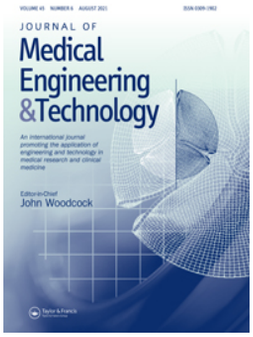

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INNOVATION

Design of an effective piezoelectric microcantilever biosensor for rapid detection of COVID-19

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ABSTRACT

Acute respiratory syndrome coronavirus 2 (SARS-CoV-2), also called COVID-19, is one of the most contagious viruses resulting in a progressive pandemic. Since specific antiviral treatments have not been developed yet and its fatal rate is almost high, early and fast detection is critical for controlling the outbreak. In this study, a piezoelectric microcantilever biosensor has been designed for detecting COVID-19 samples directly without requiring preparation steps. The biosensor acts as a transducer and is coated with the related antibody. When the SARS-CoV-2 antigens adsorbed on the microcantilever top surface through their spike proteins, a surface stress due to the mass change would be prompted leading to the measurable tip deflection and floating voltage. To obtain a biosensor with optimum parameters, different shapes and piezoelectric materials have been assessed and it was concluded that a Poly (vinylidene fluoride) (PVDF) biosensor in a shape of a holed punched form triangle, represented the best result. Therefore, the highly sensitive microcantilever biosensor can detect COVID-19 in clinical samples with various viral loads, rapidly. Also, it is selective enough to differentiate SARS-CoV-2 from other viruses with similar symptoms.

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COVID-19; microcantilever biosensor; piezoelectric; rapid detection

1. Introduction

Acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a progressive pandemic of coronaviruses in 2019 which is called COVID-19 (Coronavirus disease 2019). As the World Health Organisation (WHO) stated, COVID-19 is a Health Emergency and International Concern from February 2020 which first appeared in Wuhan, China in December 2019. There are seven kinds of coronaviruses which have different severity of symptoms. Middle-East Respiratory Syndrome – Corona Virus (MERS-CoV), Severe Acute Respiratory Syndrome -Corona Virus (SARS-CoV-1), and SARS-CoV-2 (COVID-19) have had high pathogenicity among the others. Similar to MERS and SARS-CoV-1, COVID-19 has developed unprecedented pandemics in societies [1]. Regarding the updated statistics, it was confirmed that more than hundreds of million cases have been infected which results in more than two million deaths over the world, so far. Scientist noticed that COVID-19 is one of the most contagious viruses in this era which its propagating infection ends up to severe pneumonitis that could be more fatal for whom suffering from constitutional diseases like diabetics, cardiovascular diseases, and malignancies. The majority of patients present symptoms of fever, dry coughs, sore throat, dyspnoea, and myalgia. Over 93 mutations have been found in the entire genome of SARS-CoV-2 which lead to different organ damages and develop various symptoms like diarrhoea, anosmia, ageusia, unilateral otalgia, and cutaneous lesions during the infection [1–3]. COVID-19 mostly spread through droplets by sneezing, coughing, or even talking of an infected person to a close-contacted one. Recently, the possibility of fecal-oral and vertical transmission from mother to child is suggested besides the currently confirmed droplet and direct contact transmissions [2,4]. In fact, COVID-19 is a highly contagious virus particularly in the first three days of symptom onset, and the more critical issue is that asymptomatic infections and transmissions have been reported. COVID-19 carriers could transmit the virus within the incubation period before the symptom appearance. The average incubation period for COVID-19 is 5.1 days but it varies from 0 to 12 days [5]. To decrease virus spreading speed, it is critical to distinguish symptomatic and asymptomatic patients as early as possible. Health workers have

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started to screen patients by using a thermometer or a pulse oximeter to detect the common symptoms. The screening methods do not seem significantly sensitive to distinguish patients at an acceptable level.

Recently, scientists across the world have made significant efforts to develop efficient vaccines to cease the COVID-19 crisis. Several vaccines such as Pfizer-BioNTech, Moderna, Johnson&Johnson, SputnikV, and AstraZeneca have been approved and their vaccination programs have been started in some countries [6]. However, new genetic variants of COVID-19 have being appeared continuously all over the world during its circulation. This phenomenon has raised researcher's concerns about the therapeutic method's effectiveness [7]. To be more specific, each variant contains one or more mutations in its genetic sequences affecting the neutralisation impacts of the vaccine-induced antibodies. So far three main variants arising from South Africa, the UK, and Brazil have emerged with more severity of illness followed by increased transmission and hospitalisation rates [8–10]. Some pieces of evidence have shown these variants have undergone mutations within the genes contributing to COVID-19 Spike protein that is almost the main target of the available vaccines [10–12]. Regarding the considerable confrontations of the vaccination program mentioned above, stopping the spread at the source still remains the key. In this situation, rapid diagnostic tests are required to detect and quarantine the infected cases immediately to stop the outbreak.

There are three main diagnostic tests available to detect COVID-19 infected patients; 1-real-time reverse transcription polymerase chain reaction (rRT-PCR), 2-Computed tomography imaging, and 3-Antigen-antibody conjugation test. At present, the gold standard diagnostic test is rRT-PCR which detects viral RNA in human liquid specimens but it has several drawbacks. RT-PCR is a high-risk transmitting and a time-consuming process that requires 1 to 3 days for the result preparation. Consequently, it makes an uncertain condition for individuals whether they should be quarantined or not, leading to the further spread of the disease. Moreover, PCR test results might be falsely negative due to a lack of technician knowledge about how to collect a sample from which part of the respiratory tracts. Sensitivity is not high as it is ranged between 45 and 60 percent. With all taken into account, RT-PCR is a complex operation that needs experienced technicians and expensive equipment [13,14]. Chest Computed tomography (CT) imaging has been utilised as another diagnostic tool to show the respiratory involvement of COVID-19. In some symptomatic patients, CT imaging could not show the radiographic features (bilateral ground glass opacity) of COVID-19 pneumonia. As a matter of fact, CT imaging has low specificity in terms of differentiating various and identifying specific viruses. Also, it is not useful for diagnosing asymptomatic, pre-symptomatic, and mild symptomatic patients without pneumonia [13]. Besides, x-rays of CT-scanner are harmful in a long time because ionising radiation may damage cells and lead to changes in DNA sequences. The serological assay (antibody test) analyzes whether your immune system has reacted to the infection or not. An antibody test is not suitable for screening early phase cases but also it is useful to confirm past SARS-Cov-2 infections in convalescent-phase cases. In fact, it shows that one has been infected with the virus at some point in the past and could be recovered or still be contagious. Several kinds of research have been done to measure and estimate the sensitivity, specificity, and accuracy of these tests to identify the best way they can be used in different clinical settings [15,16]. A research done by Pan *et al.* assessed IgM and IgG positive ratio and the sensitivity of the colloidal gold-based immunochromatographic assay in PCR confirmed cases. This study indicated that the test was not sensitive (11.1%) in the patients whose samples were taken at the early stage (1–7 days after onset) [16]. Hence, immunologic assays are not recommended at the first stages of the disease due to its low accuracy under two weeks of getting infected.

