TECHNICAL NOTE

P-FAB: A Fiber-Optic Biosensor Device for Rapid Detection of COVID-19

Divagar Murugan[1] · Himanshu Bhatia[2] · V. V. R. Sai[1] · Jitendra Satija[3]

Abstract

Rapid and low-cost diagnosis of COVID-19 is essential to identify the infected subjects, particularly the asymptomatic cases, primarily to arrest the spread of the disease through local transmission. Antibody-based chromatographic serological tests, as an alternative to RT-PCR, offer only limited help due to high false positives. We propose to exploit our field-deployable/portable plasmonic fiber-optic absorbance biosensor (P-FAB) platform for one-step, wash-free detection of SARS-CoV-2 virus particles directly in saliva sample with minimal sample pre-processing.

Keywords COVID-19 antigen test · SARS-CoV-2 · Nucleocapsid protein · Point-of-care device · Plasmonic fiberoptic absorbance biosensor (P-FAB)

Introduction

The coronavirus disease-2019 (COVID-19) outbreak, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), already has a major impact globally since the first case reported from China in December 2019 (To et al. 2020). The high infectivity and rapid spread of the virus have posed a serious threat across the globe which can be witnessed as a steep rise in the mortality rate in the last four months. To tackle this problem, the transmission of the virus at the community level can be reduced/prevented through social distancing and some other measures including personal hygiene. Further, mass screening for the identification of infected people (with or without symptoms) and their isolation and appropriate treatment have shown positive progress to break the chain of community transmission. Currently, the reverse transcription-polymerase chain reaction (RT-PCR) technique is widely used to detect SARS-CoV-2, which usually takes a few hours for the analysis. However, its wide-scale deployment in resource-constrained settings is limited as it needs expensive equipment and trained personnel. Considering a large population with suspected or confirmed COVID-19, there is an urgent need for a rapid diagnostic tool (Liu et al. 2020).

For rapid detection of COVID-19, typically antibody-based diagnostic assays are preferred due to their convenient field application (Udugama et al. 2020). Current strategies are based on the serological determination of the neutralizing antibodies produced in the host as a defense response against SARS-CoV-2. However, delayed immune response along with large variations in the serum IgM/IgG antibody level in the infected persons may pose the risk of false-positive/negative results (Tang et al. 2020). An efficient alternate bioanalytical approach could be a specific detection of the nucleocapsid (N) protein, an important protein of the SARS-CoV-2 virus. Few clinical investigations have reported that this N-protein is expressed in abundance at the early stage of infection (Kang et al. 2020). In addition, the posterior oropharyngeal saliva has been shown to have a median viral load of ~ 5.2 $\log_{10}$ copies/mL, which correspond to ~ 10^3–10^4 copies in the proposed sample volume of 0.1 mL. On the other hand, a simpler means of saliva collection and processing are available (Oasis Diagnostics), where a porous swab mounted on a syringe piston is chewed and pressed through a

✉ Himanshu Bhatia
  himanshu.bhatia@ricovr.com

✉ V. V. R. Sai
  vvrsai@iitm.ac.in

✉ Jitendra Satija
  jsatija11@gmail.com

[1] Biomedical Engineering Laboratory, Department of Applied Mechanics, Indian Institute of Technology Madras, Chennai 600036, India

[2] Ricovr Healthcare Inc, 252 Nassau St., Princeton, NJ 08542, USA

[3] Centre for NanoBioTechnology, Vellore Institute of Technology, Vellore, Tamil Nadu 632014, India

syringe to extract a neat sample without interferants such as mucus and cellular components. Therefore, a saliva sample from a subject, containing the whole SARS-CoV-2 virus particles and/or N-protein, can be used for the detection of COVID-19 with a simpler sample preprocessing (Azzi et al. 2020; Braz-Silva et al. 2020; To et al. 2020; Williams et al. 2020; Wyllie et al. 2020).

