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Development of a spectroscopic technique that enables the saliva based detection of COVID-19 at safe distances

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ABSTRACT

Research activities are in full swing globally to translate the use of saliva as a non-invasive and highly potential specimen for clinical diagnostics, particularly for COVID-19 detection. Being comprised of a pool of biomarkers also enriched with ACE-2 receptors, saliva can provide vital information regarding the state of the human body. Advancements in biophotonics tools for saliva investigation may offer promise for developing rapid, highly objective, optical modalities for COVID- 19 detection. This article presents concept/design study, which propose the use of Raman/laser induced fluorescence spectroscopic device that have the potential for viral detection via saliva from a safer distance. Noticeable changes of biomarkers present in saliva in response to viral infection can reflect the pathological state, thus can altogether affect the Raman spectral pattern. Monitoring these spectral patterns of saliva, which are further enhanced by using cost effective and reproducible Surface Enhanced Raman Spectroscopy substrates can be a viable option for sensitive and non-invasive viral detection. The spectral information acquired from the optical device can be processed using various multivariate statistical analytical tools, which ultimately facilitate effective viral detection in few minutes. This method doesn't demand the necessity of qualified professionals and sample processing with reagents unlike in RT-PCR test. The proposed optical device can be further modified into a portable form, which can be easily transported for field applications. The stand-off observation, contactless and highly non-invasive technique can be of paramount importance in the current context, where the safer screening of a large population for viral infection by maintaining social distances is a necessity. The proposed stand-off spectroscopic technique can also address the major concern of nosocomial viral transmission amongst healthcare workers during sample collection in a pandemic scenario.

Discussion

COVID-19 outbreak has resulted in 4,627,540 deaths as on 13 September 2021 and the infected percentile is still going up [1]. The present strategies of using nasopharyngeal/oropharyngeal swabs, bronchoalveolar lavage as clinical specimens for SARS-CoV-2 detection are invasive, may cause discomfort to the subjects, and can lead to transmission to the health-workers carrying out the tests [2]. The current pandemic scenario demands portable devices that can be installed and used in "as Is, Where Is" conditions to facilitate fast sample collection process and rapid detection, and assurance of safety for the health workers. Spectroscopic techniques have been the potential candidates, which can facilitate rapid, sensitive and non-invasive mode of SARS-CoV-2 detection as detailed in our recent literature [3]. Advancements in the area of optical instrumentation, data transmission from a field-station to a central facility, and efficient data processing using Artificial Intelligence and Machine Learning techniques have made optical pathology a suitable method for non-invasive, non-destructive, and remotely applicable, cost-effective technique for screening of large numbers of people at multiple locations. The development of miniature diode laser sources, portable spectrometers, charge coupled detectors, optical fibre based probes and stand-off detection methods enable remote measurements of any kind of samples. In addition, techniques like surface enhanced Raman spectroscopy (SERS) and ultra-sensitive laser-induced fluorescence (LIF), and multi-modal instrumentation (Raman + LIF), have opened up new avenues for biological analytes detection at trace concentrations [4,5].