Newly, a lot of researches are inclined to the potential of antigen-antibody interaction biosensors which are designed for early and fast detection [13,17]. For this purpose, the desired biosensor should be highly sensitive and have the capability to detect the COVID-19 antigens directly from swab samples without requiring sample preparation steps. Basically, antigen-antibody based biosensors can be classified into four types regarding the transducer used, which are: 1) electromechanical, 2) optical, 3) piezoelectric; and 4) micromechanical. The transducer functionality is to transform the antigen-antibody reaction into a measurable electrical, optical, colorical, and mechanical signal. Electromechanical and optical detections are the popular ones that are named conventional biosensors. They have some problems like requiring routine maintenance, considerable packaging, and complicated electronic interfacing. Micromechanical biosensors also have been utilised with a simpler structure without the above-mentioned drawbacks. Cantilevers are one of the subsets of mechanical biosensors that have been emerged since the early nineties of the last

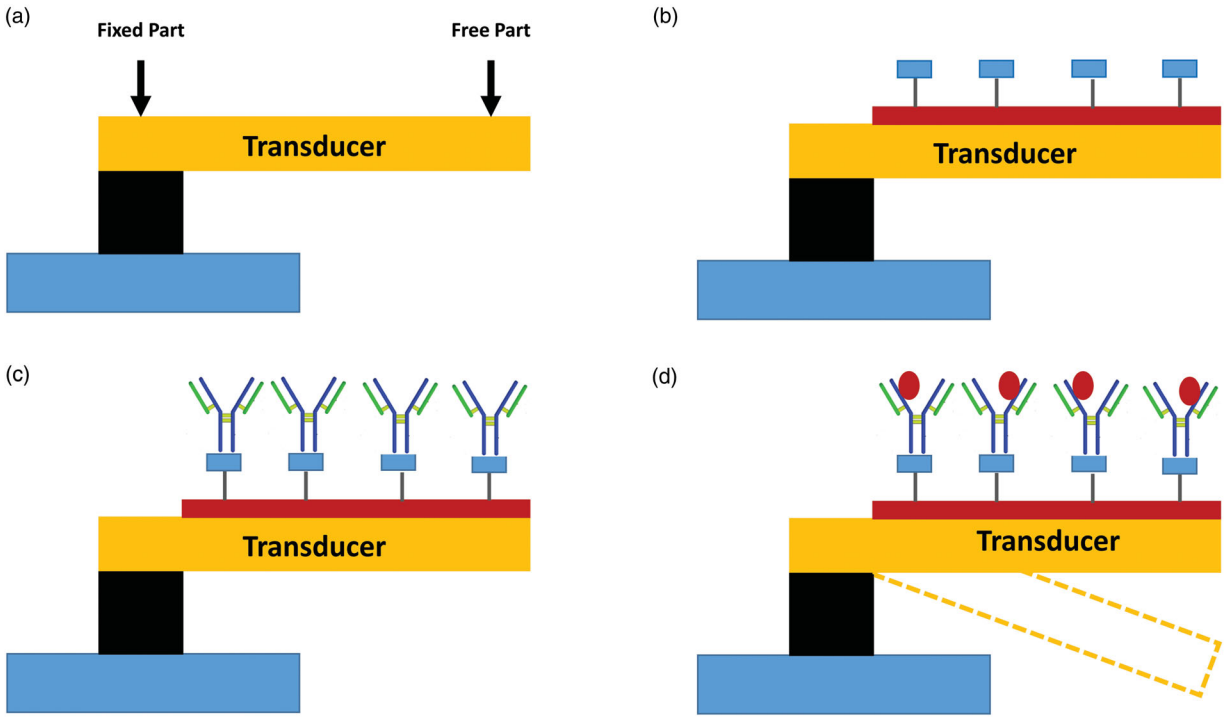

Figure 1. Schematic illustration of the microcantilever biosensor structure (a), Surface coating and functionalization (b), Antibody immobilisation (c), Antigen-antibody conjunction leading to the microcantilever deflection due to the mass change (d).

century. Given the importance of the rapid and acute diagnosis, a selective and sensitive piezoelectric microcantilever as an antibody-coated biosensor is suggested in this study. This accurate diagnostic test is convenient, available, and low-cost which can differentiate COVID-19 from other similar viruses [18]. Practically, microcantilever biosensors are swiftly becoming more and more applicable as they can detect a wide range of chemical and biological agents and screen a variety of diseases [19]. These detectors are very sensitive although they are fabricated easily and their materials and equipment don't cost so much during the production [20]. Real-time detection, the ability to detect more than one analyte accurately, and being label-free are other benefits [21]. Sensitivity and selectivity are key factors that notably affect the biosensor quality [22].

2. Structure and action mechanism of the microcantilever

Microcantilever biosensors work based on the mass changes during the adsorption of analytes on their functionalised surface. The interaction between the antibody and antigen causes this deflection [23] (Figure 1(d)). These sensors are manufactured in micro scales, which are fixed at one end, while are free at the other one (Figure 1(a)). Their selectivity can be adjusted by functionalising the surface of the microcantilevers, so that the chemically or biologically intended species can be identified as they bind to them (Figure 1(b,c)) [24]. Regarding the above-mentioned features, microcantilever biosensors could be promising tools for analysing and detecting the COVID-19 pandemic fastly and effectively.