Our Biosensors research group in collaboration with other associates, has invented 'plasmonic fiber-optic absorbance biosensor (P-FAB)', which is a hand-held diagnostic device based on U-bent optical fiber probe. This device has been successfully demonstrated to detect various types of analyte moieties, including proteinaceous antigens and endotoxins (Ramakrishna and Sai 2016; Manoharan et al. 2019). By employing various bioassay formats, we have successfully achieved the limit of detection down to an attomolar (10^{-18} M) concentration of protein (Ramakrishna et al. 2018). Considering the flexibility of the biosensor matrix design without compromising the analytical performance, this technology can be adopted for the specific detection of SARS-CoV-2 in the suspected persons with minor modifications. Here in this paper, we envisage to develop two different types of immunoassays for the detection of N-protein for COVID-19 confirmation. Also, the fundamentals of the P-FAB technology and the time-plan and resources required for its adaptation are described.

Fiber-Optic Biosensor Technology

Over the past decade, the Biosensors Laboratory at IIT Madras, with kind cooperation from his colleagues in India and abroad, has been involved in the development of simple optical absorbance based fiber-optic biosensor technologies for proteins, nucleic acids, bacteria, toxins, and heavy metals. In the past 5 years, several relevant technologies have been developed and four patents have been filed, which include novel techniques for fabrication of inexpensive silica and plastic optical fiber probes and cartridges, point-of-care device hardware, and assay development for a given analyte of interest such as protein-based disease markers. The P-FAB with gold nanoparticles as labels has the ability to detect down to a few hundred analyte molecules in 25 µL of a sample (Fig. 1). This sensing strategy has been adopted to realize a urine sample-based rapid diagnosis of tuberculosis (TB) in 15, min and for the same, a laboratory-level proof-of-concept is already established. The TB diagnosis has been established by detecting a mannose capped lipoarabinomannan (a glycolipid of 17–18 kDa), which is an integral component of the cell wall and cell membrane of *Mycobacterium tuberculosis*. ChemBioSens Pvt. Ltd., a start-up spun out of the Biosensors laboratory and incubated at IITM Research Park, is looking towards commercializing these technologies. The Biosensors Laboratory is also working with Ricovr Inc., a USA based oral diagnostics company, to develop application-specific diagnostic kits on the basis of this technology.

Plasmonic Fiber-Optic Absorbance Biosensor (P-FAB) Platform

The P-FAB technology principally monitors the optical power loss in the light (or change in the absorbance/intensity count) propagating in a multimode U-bent fiber-optic probe using a pair of green LED and photodetector. The green LED was used mainly because the absorption peak of the AuNP labels lies between 520–545 nm for the AuNP
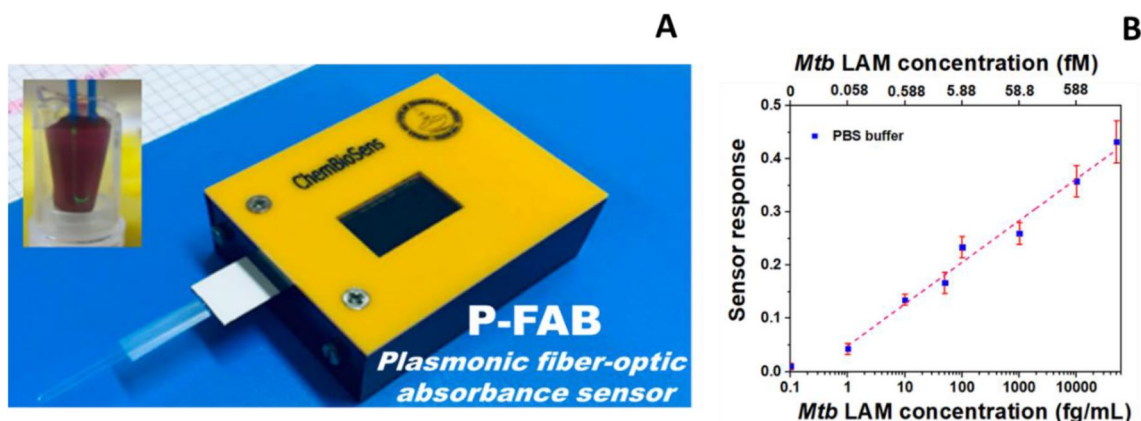