It is well recognized that any deviation from the normal state in a

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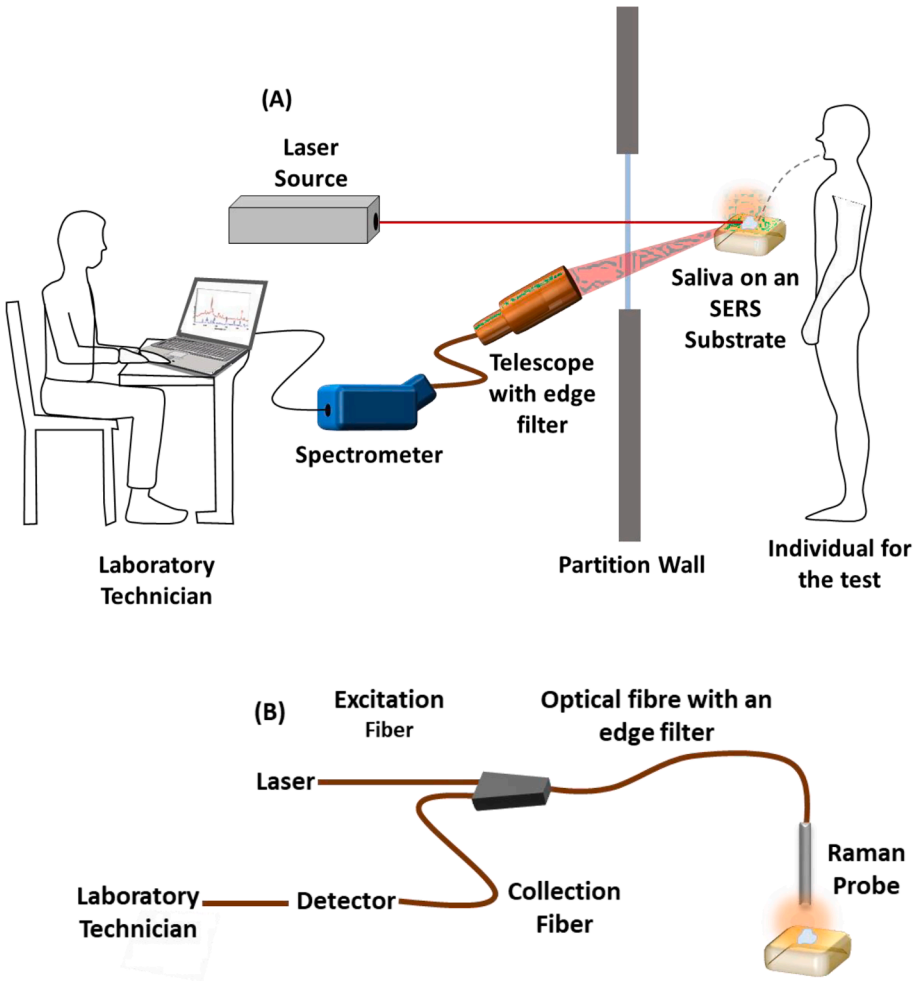

Fig. 1. Schematic of the proposed devices (A) Stand-off mode and (B) Optical fibre based.

living system leads to many bio- molecular interactions in the body, leading production of new molecular species (antigens, antibodies), and changes in normal components (IgG, IgM, CPK, etc). In a condition like viral infection - COVID- 19, in addition to the presence of the virions, these molecular species also will be present in varying amounts in body fluids like saliva, blood, and urine [6–9]. Saliva has been already recognized as a very rich source of biomarkers for screening and early detection of communicable and non-communicable diseases like various pre-malignant and malignant conditions, coronary diseases, diabetes, malaria, viral infections, stress, neurological disorders etc [10–13]. The existence of high viral load was reported in saliva since the epithelial cells in the tongue and oral mucosa were enriched with ACE2 receptors, which serve to be the host for SARS-CoV-2 [14–16]. In addition to the detection of the presence of the virus, the various "marker" molecules can also serve as suitable candidates for screening and early detection of COVID-19 infection. In response to viral infection, antibodies such as IgA will be generated which is easily available in the saliva. The presence and accumulation of these markers are highly dependent on the pathological condition of the individual, which can thus provide insights not only on the onset of infection, but also the prognosis and efficacy of medical treatment. In addition, the abundant presence of spectroscopically relevant amino acids (Tyr, Trp, and Phe) in the gene sequence of coronavirus Spike protein recommends the possibility of spectroscopic detection modalities [17]. Raman spectroscopy can be exploited to target biomarkers triggered in response to viral infections or probe the viral RNA itself from the saliva sample collected from suspected cases. Micro-Raman spectroscopy has been effectively employed for the identification of pathogens, as reported in our recent work [18]. Even minor changes in the composition of body fluid can be detected with high sensitivity with fluorescence spectral patterns. Routine detection of many proteins can be done at femto-atto moles levels by laser induced fluorescence technique [19].