Microcantilever sensors can operate in both liquid mediums and air. One of the methods that might be used for electrical signalling is piezoelectric [25]. The piezoelectric effect is the ability of certain materials to an electric charge in response to applied mechanical stress. This property is often seen in crystalline or semi-crystalline materials and there are many materials, both natural and man-made, that exhibit a range of piezoelectric effects. When piezoelectric material is placed under mechanical stress, a shifting of the positive and negative charge centres in the material takes place, which is due to the alignment of bipolars in crystalline phases of its material that results in an external field [26]. In this work, a multilayer piezoelectric microcantilever will be designed and modelled. Its mechanism of action has been depicted in Figure 2. As can be seen from the figure, the microcantilever consists of two layers; the elastic layer which is located at the bottom, and the piezoelectric on that is situated on top. When the chemical agent adsorbed on the top of the microcantilever, surface stress due to the

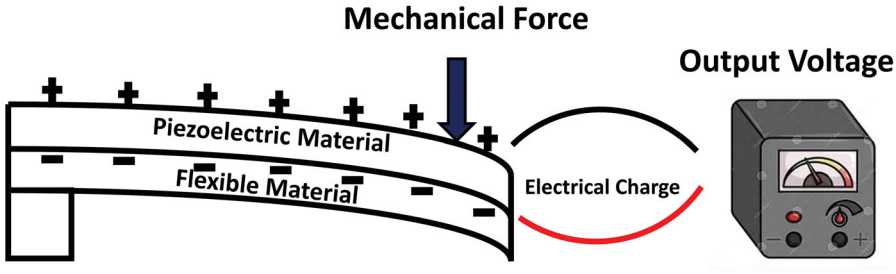

Figure 2. Schematic illustration of the piezoelectric microcantilever signal processing.

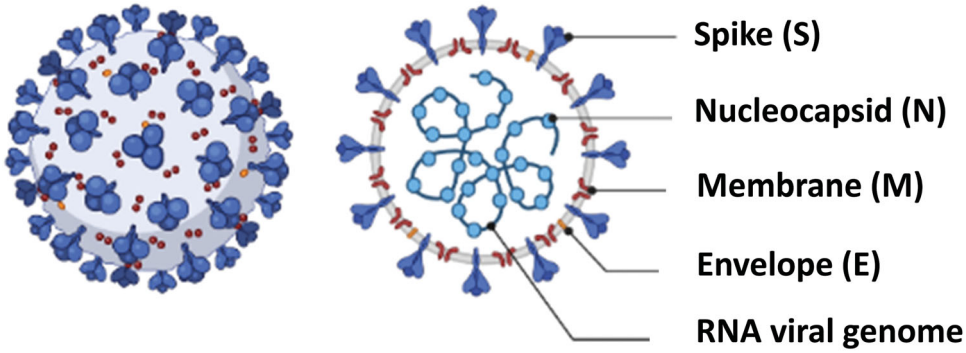

Figure 3. Structure of the COVID -19.

mass change would be prompted leading to its tip deflection. This mechanical force causes strain on the piezoelectric layer. The strain, operating as an external force, produces a voltage because of electrical charge accumulations on the outer surfaces.

3. Antigen and antibody

3.1. Antigen (COVID-19)

COVID-19 is one type of coronaviruses with RNA-positive strands which has emerged as a devastating infectious disease. COVID-19 molecular weight is about (5.5 to 6.1) $*10^6$ and its diameter is placed in the range of 75 to 160 nanometres. It is described as a spherical crown-like shape virus with some projections on its envelope [27]. The envelope contains a lipid bilayer membrane with four types of structural proteins; Membrane protein (M) which is the predominant one, Nucleocapsid protein (N), Spike glycoprotein (S) which adheres to host cells and interacts with Angiotensin-Converting Enzyme 2 (ACE2) receptors and Envelope small protein (E) (Figure 3) [28]. E and M proteins have essential roles in assembling the viral particles. Previous studies showed that N protein is beneficial to be applied as a diagnostic antigen due to its relatively conservative property in coronavirus structure. It plays an important role in viral pathogenesis, replication, and RNA packaging [29]. COVID-19 infects target cells through its spike antigen binding to ACE2 receptors expressed on different cells of tissues. It attaches to ACE2 receptors on epithelial cells of the nasopharyngeal region and type2 pulmonary cells in the lower respiratory tracts. Viral envelope fuses with the cell membrane of host cells which results in the release of viral RNA into the host cells and eventually its replication [13,28,30]. Based on the neutralising property of the anti-S protein antibody, it has the potential to be utilised for diagnosing the disease and designing the vaccines. It is the major transmembrane protein of COVID-19 and has higher immunogenicity than the others [13,17]. Regarding the mentioned features, S protein has been considered the major antigen that binds to the coated antibodies on the biosensor surface.

3.1.1. Quantification of COVID-19 in a sample

Some studies measured the COVID-19 viral load in nasal and throat swabs, oropharyngeal saliva, and sputum samples. Pan *et al.* by analysing 82 infected patients stated that the viral load differed in various specimens which were about 79,000 per ml in the throat and around 752,000 copies/ml in sputum 5–6 days after the first symptom appeared [13]. Yu and co-workers by examining 323 samples taken from 76 patients illustrated that the mean viral load in sputum is about 17,429 copies/test which is much higher

than in throat and nasal swabs that are 2552 copies/ml and 651 copies/ml, respectively [31]. The viral load of SARS-CoV-2 reaches its peaks swiftly (after 3 days of symptom onset) which is the same as the influenza virus [13]. From the experimental perspective, positive rates of rt-PCR were compared in different samples from respiratory tracts, including sputum species, nasopharyngeal swabs, and throat, which were reported as 74.4–89.9%, 53.6–73.3%, and 44.2%, respectively. COVID-19 viral load is higher in early and advancing stages than in the recovery period. According to the pieces of evidence, viral particles have been found in different human liquids including faces, urine, blood (viremia in severe disease). However, the viral count is too low in these specimens to be detected for diagnosing the infection [14].

