Development of COVID-19 Detection Using SPR Sensors: A Preliminary Result

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Abstract. Coronavirus disease or COVID-19 is a virus from the Coronaviridae family that has caused pandemics throughout the world since the end of 2019. The virus spreads ten times faster through human interaction than SARS-CoV. The RNA sequence of COVID-19 has a 79.5% similarity with SARS-CoV. Fast and specific detection of COVID-19 is needed so that patient detection can be done quickly and accurately. One method that can be developed as a COVID-19 biosensor is aptamers-based biosensors. The aptamer is an artificial oligonucleic acid that can specifically bind to target molecules. The aptamer is easily and chemically modifiable for increasing stability and reducing toxicity. It shows a comparable affinity for the target virus and better thermal stability than monoclonal antibodies. This advantage makes aptamer a promising candidate in diagnostic and detection applications. The goal of this research is to use an RNA aptamer as the specific recognition element in a portable surface plasmon resonance (SPR) biosensor for the detection of COVID-19 in humans. An aptamer RNA 1 COVID-19 was designed using the COVID-19 sequence from GISAID using the in-silico method. End of 3’ aptamer RNA 1 was modified with dithiol. Then, the aptamer was immobilized on the gold nanoparticle sensor surface via Cysteine-dithiol binding. The RNA solution, that had been extracted from swab samples, was diluted ten times before being used as a sample. The immobilized aptamer RNA 1 captured COVID-19 in RNA solution, causing an increase in refraction index (r.u). An aptamer RNA 1 was found to bind RNA virus of COVID-19 where the positive sample of COVID-19 has refraction index (r.u) between 3 r.u – 10 r.u for various Ct values.

1. Introduction
Coronavirus disease or COVID-19 is a virus from the Coronaviridae family first discovered at the end of 2019 in Wuhan, China. The genetic material for COVID-19 is ribonucleic acid or RNA. The RNA sequence of COVID-19 shares 79.5% similarity with SARS-CoV, which we know as SARS, which
broke out in 2002 in China. The COVID-19 spreads ten times faster through human interaction than SARS-CoV. Symptoms of COVID-19 include cough, fever or chills, shortness of breath or difficulty breathing, runny nose, and many others [1–4]. To prevent a higher death rate, a detection tool that has accuracy is needed and high sensitivity. Currently, PCR is the most widely used method to detect coronavirus infection. However, PCR has several disadvantages, such as tools and equipment qPCR reagents are expensive, require specialists to analyze and interpret results, and relatively long processing time. In addition, different qPCR kits often give different results. This can be caused by the presence of mutations in the primers used in the qPCR kit. The TaqMan probe used as probes in qPCR kits has a minimum detection limit of 4 to 10 copies of RNA template per reaction [5, 6]. TaqMan probe quality will decrease if stored too long, which results in the sensitivity and specificity of the TaqMan probe being low, resulting in less good if used for routine detection. While other alternative methods are rapid tests, which have a weakness, namely the sensitivity is only 70%, so it regularly causes false positive. Therefore, we need a new method that is efficient, inexpensive, has sensitivity, high degree of accuracy without being disturbed by mutations [5–6]. One of the methods that can be developed as a COVID-19 biosensor is an aptamers-based biosensor. Aptamers are artificial oligo nucleic acid-like DNA or RNA that can specifically bind to target molecules [7]. Aptamers are easily chemically modifiable for increased stability and reduced toxicity. It shows a comparable affinity for the target virus and better thermal stability than monoclonal antibodies [8]. Therefore, COVID-19 sample detection will be able to be carried out in a shorter time, at a lower price, cheaper, and more accurate results.

2. Materials and Methods

2.1. Fabrication of AuNPs Substrate
The gold nanoparticle substrates used in this study were manufactured by NanoSPR. The dimensions of the gold nanoparticle plate are 20 mm x 20 mm x 1 mm, the refraction index is 1.61, Au layer and Cr underlayer thicknesses are 45 nm and 5 nm, respectively.

2.2. Design Aptamer RNA 1
The aptamer was designed using the COVID-19 sequence from the GISAID database. The alignment of the COVID-19 database was carried out using Clustal W. The aptamer was designed from the COVID-19 conserved region based on the Clustal W result. Secondary structure analysis of the aptamer design was performed using the mFold Web server [9]. Only aptamers that have looped secondary structures are tested.

2.3. Immobilization Aptamer RNA 1 to AuNPs Substrate
The first step in the RNA aptamer immobilization process was rinsing the NanoSPR substrate using 90 L NFW (Nuclease Free Water). This is done so that the surface of the NanoSPR is clean from impurities such as dust. Furthermore, 100 L of NFW 50 mL of 5 M aptamer RNA was dripped and flattened on the surface of the NanoSPR substrate, especially in the area to be used for testing, then incubated at room temperature for 30 minutes. Then rinsed the NanoSPR substrate using 90 L NFW and dried using a dust blower. Then 50 L of 1% BSA (BSA dissolved in NFW) was added for 10 minutes. Rinsed again using 90 L NFW and dried using a dust blower.

2.4. NanoSPR Substrate Test
The NanoSPR used has the following specification: optical 2, electrochemical 1, refractive index measurement ranging 1.0–1.5, refractive index sensitivity 0.00002, maximum angular scan 17°, single resonant curve measurement time 3 sec, thermostabilization up to 60°C, deviation from set temperature 0.2°C, tracing measurement mode 2 sec, slope measurement mode 0.2 sec, angle of incidence setting precision 5 angular sec and overall dimensions of the measurement unit 8.6x5.2x4 in. (215x130x100).
Before being used as samples in the NanoSPR assay, the RNA samples extracted from the nasopharyngeal swab were diluted ten times using nuclease-free water (NFW). The immobilized NanoSPR substrate was then tested using an SPR device using COVID-19 RNA with multiple qPCR-confirmed CT as samples with flow rate is 0.25 uL/minute, NFW (Nuclease Free Water) for 10 minutes, sample for 10 minutes, and washing NFW (Nuclease Free Water) for 10 minutes.