Fig. 1 Photographic image of the P-FAB device with an integrated U-bent fiber probe cartridge for one-step, wash-free analyte detection. The inset image shows **a** U-bent probe dipped in a mixture of gold nanoconjugates and sample solution for analyte detection. **b** The representative P-FAB response for mannosylated LAM based TB detection obtained by means of a sandwich plasmonic immunoassay with AuNP labels using anti-LAM IgM and IgG as capture and detector antibody, respectively

size of interest (20–60 nm). Any specific receptor-analyte interaction at the bio-functionalized U-bent sensing region of the fiber-optic probe leads to modulation in the effective refractive index (RI) and/or evanescent wave absorbance at the biosensor matrix as a function of analyte concentration. The use of gold nanoparticles further amplifies the interaction signal that eventually leads to a greater loss in the light and is measured usually as an increase in the absorbance value (or decrease in the intensity count). This P-FAB technology is already optimized for various parameters including the fiber core size and its numerical aperture, bent diameter, and probe length (Gupta et al. 1996; Khijwania and Gupta 1999; Verma and Gupta 2008; Sai et al. 2009; Gowri and Sai 2016; Divagar and Sai 2018; Danny et al. 2020).

P-FAB Platform for Rapid Diagnosis of COVID-19

To realize the bioassay of N-protein detection using the P-FAB platform, we propose to develop two different bioanalytical approaches for the detection of N-protein as illustrated in Fig. 2.

(i) Label-free bioassay: in this strategy, the biosensor matrix needs to be developed by first immobilizing the gold nanoparticles on the U-bent fiber-optic probe (Satija et al. 2014), followed by covalent conjugation of anti-N protein monoclonal antibodies

through a suitable thiol-PEG-NHS based coupling chemistry. The antibodies with high affinity will be procured for this purpose. Furthermore, the quality of the antibodies will be assessed through standard techniques such as SPR and ELISA to estimate the binding affinity and activity. Subsequently, the antibody immobilized probes will be treated with bovine serum albumin. (BSA) solution to prevent non-specific interactions. Thereafter, these biofunctionalized probes will be used for the diagnosis of COVID-19 by introducing the patient's saliva sample. The binding of SARS-CoV-2 or free N-protein gives a drop in the light intensity. The results can be obtained in 15 min (Sai et al. 2009).

(ii) Labeled bioassay: this strategy is based on the sandwich immunoassay and employs capture and detector antibodies which will be labeled with gold nanoparticles (Ramakrishna and Sai 2016). First, the biosensor matrix needs to be developed by immobilizing anti-N protein monoclonal antibodies (i.e. capture antibodies) on the U-bent fiber-optic probe followed by treatment with BSA solution to minimize the non-specific interactions. Thereafter, the sample obtained from an infected person will be mixed with the anti-N protein monoclonal antibodies (detector antibody) conjugated gold nanoparticles for 5 min followed by the introduction of this complex to the sensing region of the bent probe. This will promptly generate a signal response in the form of absorbance
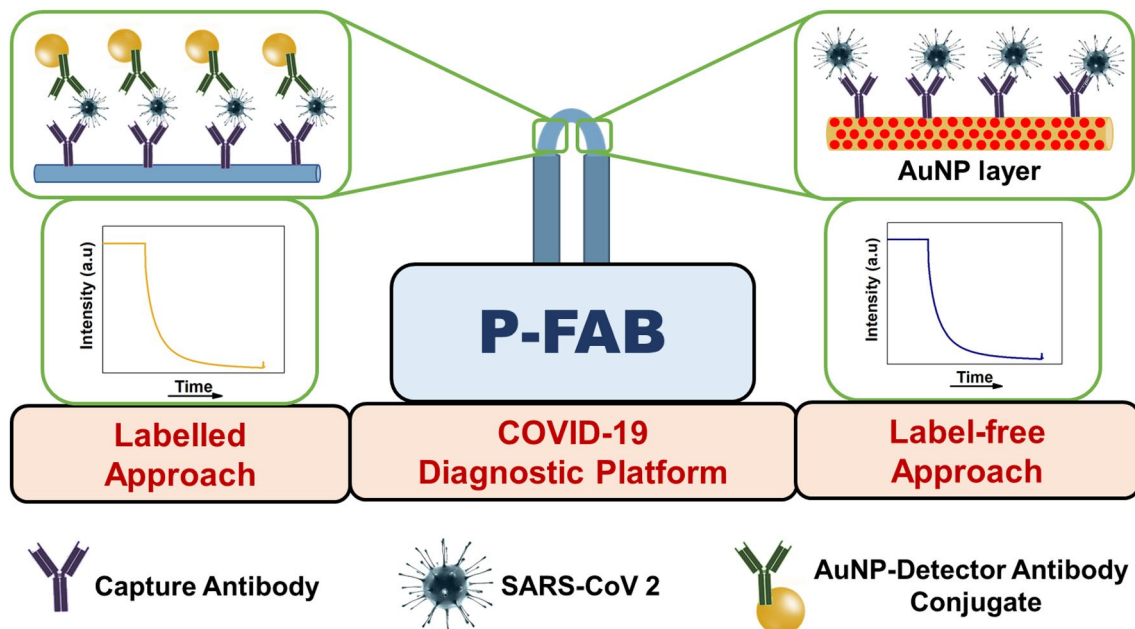