3.2. Antibody

The human immune system responds to virus invasion by producing immunoglobulin molecules (antibody) to neutralise the viral antigen. There are three types of antibodies created in response to infection; IgA, IgG, and IgM; antibodies rise and fall at different times after the onset of infection. In usual, IgG is the choice to be measured in most antibody tests as it lasts for the longest time and may reflect longer-term immunity. Like other infectious diseases, B-lymphocytes secrets some types of antibody especially IgM in the acute phase which is found in lymph fluid, blood, and plasma cells as the first antibody. Its protective function is temporary and remains for a short time [1]. Scientists demonstrated that about 48% of the patients, produced IgM antibody in the first week of infection while nearly 96% of them, have it two weeks later [15]. Therefore, it is not an appropriate agent for diagnosing patients in the early phase, particularly in two weeks of symptoms onset. According to the above-mentioned features and for simpler modelling, IgG has been selected to be coated on the biosensor surface and binds the COVID-19 virus through its antigen. IgG is the most abundant and the smallest antibody that is produced by white blood cells. IgG is secreted and identified in serum 7 days after symptom onset. Its concentration begins to rise gradually and peaks during 21th–25th day at 16.47 μg/ml and remains at its high level for about 4 weeks [32,33]. It is a Y-shaped monomer with a molecular weight of 150 kDa [34]. In the current study, the specific COVID-19 antibody could be collected in two ways; 1-Convalescent plasma, 2-Recombinant antibodies (rAbs). Convalescent plasma contains high specific antibodies against antigens of SARS-Cov-2. Now, it has therapeutic uses in COVID-19 patient treatment and it could be utilised as diagnostic agents in the future, as well [35–37]. Monoclonal antibodies (mAbs) are homogenous collections of antibodies that are identical in their protein sequences. Recombinant antibodies are monoclonal antibodies that are produced *in vitro* by using synthetic genes. Once mAbs with the desired specificity has been developed, it must be produced in a large amount as practical medicines for therapeutic uses. Recently, scientists have developed recombinant monoclonal antibodies that recognise S and N protein of SARS-Cov-2, specifically [38–40].

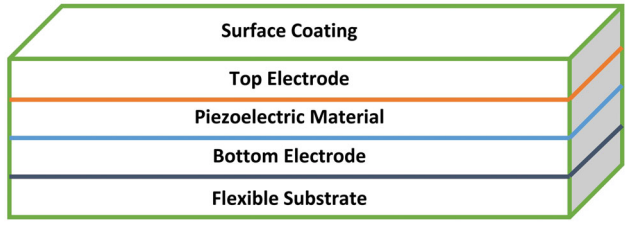

Figure 4. The proposed microcantilever structure.

4. Proposed structure and material for the microcantilever

The proposed biosensor consists of the following layers from bottom to top: A flexible substrate, a piezoelectric material which is enveloped with two electrodes for sensing and measuring the external charges, and the surface coating for recognition of the biological agent (Figure 4).

Silicon-based materials like Silicon (Si), Silicon (Si_3N_4), and Silicon Oxide (SiO_2) are used commonly for biosensor fabrication. Based on the previous studies, SiO_2 is one of the best materials that represented higher deflection in response to a mechanical force in comparison with two others [20]. Thus, SiO_2 is recommended for the flexible layer. Both ceramics and polymers that have crystalline and semi-crystalline structures demonstrate piezoelectricity. The piezoelectric coefficient is the amount of electric charge created by the application of mechanical force. Lead zirconate titanate (PZT), barium titanate ($BaTiO_3$), and zinc oxide (ZnO) are among the piezoelectric ceramics with high piezoelectric coefficients which are 110 pC/N, 78 pC/N, and 5 pC/N, respectively. Nowadays, lead-free materials have been attracting more researches because they are friendlier to the environment when their wastes are disposed. Polymers have different properties

compared to minerals [41]. Polymers can fill the depressions, while crystals and ceramics cannot. The piezoelectric strain coefficient (d_{31}) for a polymer is less than that of a ceramic; however, its piezoelectric stress coefficient (g_{31}) is higher. This means that polymers perform better in terms of sensor construction. Sensors and actuators made of piezoelectric polymers are more flexible because they are light and hard and can be turned into different shapes. Polymers also show a great impact on strength and durability. Having dielectric constant, elastic stiffness, low density, and high voltage sensitivity are other polymer properties [42]. Polyvinylidene fluoride (PVDF), Poly (trifluoroethylene) (PTrFE), Polyamide-11 (Nylon-11) are among the polymers with the highest piezoelectric properties with a piezoelectric coefficient of 23 pC/N, 12 pC/N, and 8 pC/N, respectively [43]. In this study, the function of PZT, $BaTiO_3$, and PVDF that have the highest d_{31} among ceramics and polymers as a piezoelectric layer of the microcantilever of the biosensor has been investigated and the results are represented in the following sections. The material properties of the flexible and piezoelectric layers are shown in Table 1.

5. Model analysis and simulation

The deflection amount of the microcantilever tip can be calculated, using Stoney's equation as [44]:

$$\Delta h = \frac{3(1-\nu)L^2}{T^2 E \Delta\delta} \tag{1}$$

where Δh is the tip displacement, L is the microcantilever length, T is the microcantilever thickness, $\Delta\delta$ is the surface stress, E is the Young's Modulus and ν is the Poisson ratio. The following equation also demonstrated the relationship between the tip deflection of the microcantilever and the generated voltage [45]:

$$\Delta h = d31 \frac{3\ L^2\ Ep}{T^2 Ee} V \tag{2}$$

where V is the generated voltage, d_{31} is the piezoelectric constant of the piezoelectric layer, Ep is the Young's Modulus of the piezoelectric layer, Ee is the Young's Modulus of the elastic layer, and V is the engendered voltage. Placing the Equation (2) in Equation (1) gives:

$$V = \frac{Ee\ (1-\nu)}{d31\ Ep\ E} \Delta\delta \tag{3}$$

Equation (3) shows the relation between the surface stress and the generated voltage. It should be noticed that E is considered as the overall Young's Modulus [46].

