 3−5 days, but recent advances have reduced this to hours [(23)].	Determination of neutralizing antibodies is important in the short term for the therapeutic application of convalescent plasma and, in the long term, for vaccine development [(23)].	This type of testing requires cell culture facilities, and in the case of SARS coronavirus, Biosafety Level 3 (BSL3) laboratories [(23)].
Luminescent Immunoassay	Luminescent immuno-assays comprise methods that lower the limits of detection for antibody-based reagents. Generally, they involve chemiluminescence and fluorescence [(23)].	IgG was detected in 71.4% (197/276) of all the sera, 192 higher than the detection rate of IgM (57.2%, 158/276). A combination of the two 193 antibodies enhanced the detection rate to 81.5% (225/276). Different sensitivity of the 194 detections of IgG and IgM had been reported in SARS [(22)].	Diazyme Laboratories Inc. announced the availability of two new fully automated serological tests for SARS-CoV-2 that are run on the fully automated Diazyme DZ-lite 3000 Plus chemiluminescence analyzer [(23)].	Currently, was approved for its use in the USA, China, and Brazil [(23)].
Biosensor Test	Biosensor tests rely on converting the specific interaction of biomolecules into a measurable readout via optical, electrical, enzymatic, and other [(23)].	The surface plasmon resonance (SPR) chip had a lower limit of detection of 200 ng/mL for anti-SCVme antibodies within 10 min [(23)].	Most recently, PathSensors Inc. announced a CANARY biosensor to detect the novel SARS coronavirus. This platform utilizes a cell-based immunosensor that couples capture the virus with signal amplification to provide a result in 3−5 min [(23)].	The biosensor is slated to be available for research purposes in May 2020 [(23)].

functional groups (—COOH) to the surfaces, useful for antigen immobilization. Once the optic fiber surface is functionalized, follows the activation of —COOH groups using EDC and NHS and the covalent immobilization of the antigen on the optical fiber surface by pumping a solution of antigen in PBS. Then, surface passivation with BSA in PBS to achieve surface passivation is deposited.

The deposition procedure, solution concentrations, and times involved were followed as presented previously reported in reference [(13)]. Figure 1 presents the final scheme of the proposed biosensor based on an optical fiber. Once the biolayer is deposited, several solutions in PBS with increasing concentrations of the antibody are put in contact with the biosensor. A washing stage using PBS buffer (phosphate-buffered saline) between each new antibody concentration is

necessary to determine wavelength shift due to new antibody binding to the biolayer.

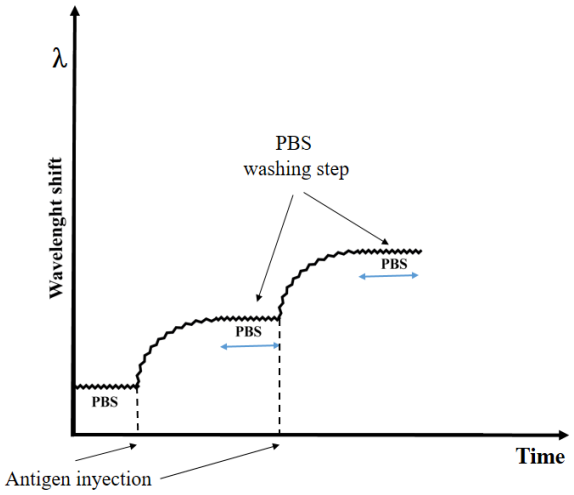

Figure 2. *Direct immunoassay scheme with an optical fiber biosensor*

The variation of the previous parameters for an attenuation band of the optical fiber biosensor as a function of the antibody concentration could be fit through a logistic curve that described the sigmoidal response of a biosensor [(14)]. The Hill equation is well known to characterize this sigmoidal behavior [(15,16)].

From the calibration curve, it is possible to determine parameters such as the dynamic signal range (DSR), the working range (WR), and the limit of detection (LOD) of the biosensor. The biosensor using antibodies could among other possibilities, a using monoclonal antibody that neutralizes SARS-CoV-2 (47D11) [(17)] detecting the virus in infected people and using the EUROIMMUN Anti-SARS-CoV-2 antibodies [(18)], Used for ELISA detection of *in vitro* determination of human antibodies of immunoglobulin classes IgA and IgG against SARS-CoV-2.

Therefore, it is necessary and urgent, to perform approximations to detect the presence of virus as SARS-CoV 2. This technology cannot remain only in researching, it must be implemented for diagnosis now, taking advantage of its multiple advantages over other diagnostic technologies.

3. Conclusions and future work

To conclude, this work demonstrates the biosensors based on fiber-optic technology applicability for SARS-CoV-2 virus diagnosis. As work in the future, the fiber-optic biosensors could be implemented in Colombia in the clinical diagnosis for SARS-CoV-2 disease. With the research is expected that as the telecommunications paradigm changes to the use of fiber-optic technology the same happen with the COVID-19 diagnostic methods, in that order, the article impacts the academic community lighting an important and available technology for development**.**

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