Fig. 2 Labeled and label-free approaches proposed for the development of a fiber-optic biosensor based point-of-care device for SARS-CoV 2 detection from saliva samples

Fig. 3 Timeline for establishing the P-FAB technology for COVID-19 diagnosis

change (or intensity count) leading to saturation in 10–15 min. Viral load as small as 10^6 particles/mL, which is sufficient to cause infection, can be detected using this technique.

The choice among these two approaches for the clinical studies is determined mainly by their sensitivity and specificity. Label-free sensors are highly desirable due to the one-step response without any need for reagents, however suffer from poor specificity because of high non-specific binding. Labelled assays remain as most preferred due to their best possible sensitivity and specificity, however involve a multistep process necessitating either microfluidic integration or manual intervention. Hence, both approaches will be evaluated and compared to choose one for clinical testing. In comparison to the existing LFA based PoC devices, while unprecedented LoDs is a clear advantage, some of the shortcomings include handling of the labelled reagent separately and many unforeseen bottlenecks in establishing the chemical processes and device fabrication.

Timeline

To tweak the P-FAB technology for COVID-19 diagnosis, we need to optimize a few parameters followed by analytical validation and pilot level scale-up and this may take approximately nine months as illustrated in Fig. 3.

Resources Envisaged for the Conversion

To develop the P-FAB based rapid, portable COVID-19 diagnostic tool, there will be immediate requirements of financial support, BSL-III laboratory infrastructure and industrial collaboration. The financial aid will facilitate the establishment of bioassay, mass fabrication of fiber-optic probes & cartridges and point-of-care (PoC) devices. To handle the viral particles and clinical samples and to further validate the assay and PoC device for SARS-CoV-2 detection in the saliva sample, a BSL-III laboratory set-up might be required or collaboration with any institute equipped with such facility may be sought. Industrial support is needed (i) to design and fabrication of user-friendly devices, probes & cartridges, (ii) to establish process flow lines for the above constituents of the device, (iii) for third party validation of

the PoC devices for SARS-CoV 2 and (iv) to establish distribution channels for maximum reach.

Conclusion

The P-FAB, which is based on the U-bent optical fiber sensor system, is a very useful and sensitive diagnostic platform and well-established for the detection of various biomolecular analytes. Since the technology serves as a universal sensing platform, it can be adapted conveniently for the diagnosis of COVID-19 by making some minor alterations in the biosensor matrix. Although COVID-19 is an emerging infection with limited scientific information/evidence, its early and rapid detection is possible by determining the N-protein present in the saliva sample, however, at very low concentrations. The two plasmonic labeled and label-free immunoassays on the highly sensitive P-FAB platform as proposed here can be an ideal candidate for COVID-19 diagnosis within 15 min. We believe that P-FAB technology has a great potential to play a major role in the diagnosis and tackling of coronavirus pandemic.