The piezoelectric microcantilever biosensor is simulated using the finite element method through COMSOL Multiphysics 5.4. A simple three-layered silicon microcantilever model which consists of a flexible layer, a piezoelectric material, and a surface coating from bottom to top was considered. The rectangular-shaped model with its hole punched form and the triangle-shaped one with its hole punched form have been designed in order to find the highest sensitivity. A rectangular form has the dimensions of 2 mm X 0.25 mm (L X W) and the triangle form also has the dimensions of 2 mm X 0.25 mm (H X W). In all geometries, the thickness of the flexible layer, the piezoelectric material, and the surface coating is 0.03 mm, 0.05 mm, and 0.01 mm, respectively (Figure 5). IgG antibodies are immobilised to the top silicon layer. According to studies assessing COVID-19 viral load in samples, it is assumed that at least 1000 antigens will bind to these antibodies and created a force nearly to 9.6e-17 N due to COVID-19 molecular weight to the top surface. It must be noted that all stresses are in Z-direction and one end of the cantilever is fixed. Calculation of the biosensor deflection and the voltage generated in piezoelectric material would evaluate the amount of antibody-antigen conjugations and as a result, the biosensor prosperity in detecting COVID-19 antigens.

6. Results and discussion

In order to find the optimum piezoelectric microcantilever biosensor which possesses the best shape and material for obtaining maximum output voltage, 12 models were studied (four shapes with 3 different piezoelectric materials). The same force per area was applied to each of them. According to relations (1) and (3), by knowing the intended parameters from the materials, the floating potentials of the above experimental design models were calculated and the results are depicted in Table 2.

It can be seen from the table that the microcantilever which is in the shape of a holed punched form triangle

Table 1. Material properties of the flexible and piezoelectric layers.

Properties Material	Density (kg/m^3)	Young's Modulus (Gpa)	Poisson's Ratio	Piezoelectric strain coefficient (d31) (pC/N)
SiO_2	2200	70	0.17	–
PZT	7600	63	0.31	78
PVDF	1760	2	0.34	23
$BaTiO_3$	6020	67	0.35	110

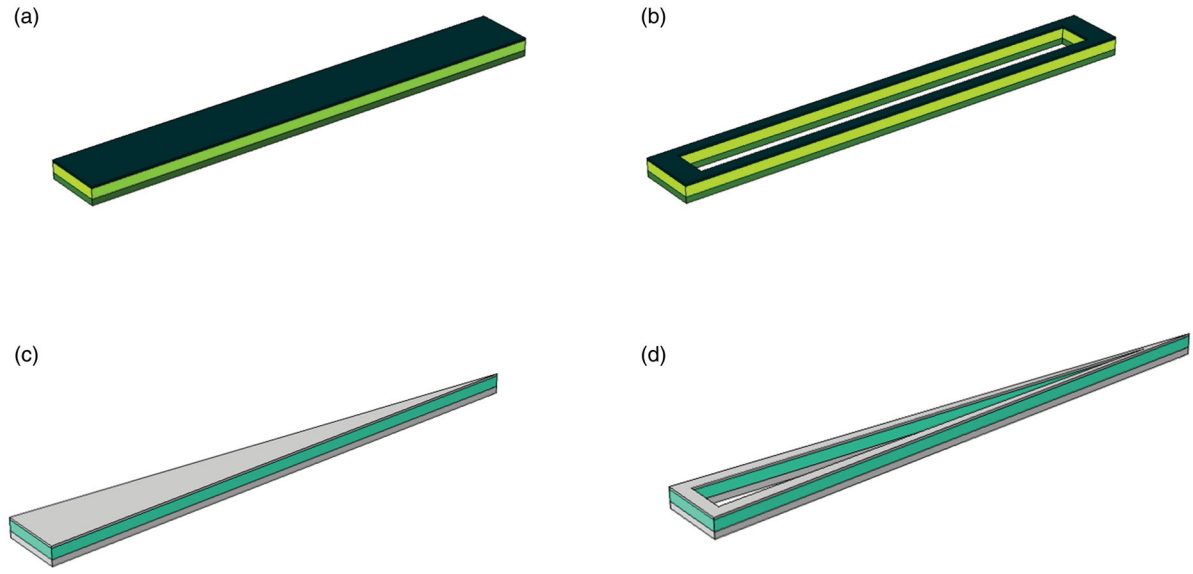

Figure 5. Different geometries of the microcantilevers (a) Rectangular, (b) Rectangular (holed punched form), (c) Triangle, (d) Triangle (holed punched form).

Table 2. Floating potential attained by various microcantilever shapes with different materials having comparable dimensions.

Geometry material	Rectangular (v)	Rectangular (holed punched form) (v)	Triangle (v)	Triangle (holed punched form) (v)
PZT	$3.3*10^{-16}$	$6.78*10^{-16}$	$4.75*10^{-16}$	$1.95*10^{-15}$
$BaTiO_3$	$5.1*10^{-16}$	$9.05*10^{-16}$	$7.5*10^{-16}$	$2.75*10^{-15}$
PVDF	$3.55*10^{-15}$	$7.6*10^{-15}$	$6.3*10^{-15}$	$2.4*10^{-14}$

and its piezoelectric layer is made of PVDF, exposed the highest floating voltage. Although PVDF has a lower d_{31} and Young's Modulus, according to relation (3), these parameters are in the denominator of the relation which leads to a higher voltage in response to the specific surface stress. As it was mentioned before and concluded from the modelling, polymers have better functionalities for constructing biosensors.

To assess the influence of different parameters on the piezoelectric performance, the effect of the microcantilever length and the piezoelectric layer thickness on the output voltage have been investigated.