3. Results and Discussion

3.1. Secondary Structure Prediction

The design of aptamer RNA 1 sequences was analyzed using the mFold Web server to predict bond strength. The analysis result is provided as follows:

![Figure 1. Secondary Structure Aptamer RNA 1. red: optimal energy, green: second optimal energy, blue: third optimal energy, black: lowest energy [9]](image)

Based on the analysis using mFold, it is known that $\Delta G = -3.60 \text{(kcal.mole}^{-1})$ when calculated at 37°C. It means the secondary structure of the aptamer RNA 1 will fold spontaneously. There are two loops at each end of the secondary structure of the aptamer RNA 1, meaning that the bond between the aptamer and the COVID-19 RNA will occur at both ends. Therefore, the bond strength between aptamer RNA 1 and COVID-19 RNA will be high.
3.2. SPR Detection

SPR utilizes surface plasmon waves (electromagnetic waves) in the form of oscillations of charge density propagation at the dielectric and metal interface, which spontaneously propagate in a perpendicular direction. The electromagnetic wave is used for detecting changes when the target analyte interacts with the biorecognition component of the sensor. Therefore, when the chip that has been immobilized using a biosensor is exposed to the sample, it will cause changes that affect the refractive index which is then captured by the SPR sensor. SPR data retrieval using the nanoSPR tool was carried out using the slope mode. The resulting data is a change in the RU reflection index taken from a fixed incidence angle. In this mode, the angle setting or selection can be determined from the plasmon curve. The tool will read changes in the reflection index captured by the photodetector without the need to scan the angle.

Testing of immobilized SPR substrates was carried out using samples that have been confirmed to be positive or negative for COVID-19. Nasopharyngeal SWAB samples were taken from patients who had COVID-19 symptoms, then extracted to obtain viral RNA. Before being used as samples in the SPR assay, the samples were tested using a qPCR assay. The confirmed positive and negative viral RNA was then assayed by SPR. The number of samples used in this study was 7 samples consisting of one positive control, three negative samples, three positive samples with various variants of Ct. By using the SPR test, it can be seen that the interaction between the aptamer RNA 1 with samples that are confirmed to be positive or negative for COVID-19.
Figure 2. (A) Positive Control of Aptamer RNA 1, (B) RNA Negative Sample

Based on Figure 2A, it can be seen that the positive control aptamer RNA 1 gave a very good response to the positive control, which was around 30 r.u. The result indicates that there is an optimal binding between the aptamer RNA 1 as bio-receptors and the positive control. At the time of the last flush, in twenty to thirty minutes, there is no sign of a drop, showing that the bond between the aptamer RNA 1 and the positive control is very strong. While the negative samples gave a very small response, ranging from 2-3 r.u. (Figure 2B) which means that almost no binding occurs between the aptamer RNA 1 and the sample.

Furthermore, in Figure 3 below, we have compared the qPCR and the nanoSPR measurement to determine the accuracy and specificity of the aptamer RNA. Figure 3 shows the SPR detection to several positive COVID-19 RNA samples whose Ct value is known using the qPCR assay.
Figure 3. Positive Sample Response (A) Positive Sample with Ct 12.1, (B) Positive Sample with Ct 30.3, (C) Positive Sample with Ct 37.9

The samples used in this study were confirmed using the qPCR assay. The qPCR is a PCR method that allows the quantification and analysis of PCR results in real-time. It has been used as a more sensitive and accurate test to detect COVID-19 compared to antigens or antibodies. Positive or negative depends on the Ct (cycle threshold) value or Cq. The Ct or Cq indicates the number of cycles required for the fluorescent signal to pass the threshold. The Ct value is inversely proportional to the number of target RNA in the sample (a low Ct value indicates many target RNA). If the Ct value is greater than or equal to 40 then the sample is negative for COVID-19, if the Ct value is less than 40 then the sample is positive for COVID-19. The smaller the Ct value, the greater the amount of target RNA detected in the qPCR assay.

Based on Figure 3, it can be seen that the increase in the binding response between the aptamer RNA 1 and the positive sample was 10 r.u for the RNA sample Ct 12.1; 8 r.u for the RNA sample Ct 30.3 and 3 r.u for the RNA sample Ct 37.9. The higher the Ct (Ct 12) value, the more COVID-19 virus RNA has been extracted from the swab sample. The positive sample response with CT 37.9 is 3 r.u close to 2 r.u which is the negative sample response value. This shows that the SPR results are according to the qPCR assay result which the higher the Ct value, the higher the response generated, vise-versa.

The RNA extracted from the nasopharyngeal swab contained various kinds of RNA (not only COVID-19 RNA). If in this study a good response was obtained between the aptamer RNA 1 with a positive sample of COVID-19, then this means the stronger the binding between the aptamer RNA 1 and the sample. In other words, the binding of the aptamer RNA 1 is specific to the COVID-19 RNA sample.

4. Conclusion
We have evaluated to assess the use of an RNA aptamer as the specific recognition element in a portable surface plasmon resonance (SPR) biosensor for the detection of COVID-19 in humans, which resulted in a positive outcome. From the study, it can be concluded that SPR can detect COVID-19 samples well with satisfactory outcomes and gives the same results as the qPCR test results.

5. Suggestion
We suggest for future research to carry out further tests using samples with extra variations in the value of Ct.
Acknowledgements
The authors gratefully acknowledge the financial support provided by PMDSU funding to carry out the research.

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