References

Azzi L, Carcano G, Gianfagna F et al (2020) Saliva is a reliable tool to detect SARS-CoV-2. J Infect. https://doi.org/10.1016/j.jinf.2020.04.005

Braz-Silva PH, Pallos D, Giannecchini S, To KK (2020) SARS-CoV-2: what can saliva tell us? Oral Dis. https://doi.org/10.1111/odi.13365

Danny CG, Raj MD, Sai VVR (2020) Investigating the refractive index sensitivity of U-bent fiber optic sensors using ray optics. J Light Technol 38:1580–1588. https://doi.org/10.1109/JLT.2019.2958044

Divagar M, Sai VVR (2018) Fiber optic plasmonic sandwich immunosensor: influence of AuNP label size and concentration. In: Proceedings of IEEE Sensors. IEEE, pp 1–4

Gowri A, Sai VVR (2016) Development of LSPR based U-bent plastic optical fiber sensors. Sensors Actuators B Chem 230:536–543. https://doi.org/10.1016/j.snb.2016.02.074

Gupta BD, Dodeja H, Tomar AK (1996) Fibre-optic evanescent field absorption sensor based on a U-shaped probe. Opt Quantum Electron 28:1629–1639. https://doi.org/10.1007/BF00331053

Kang S, Yang M, Hong Z, et al (2020) Crystal structure of SARS-CoV-2 nucleocapsid protein RNA binding domain reveals potential unique drug targeting sites

Khijwania SK, Gupta BD (1999) Fiber optic evanescent field absorption sensor: effect of fiber parameters and geometry of the probe. Opt

Quantum Electron 31:625–636. https://doi.org/10.1023/A:1006956824722

Liu W, Liu L, Kou G et al (2020) Evaluation of nucleocapsid and spike protein-based ELISAs for detecting antibodies against SARS-CoV-2. J Clin Microbiol. https://doi.org/10.1128/JCM.00461-20

Manoharan H, Kalita P, Gupta S, Sai VVR (2019) Plasmonic biosensors for bacterial endotoxin detection on biomimetic C-18 supported fiber optic probes. Biosens Bioelectron 129:79–86. https://doi.org/10.1016/j.bios.2018.12.045

Ramakrishna B, Sai VVR (2016) Evanescent wave absorbance based U-bent fiber probe for immunobiosensor with gold nanoparticle labels. Sensors Actuators B Chem 226:184–190. https://doi.org/10.1016/j.snb.2015.11.107

Ramakrishna B, Janakiraman V, Sai VVR (2018) A wash-free, dip-type fiber optic plasmonic (DiP) assay for sub-zeptomole analyte detection. arXiv Prepr arXiv181006437 1–6

Sai VVR, Kundu T, Mukherji S (2009) Novel U-bent fiber optic probe for localized surface plasmon resonance based biosensor. Biosens Bioelectron 24:2804–2809. https://doi.org/10.1016/j.bios.2009.02.007

Satija J, Karunakaran B, Mukherji S (2014) A dendrimer matrix for performance enhancement of evanescent wave absorption-based fiber-optic biosensors. RSC Adv 4:15841–15848. https://doi.org/10.1039/c4ra00198b

Tang Y-W, Schmitz JE, Persing DH, Stratton CW (2020) The laboratory diagnosis of COVID-19 infection: current issues and challenges. J Clin Microbiol. https://doi.org/10.1128/JCM.00512-20

To KK-W, Tsang OT-Y, Leung W-S et al (2020) Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis 3099:1–10. https://doi.org/10.1016/s1473-3099(20)30196-1

Udugama B, Kadhiresan P, Kozlowski HN et al (2020) Diagnosing COVID-19: the disease and tools for detection. ACS Nano. https://doi.org/10.1021/acsnano.0c02624

Verma RK, Gupta BD (2008) Theoretical modelling of a bi-dimensional U-shaped surface plasmon resonance based fibre optic sensor for sensitivity enhancement. J Phys D Appl Phys. https://doi.org/10.1088/0022-3727/41/9/095106

Williams E, Bond K, Zhang B et al (2020) Saliva as a non-invasive specimen for detection of SARS-CoV-2. J Clin Microbiol. https://doi.org/10.1128/jcm.00776-20

Wyllie AL, Fournier J, Casanovas-Massana A et al (2020) Saliva is more sensitive for SARS-CoV-2 detection in COVID-19 patients than nasopharyngeal swabs. medRxiv. https://doi.org/10.1101/2020.04.16.20067835

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.