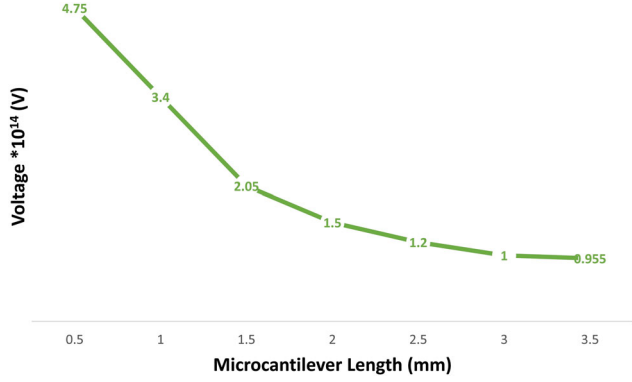

Figure 6. The impact of the biosensor length changes on the output voltage.

6.1. The effect of the microcantilever length on the output voltage

In this part, the length has been increased from 0.5 mm to 3.5 mm, linearly and the output voltage has been calculated. The results are depicted in Figure 6. As it is shown, by decreasing the microcantilever length, the floating voltage increases. This result is due to the fact that, by decreasing the length, the microcantilever senses greater force per area which leads to a higher output voltage.

6.2. The effect of the piezoelectric layer thickness on the output voltage

Another consideration is to evaluate whether the piezoelectric layer thickness affects the floating voltage or not. In this section, the thickness has been increased linearly from 0.02 mm to 0.08 mm. The results are depicted in Figure 7. As shown, by increasing the layer thickness, the output voltage fluctuates and doesn't follow a specific trend. It is concluded

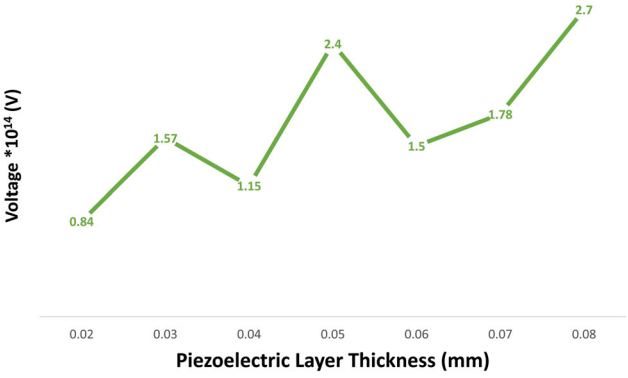

Figure 7. The impact of the piezoelectric layer thickness on the output voltage.

that the output voltage doesn't depend on the piezoelectric layer thickness, directly.

6.3. Covid-19 microcantilever functionality when Cross-Reactivity occurs

When a specific agent reactivity stimulates reactions outside the main supposed reaction, cross-reactivity happens. In immunology, this event can be observed between the immune system and antigens when an antibody conjugates with antigens of two different pathogens [47]. The designed biosensor in this study should possess a good functionality in response to cross-reactivity and must be selective enough to differentiate antigens binding to the same antibody. Co-occurrence of COVID-19 pandemics and influenza viruses (flu) which become widespread in fall and winter, might affect a lot of people and must be considered seriously. Influenza viruses are divided into four types of A, B, C, and D. Influenza A and B are the flu seasons causing seasonal epidemic disease. Among these types, only influenza A leads to a flu pandemic. H_1N_1 is one of the main subtypes of influenza A spreading between people, routinely [48]. Although COVID-19 and H_1N_1 are originated from different viruses, they cause respiratory disease with a wide range of analogous symptoms from mild to severe disease and death. As they have similar presentations, discriminating between them is difficult which makes testing inevitable. Since COVID-19 has a high speed of transmission, the patients must be distinguished and isolated rapidly in order to control the rate of spreading. In the following, by assessing the H_1N_1 experimental data, it will be approved that the designed biosensor has the capability to diagnose appropriately if both of the viruses' antigens tend to pair the same antibody immobilised to the top surface of the microcantilever. Lee and co-workers introduced 32D6 as a human H_1N_1 neutralising antibody through an Epstein-Barr virus – immobilised B cell-based technology. According to their experimental data, 0.036 µg/ml of 32D6 was needed to neutralise H_1N_1. Table 3 also displayed the binding kinetics of 32D6 towards HA of H_1N_1. As it is shown, the dissociation constant (K_D) was reported $3.528^* 10^{-10}$ M. K_D is the ratio of K_{off}/K_{on}. K_{on} means how quickly the antibody binds to the antigen and K_{off} is used to determine how quickly an antibody dissociates from its target. A low K_D value discloses a high speed reaction, while a high K_D value relates to a low speed interaction. In this research, K_{on} and K_{off} were measured as 2.585^*10^5 $M^{-1}S^{-1}$ and 9.121^*10^{-5} S^{-1}, respectively. The low K_D value represents a high affinity interaction in this research [49].

Table 3. The binding kinetics of 32D6 towards HA of H1N1 that was measured using the Biacore systems [41].

Binding kinetics	Value
K_{on}	2.585^*10^5 $M^{-1}S^{-1}$
K_{off}	9.121^*10^{-5} S^{-1}
K_D	$3.528^* 10^{-10}$ M

6.3.1. Implementing Experimental data for the biosensor validation

The above-mentioned experimental data should be implemented to the current modelling to assess its functionality when cross-reactivity occurs. Due to their results, it is calculated that about 1.45e11 antigens were neutralised up to 1500s. This number of H_1N_1 viruses will apply a force nearly to 9.39e-19 N on the top surface of the designed biosensor when binding to immobilised antibodies. By using the presented simulation, the optimum microcantilever which is in a shape of a holed punched form triangle and its piezoelectric layer is made of PVDF exposed the floating voltage equal to 2.4e–16 V. COVID-19 experimental data is also obtained from Seo *et al.* study. They fabricated a sensor by coating graphene sheets of the field-effect transistor with the related antibody to detect the COVID-19 spike protein. It was declared that about 1.6e4 pfu/ml of the viruses were recognised in about 250 s [17]. This number of antigens will create a load equal to 1.54e–15 N to the microcantilever top surface leading to the output voltage of 3.85e–13 V. The result indicates the COVID-19 floating voltage is about 3 orders higher than H_1N_1 voltage. As it was mentioned in previous sections, the designed microcantilever works based on the mass changes during the adsorption of analytes on its functionalised surface. Furthermore, COVID-19's molecular weight is a key feature to distinguish it from others. As a matter of fact, COVID-19 is one of the heaviest ones among