The present manuscript is based on our vast expertise in developing optical devices for disease diagnosis by analyzing clinical samples (tissue, body fluid and breath analysis) in in situ, in vivo and stand-off modes [20–22]. Our group has developed a Raman system combined with LIF and laser-induced break down spectroscopy device for the stand-off detection of materials [23]. The system can also be coupled to a fiber optic probe which can take the excitation laser to a sample several meters away, and transmit back to the spectrometer the Raman or fluorescence signal. It is to be noted that the fiber optic probe will also be a non-contact probe, and samples can be kept several centimeters away from the probe tip. Thus, stand- off Raman/LIF can be a highly desirable technique to monitor viral infections by investigating saliva or other body fluids from a safe distance. In the proposed method, saliva collection and loading of saliva sample on to the device can be performed by the individual themselves. Required instruction can be given by the Laboratory Technician/Operator stationed behind suitable partition between the laboratory and collection centre. Raman/LIF excitation of the sample can be performed using suitable sources like portable multi-wavelength lasers a nanosecond Q-switched Nd:YAG pulsed laser radiation (266, 532, & 1064 nm) which are commercially available now. The Raman/LIF signal can be collected via a telescopic arrangement (Fig. 1(A) or by an optical fibre probe (Fig. 1(B)). As per the instruction of the operator, the saliva sample can be placed on the nanoplasmonic sensor chip (SERS substrate) for Raman or on a simple

microscope slide for LIF. The spectral features can be monitored by the Operator at a safe distance (~5 m distance). All data processing for the diagnosis can be done on a laptop or data can be transmitted to a central lab, to give diagnosis immediately, so that the test can be repeated if necessary.

At present, suitable methods for fabrication of reproducible SERS substrates are available (e.g. Hierarchical structures created on the surface of biocompatible polymer followed by the reduction of noble metallic nanoparticles on structures) and the spectral pattern of the biomolecules/biomarkers in the sample can be obtained with very high sensitivity, which has been demonstrated in our previous work [24]. As mentioned in our earlier studies related to stand-off experiment, the Raman spectra are recorded with pulsed laser excitation and gated detection (20 ns gate width), with an integration time of 10 s [23]. It is evident from the above literature that spectra taken at a distance of 5 m, are as good as any spectrum taken under laboratory conditions. Also, as indicated in the figure in the proposed system, in case of any difficulty that may be encountered in recording the stand-off Raman or fluorescence spectrum, a fiber optic probe can be used. This probe has been used by us for both laboratory measurements and in vivo spectral recording with portable instruments [21,25–26]. The whole process of data acquisition and analysis can be performed within a few minutes. In all these instances, there is no need to even know the identities of the markers in these conditions. What is done is pattern analysis of the spectrum (fluorescence, Raman, Laser Induced Breakdown Spectroscopy [22,27–28], choosing appropriate regions or the whole spectrum and establish the standard pattern for each class possible for the sample -premalignant, malignant, infection, etc. In all these studies no special precautions are necessary in recording the spectra. Present-day data processing techniques like PCA, LDA, SVM, Cluster Analysis etc. can classify samples from small spectral changes in the total data, and do not require specific spectral lines or bands to identify a specific marker or molecular species. As is described in the various pattern analysis methods, the individual concentrations or identities of any of the markers do not play a significant role in the pattern analysis, since these will vary depending on the disease stage/status. In practice, the whole data will thus be normalized with respect to a standard spectral peak or point, and the variations in the relative concentrations and patterns are used to decide on the stage and type of disease. This highly non-invasive method proposed for COVID-19 detection doesn't require qualified professionals and sample processing with reagents unlike in the case of swab- based RT-PCR test. As mentioned earlier the system can be scaled down to a portable device or used as a table top model, which can be easily transported for hospitals and other field applications.

Conclusions

Coronavirus disease-2019 (COVID-19) pandemic has kept the whole world to a standstill by posing a great threat to human life. In view of the probable nosocomial viral transmission during sample collection, the need for safer viral detection technologies is of paramount importance as preventive measures to reduce the spread in the current pandemic. This paper discusses the concept of a potential spectroscopic device, which can facilitate stand-off detection of viral infection from human saliva. The proposed system relies on the optical spectroscopy concepts of Raman spectroscopy and laser induced fluorescence, can enable non-invasive viral detection in a contactless mode. In addition, this instrument can be easily transformed to a portable device, which can be highly beneficial to be transported to clinics and screening centers. The proposed system can be extended further for the detection of any pathogen, where the possibility of nosocomial transmission is a concern.

CRediT authorship contribution statement

Jijo Lukose: Conceptualization, Methodology, Writing – original draft. **Ajaya Kumar Barik:** Conceptualization, Methodology, Writing – original draft. **V.K. Unnikrishnan:** Review & editing. **Sajan D. George:** Review & editing. **V.B. Kartha:** Review & editing. **Santhosh Chidangil:** Conceptualization, Methodology, Writing – original draft.

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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