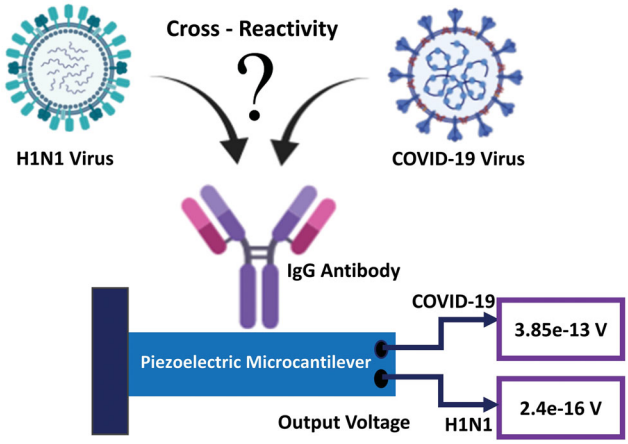

Figure 8. COVID-19 piezoelectric microcantilever functionality in distinguishing COVID-19 from other viruses when cross-reactivity occurs.

the viruses with the same symptoms. Therefore, the biosensor significantly displays a higher voltage when the swab sample contains the COVID-19 in comparison with other viruses like H1N1 (Figure 8). As a result, a borderline can be identified readily to decrease negative responses and to make the biosensor work precisely. Voltages upper than the borderline value attributes to COVID-19 while lower voltages ascribe to other viruses or agents settling on the coating surface.

In comparison with a number of COVID-19 antigen rapid test devices (Ag-RTDs) that are in development or have been recently commercialised, the suggested biosensor would surpass the available Ag-RTDs in terms of sensitivity and specificity. In fact, regarding the cross-reactivity issue, this piezoelectric microcantilever biosensor that works based on the mass changes would show fewer false results compared to Ag-RTDs simply using immunochromatographic (lateral flow) test format [50].

7. Conclusion

As COVID-19 has become a fatal and progressive pandemic, developing a fast and highly responsive method is inevitable for controlling the outbreak. In this study, a piezoelectric microcantilever biosensor which works based on SARS-CoV-2 antigen-antibody interactions was designed. The biosensor has the capability to detect COVID-19 in clinical samples without any pre-treatment and labelling. Moreover, the sensor has an appropriate functionality when cross-reactivity occurs. Therefore, the proposed biosensor suggests a low cost, simple, selective, and highly sensitive detection of COVID-19. In addition, this mechanism of biosensing could be applied to other contagious viruses.

Disclosure statement

The authors declare they have no conflicts of interest relevant to the content of this article.

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References

[1] Olwenyi OA, Dyavar SR, Acharya A, et al. Immuno-epidemiology and pathophysiology of coronavirus disease 2019 (COVID-19). J Mol Med. 2020;98(10):1315–1369.
[2] Bulut C, Kato Y. Epidemiology of COVID-19. Turk J Med Sci. 2020;50(SI-1):563–570.
[3] Nguyen TT, Pathirana PN, Nguyen T, et al. Genomic mutations and changes in protein secondary structure and solvent accessibility of SARS-CoV-2 (COVID-19 virus). Sci Rep. 2021;11(1):1–16.
[4] Kotlyar A, Grechukhina O, Chen A, et al. Vertical transmission of COVID-19: a systematic review and meta-analysis. Am J Obstet Gynecol. 2020;224(1):35–53.e3.
[5] Lauer SA, Grantz KH, Bi Q, et al. The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. Ann Int Med. 2020;172(9):577–582.
[6] Forni G, Mantovani A. COVID-19 vaccines: where we stand and challenges ahead. Cell Death Differ. 2021;28(2):626–639.
[7] Dos Santos WG. Impact of virus genetic variability and host immunity for the success of COVID-19 vaccines. Biomed Pharmacother. 2021;136:111272.
[8] Wang P, Nair MS, Liu L, et al. Antibody resistance of SARS-CoV-2 variants B. 1.351 and B. 1.1. Nature. 2021;7:10.
[9] Shen X, Tang H, McDanal C, et al. SARS-CoV-2 variant B. 1.1. 7 is susceptible to neutralizing antibodies elicited by ancestral Spike vaccines. Cell Host Microbe. 2021;29(4):529–539.e3.
[10] Heinz FX, Stiasny K. Profiles of current COVID-19 vaccines. Wiener Klinische Wochenschrift. 2021;133(7):271–283.
[11] Dai L, Gao GF. Viral targets for vaccines against COVID-19. Nat Rev Immunol. 2020;21(2):73–82.
[12] Badua CLD, Baldo KAT, Medina PMB. Genomic and proteomic mutation landscapes of SARS-CoV-2. J Med Virol. 2021;93(3):1702–1721.
[13] Cui F, Zhou HS. Diagnostic methods and potential portable biosensors for coronavirus disease 2019. Biosens Bioelectron. 2020;165:112349.
[14] Li C, Ren L. Recent progress on the diagnosis of 2019 Novel Coronavirus. Transbound Emerg Dis. 2020;67(4):1485–1491.
[15] Imai K, Tabata S, Ikeda M, et al. Clinical evaluation of an immunochromatographic IgM/IgG antibody assay

and chest computed tomography for the diagnosis of COVID-19. J Clin Virol. 2020;128:104393.
[16] Pan Y, Li X, Yang G, et al. Serological immunochromatographic approach in diagnosis with SARS-CoV-2 infected COVID-19 patients. J Infect. 2020;81(1): e28–e32.
[17] Seo G, Lee G, Kim MJ, et al. Rapid detection of COVID-19 causative virus (SARS-CoV-2) in human nasopharyngeal swab specimens using field-effect transistor-based biosensor. ACS Nano. 2020;14(4): 5135–5142.
[18] Kim HH, Jeon HJ, Cho HK, et al. Highly sensitive microcantilever biosensors with enhanced sensitivity for detection of human papilloma virus infection. Sens Actuators B. 2015;221:1372–1383.
[19] Gopinath PG, Anitha VR, Mastani SA. Design and simulation of high sensitive paddle microcantilever sensor for biosensing. Proceedings of the2017 International Conference on Energy, Communication, Data Analytics and Soft Computing (ICECDS) (pp. 2907–2910). IEEE; 2017.
[20] Wee KW, Kang GY, Park J, et al. Novel electrical detection of label-free disease marker proteins using piezoresistive self-sensing micro-cantilevers. Biosens Bioelectron. 2005;20(10):1932–1938.
[21] Rasmussen PA, Thaysen J, Hansen O, et al. Optimised cantilever biosensor with piezoresistive read-out. Ultramicroscopy. 2003;97(1–4):371–376.
[22] Sepaniak M, Datskos P, Lavrik N, et al. 2002. Peer reviewed: microcantilever transducers: a new approach in sensor technology. Anal Chem. 74(21): 568A–575A.
[23] Senesac LR, Dutta P, Datskos PG, et al. Analyte species and concentration identification using differentially functionalized microcantilever arrays and artificial neural networks. Anal Chim Acta. 2006; 558(1–2):94–101.
[24] Saurabh NK, Badri D, Rudraswamy SB. Design of microcantilever sensor for detection of P24 antigen in HIV diagnoses. Int J Sci Eng Sci. 1(12):44–47.
[25] Velanki S, Ji HF. Detection of feline coronavirus using microcantilever sensors. Meas Sci Technol. 2006; 17(11):2964–2968.
[26] Katsouras I, Asadi K, Li M, et al. The negative piezoelectric effect of the ferroelectric polymer poly (vinylidene fluoride). Nature Mater. 2016;15(1):78–84.
[27] Mousavizadeh L, Ghasemi S. Genotype and phenotype of COVID-19: their roles in pathogenesis. J Microbiol Immunol Infect. 2021;54(2):159–163.
[28] Azkur AK, Akdis M, Azkur D, et al. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. Allergy. 2020;75(7): 1564–1581.
[29] Zhang P, Gao Q, Wang T, et al. Evaluation of recombinant nucleocapsid and spike proteins for serological diagnosis of novel coronavirus disease 2019 (COVID-19). MedRxiv. 2020:2020.03.17.20036954.
[30] Giron CC, Laaksonen A, da Silva FLB. On the interactions of the receptor-binding domain of SARS-CoV-1 and SARS-CoV-2 spike proteins with monoclonal antibodies and the receptor ACE2. Virus Res. 2020;285: 198021.
[31] Yu F, Yan L, Wang N, et al. Quantitative detection and viral load analysis of SARS-CoV-2 in infected patients. Clin Infect Dis. 2020;71(15):793–798.
[32] Liu X, Wang J, Xu X, et al. Patterns of IgG and IgM antibody response in COVID-19 patients. Emerg Microbes Infect. 2020;9(1):1269–1274.
[33] Ma H, Zeng W, He H, et al. COVID-19 diagnosis and study of serum SARS-CoV-2 specific IgA, IgM and IgG by chemiluminescence immunoanalysis. medRxiv. 2020.
[34] Hou H, Wang T, Zhang B, et al. Detection of IgM and IgG antibodies in patients with coronavirus disease 2019. Clin Transl Immunol. 2020;9(5):e1136.
[35] Wu Y, Wang F, Shen C, et al. A noncompeting pair of human neutralizing antibodies block COVID-19 virus binding to its receptor ACE2. Science. 2020;368(6496): 1274–1278.
[36] Mair-Jenkins J, Saavedra-Campos M, Baillie JK, et al. The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: a systematic review and exploratory meta-analysis. J Infect Dis. 2015;211(1):80–90.
[37] Valk SJ, Piechotta V, Chai KL, et al. Convalescent plasma or hyperimmune immunoglobulin for people with COVID-19: a rapid review. Cochrane Database Syst Rev. 2020;(5):CD013600.
[38] Fan G, Wang Z, Hao M, et al. Bispecific antibodies and their applications. J Hematol Oncol. 2015;8(1): 130.
[39] Marston HD, Paules CI, Fauci AS. Monoclonal antibodies for emerging infectious diseases—borrowing from history. N Engl J Med. 2018;378(16):1469–1472.
[40] Walker LM, Burton DR. Passive immunotherapy of viral infections:' super-antibodies' enter the fray. Nat Rev Immunol. 2018;18(5):297–308.
[41] Jaffe B. 2012. Piezoelectric ceramics (Vol. 3). Elsevier Science. London: Academic Press.
[42] Ounaies Z, Young JA, Harrison JS. An overview of the piezoelectric phenomenon in amorphous polymers. Field responsive polymers. ACS Symposium Series. 726: American Chemical Society; 1999. p. 88–103.
[43] Kawai H. The piezoelectricity of poly (vinylidene fluoride). Jpn J Appl Phys. 1969;8(7):975–976.
[44] Calleja M, Tamayo J, Johansson A, et al. Polymeric cantilever arrays for biosensing applications. Sen Lett. 2003;1(1):20–24.
[45] Cheng HM, Ewe MT, Chiu GT, et al. Modeling and control of piezoelectric cantilever beam micro-mirror and micro-laser arrays to reduce image banding in electrophotographic processes. J Micromech Microeng. 2001;11(5):487–498.
[46] Zhou W, Khaliq A, Tang Y, et al. Simulation and design of piezoelectric microcantilever chemical sensors. Sens Actuators A. 2005;125(1):69–75.
[47] Jacofsky D, Jacofsky EM, Jacofsky M. Understanding antibody testing for COVID-19. J Arthroplasty. 2020; 35(7):S74–S81.
[48] Gaitonde DY, Moore FC, Morgan MK. Influenza: diagnosis and treatment. Am Fam Physician. 2019;100(12): 751–758.

[49] Lee CC, Yang CY, Lin LL, et al. An effective neutralizing antibody against influenza virus H1N1 from human B cells. Sci Rep. 2019;9(1):1–11.
[50] Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays. Interim guidance. Geneva: World Health Organization; 2020 [accessed 2020 Nov 18]. https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays.