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Trabajo Fin de Grado

Una solución no invasiva y en tiempo real para la detección de la exposición a virus respiratorios altamente contagiosos

A non-invasive solution for personalized real-time diagnosis of the exposure to highly contagious respiratory viruses

Autor
Sara Ramada Lahoz

Director
Raquel Moreno Loshuertos

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ABSTRACT

This document explains the creation of VAir, a non-invasive reusable wireless device to sense Influenza A in the air. The ambitious aim is to develop a reliable sensor that would be easy to operate and allow rapid detection of even asymptomatic conditions.

In nowaday medical diagnostics, virus recognition is performed in laboratories using conventional methods, namely polymerized chain reaction (PCR) amplification and enzyme-linked immunoassays. Being extremely sensitive and selective, these methods are common to employ multiple detection layers for several analytes, which is not quick enough to prevent the spread of the virus both within and between individuals.

VAir would detect the viral target via a specific interaction with the nanobodies. The quantification is performed automatically. When integrated in IoT-frameworks, it turns into a source of continuous health data, providing valuable information on the exposure to contagious hazards and contact tracing. The growing prevalence of infectious diseases is increasing the demand for such point-of-care (PoC) diagnostics. The crucial need in remote monitoring and connected healthcare solutions is expected to push the demand for portable devices, boosting the sales of biosensors across both PoC and home diagnostic applications.

2. INFLUENZA ANALYSIS

2.1 INTRODUCTION

Influenza viruses: A (IAV), B (IBV), C (ICV) and D (IDV) form the four genera of the Orthomyxoviridae family. Despite the overall structure is the same, only the three first mentioned affect human. The virion has 80 to 120 nm of diameter and its RNA nucleus is surrounded by an envelope formed of glycoproteins.

The Orthomyxoviridae family is formed by virus with a negative-sense RNA viruses that contain from six to eight segments of linear nucleic acids. In this case the genome is segmented into 7 to 8 segments each of them containing one or two genes including the hemagglutinin (HA), neuraminidase (NA) and neucleoprotein (NP) among others, in total 11 proteins are encoded.

Hemagglutinin and neuraminidase are large glycoproteins placed in the external part of the virions, they act as important antigens and as targets for antiviral drugs. Hemagglutinin is a lectin responsible of the cell invasion playing a crucial role in the entry into the host cell while the neuraminidase is an enzyme that beaks up sugars easing the release of the mature virions from the cells. Hemagglutinin binds the silica acid of the host cell membrane to allow the penetration of the virus in the host cell, it will be internalized in endosomes. Its acidic environment causes the binding go the virions envelope to the cell's vacuole membrane and the M2 ion channel opens and acidify the vision's nucleus degrading it. (1)

The viral RNA and proteins (like RNA polymerase) are released into the cytoplasm. They migrate to the nucleus were RNA polymerase transcribe complementary positive-stranded RNA; vRNAs either migrate to the cytoplasm and are translated or remain in the nucleus. Newly synthesized viral proteins (neuraminidase and hemagglutinin) are secreted and formed on the cell surface through the Golgi apparatus or sent to the nucleus where they combine with vRNA to create new viral genes.

In this way, the negative-stranded vRNA, RNA-dependent RNA polymerase, and viral protein constituting the new virion are generated. vRNA and viral proteins move to a compartment formed between hemagglutinin and neuramidase formed on the cell surface. The new virions are released as spheres with the glycoproteins of the formed envelope and the phospholipid layer of the cell. Virions have the property of attaching to host cells with hemagglutinin, and neuraminidase breaks this bond to help release. When the virus is released, the host cell bursts and dies spreading the infection. (2)

2.2 HOW RESPIRATORY VIRAL DISEASES SPREAD

Belonging to diverse families, respiratory viruses differ in viral and genomic structures, populations susceptible to infection, disease severity, seasonality of circulation, transmissibility and the mechanism of transmission. Transmissibility can be defined as the easy of the virus to be transmitted, it is estimated using two parameters: basic reproduction number (R_0) and secondary attack rate.

There are four mechanism or modes of transmission: direct and indirect contact (meaning physical or fomite touch respectively), droplets and aerosols. It's not fully understood how the proportional contributions of each mode to a certain virus's transmission in various contexts and how its variation impacts transmissibility and transmission dynamics.The particle size threshold between droplets and aerosols and the significance of aerosol transmission for the influenza virus and the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are topics of continuous discussion. It is still difficult to clinically differentiate respiratory viral infections. Therefore, evaluating the likelihood of transmission and the evidence for various routes of transmission can help prevent the spread of respiratory viruses. (3)

As it has been mentioned before, transmissibility refers to how easily the disease will spread from an infected person to a susceptible person.

In the previous section the infectious cycle was explained. However, how easily the illness will travel from an infected individual to a vulnerable person, or how transmissible the disease is, is one of the most crucial early considerations in the containment of a viral pandemic. Infectivity of the pathogen, contagiousness, susceptibility and contact patterns between the infected person and the exposed person, and environmental stress placed on the pathogen during transmission all play a role in determining transmissibility. These will establish the scope and degree of control measures required to stop the spread of the disease.

When a respiratory virus escapes from an infected person's respiratory system and travels through the environment to infect the respiratory tract of a susceptible and exposed person, this is known as respiratory viral transmission.

Some respiratory viruses, such us influenza viruses, coronaviruses and rhinoviruses, had been recovered from infect cells in the gastrointestinal tract, or faeces suggesting they may contribute in infection (by direct or indirect contact). Furthermore, influenza virus infection may also be possible via ocular exposure.

Air-born is a term used to describe transmission via droplets and aerosols as both can travel through air. The main difference is their size, 'aerosols' are referred to as a stable suspension of solid and/or liquid particles in air or ~30–100 μm, whereas 'droplets' as liquid particles that are maximum 100 μm (largest particle size that can

remain suspended in still air for more than 5 s from a height of 1.5). Long-distance disease transmission is amplified by the ability of bioaerosols to remain airborne for an extended length of time and move through the air, requiring additional precautions.

Volunteer challenge studies showed that influenza virus and adenovirus infection initiated by the inhalation of infectious bioaerosols required a lower infectious dose. Infection initiated by inhaling infectious influenza virus bioaerosols led to higher risk of fever compared with intranasal inoculation, although some studies alternatively suggested that infection initiated by droplets leads to severer disease due to a higher infectious dose. To sum up, viral, environmental and host are the determinants of respiratory virus transmission.

2.3 HOW IS INFLUENZA VIRUS FOUND IN THE AIR?

The majority of medical professionals concur that the major mechanism by which influenza viruses are transmitted is through the droplets or aerosols produced when an infected individual coughs or sneezes. Before hanging suspended in the air, aerosols normally reach around one meter distance. There, they can remain as aerosols for several hours. Droplets from a cough or sneeze might land the neighboring people's faces or noses, or someone could breathe them in. The fact that matter is that flu infected patients can contaminate fomite and people not only by coughing or sneezing but by breathing out; everyday items at home and in public places can easily become contaminated: from food and door handles, to remote controls or telephone handsets

This means virus particles can be constantly circulating in the airborne in different forms (inside dust particles or aerosol droplets). Usually the amount of virus particles are not enough to infect a healthy individual and cause disease. However, the danger of human infection rapidly rises at increasing levels of the virus presence in airborne making new quick diagnostic techniques necessary.

Finally, it has been proved that virus survival times in the air are improved by lower temperatures explaining why the flu is more common during the winter. Also it is linked to air’s ‘absolute humidity’ (percentage of water vapor in the air). Since the absolute humidity of cold air is lower, it is drier. When we cough or sneeze, the droplets that we exhale remain relatively big in wet air, but they fragment into tiny bits in dry air. Unlike the tiny bits, which may float for hours, the bigger droplets fall out of the air and onto surfaces more rapidly. (3)

3. MARKET ANALISYS

According to the data provided from the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO), respiratory diseases outbreaks have become more constant over the past few years. The burden of the global respiratory virus-related disease outbreaks is summarized in Table 1.

Despite the expanding recommendation of influenza vaccination, seasonal influenza remains responsible for a large disease and economic burden. The estimated average annual total economic burden of influenza to the U.S. healthcare system and society was $11.2 billion (4), consisting of direct medical costs of $3.2 billion and indirect costs of $8.0 billion. WHO estimates (5) that seasonal influenza may result in up to 650.000 deaths each year due to respiratory diseases alone.

Global Respiratory Disease Outbreak	**Year**	**Virus**	**Number of deaths**	**Number of cases**	**Financial impact**
Severe Acute Respiratory Syndrome, SARS	2003	SARS-CoV	774 (5)	8,098 (6)	$40.000 million (7)
H5N1 Avian Influenza	2004	Influenza A subtype H5N1	374 (8)	628 (8)	$20 billion (8)
H1N1 Swine Influenza	2009	Influenza A subtype H1N1	575,4(9)	60.8 million (10)	$50.000 million (11)
MERS	2012	MERS-CoV	888 (12)	2578 (12)	$860 million (12)
H7N9 Influenza	2013	Influenza A virus subtype H7N9	610 (13)	1565 (13)	$6.5 billion (14)
Coronavirus disease 2019, COVID-19	2019	SARS-CoV-2	5.1 million (15)	250.2 million (16)	> $100 trillion (16)

Table 1. The social and economic burden of the recent global respiratory virus-related diseases outbreaks

The staggering morbidity and mortality related to respiratory viral diseases highlights the importance of accurate and fast diagnosis of the causative pathogens to select the appropriate treatment, prevent the epidemics, and reduce the disease burden. In nowadays medical diagnostics, virus recognition is performed in laboratories using conventional methods, namely polymerized chain reaction (PCR) amplification and enzyme-linked immunoassays, both requiring biological labels, such as radioisotopes, enzymes, and fluorophores, which then can be readily detected using various analytical techniques. These approaches, which are exceedingly sensitive and selective, frequently use many detection layers for a variety of analytes, but they are not rapid enough to stop the transmission of the virus both within and between people. The need to engage highly trained staff, long analytical times, and the huge investment in effort and resources hamper diagnosis and make it impractical in the primary health-care setting.

For instance, to eradicate influenza viruses, infected patients have to take antiviral medicine (e.g. Tamiflu) within two days after the onset of symptoms (17). A recent study used an antibody-based chromatographic approach to qualify the Influenza A virus within 30 minutes, indicating this to be an efficient tool for PCR-free detection with the advantage of the experimental procedure being simple and fast (18). However, this test had a limited sensitivity, and samples from patients with low concentrations of the virus yielded false-negative results. Microfluidic system-based sandwich-type diagnostic assays for the Influenza A virus have been performed using mouse monoclonal antibodies, against the virus nucleoprotein hemagglutinin (HA), with a detection limit of 0.032 HA units (19). This method also suffers from drawbacks, including its relatively high cost and its sensitivity to temperature and humidity, which results in batch-to-batch variations.

Despite the fact that a considerable effort has been put in the development of biosensors able to detect respiratory viruses at an early stage with high sensitivity and low cost, none of these are commercially available on the market, and cannot be used for pandemic diseases such as COVID-19 (20).

Biosensors have found immense applications in medical diagnostics, and it offers more specific, sensitive, fast, and reproducible results as compared to the conventional techniques like biochemical assays and immunoassays.

In 2021, the biosensors market is valued at $25.5 billion and is projected to reach $36.7 billion by 2026; in other words, it is expected to grow at a CAGR[1] of 7.5% (21). With such market growth and continued innovation in the devices, an exponential increase in the amount of health-related data is to become available. Nowadays,

[1] CAGR: Compound Annual Growth Rate

portable biosensors, capable of measuring physiological parameters such as electrocardiogram, electromyogram, heart rate, body temperature, electrodermal activity, blood pressure, and breathing rate (22–23) are integrated into textile fiber, clothes, elastic bands or are directly attached to the human body (24).

The examples of biosensing devices for respiratory virus detection, produced by leading manufacturers in the industry: Abbott Point of Care Inc. (USA), Sekisui Diagnostics, LLC (USA), and Quidel Corporation (USA), are provided in Table 2.

Biosensor Name	**Producer**	**Price, EUR**	**Biomarker Measured**	**Advantages**	**Disadvantages**
PANBIO™ COVID-19 ANTIGEN SELF-TEST (25)	Abbott Point of Care	7,44	Qualitative detection of nucleocapsid protein **SARS-CoV-2** antigen	1. Rapid: results ready in 15 minutes 2. No equipment or trained staff needed to perform the analysis 3. High accuracy: 100% of negative samples and 95.8% of positive samples 4. Easy-to-read 5. Qualitative detection	1. Single-use 2. Cannot be introduced into digital monitoring system 3. Requires a nasal swab 4. Not real-time virus detection

OSOM (26)	Sekisui Diagnostics, LLC	73,34	Qualitative detection of mononucleosis heterophilic antibodies against **Epstein-Barr** virus	1. Rapid: results in 5 minutes 2. High sensitivity: > 99% 3. High specificity: 96% 4. Additional test sticks for external quality control 5. Easy-to-read 6. Qualitative detection	1. Requires serum, plasma or whole blood sample 2. Not sensitive to low antibody levels 3. Single-use 4. Not real-time virus detection
QuickVue Influenza A+B Test (27)	Quidel Corporation	601,24	**Influenza A** and **Influenza B** antigens	1. Easy-to-use 2. Rapid: results in 10 minutes 3. Clear and simple illustrations to guide the flu test procedure included 4. No need for additional equipment 5. Room temperature storage	1. High-cost 2. Single-use 3. Cannot be introduced into digital monitoring system 4. Requires a nasal swab 5. Not real-time virus detection

Table 2. Commercially available biosensors for respiratory virus detection and their advantages and disadvantages

3.1 PATENTS

At this point there are no commercially available self-use systems which can perform real-time detection of Influenza and be fused to a digital environment. However there are some alternatives in the market with similar aims. Its observation may be useful in the search of specific features or common problems.

a. NOZE. Patent number: CN111650173A (28)

An air quality device for virus detection Created by Li Yehong; Jiang Xiangyang; Yang Jiankun; Shan Hong; Yang Yakun. Permits the real-time monitoring of air quality factors that affect well-being, including virus survival index. (29)

b. uHoo. Patent number: WO2021242170A1 (30)

Other air quality device for viruses created by Dustin Onghanseng and Brian Lin. This device is associated to a mobile app that informs you about the air quality and the degree of spread (from 1 to 10) for the virus. It works measuring environmental data using sensors, determining the virus index scale, indicating environmental categories corresponding to different viral indices, and determining the risk of the space. (31)

c. Sensor for Influenza Detection. Patent number: KR20150040891A (32)

Portable device with high sensor resolution for the detection of influenza in liquid or gas. It has a determination unit that measures a virus index for the occupied space based on different environmental data (environmental conditions that allow virus survival, the ease it may be transported by air, the ease the virus may be transmitted because of its resistance on surfaces etc.) by its comparison to a Virus Index Scale they are able to determine the risk of infection. Its high resolution and sensitivity is thanks to the recognition and bounding to the sialyc group of the sialyc oligosaccharide of the virus (32)

3.2 TARGETS

In terms of targeting people, groups at higher risk of infection should be assessed first. So according to the Centers for Disease Control and Prevention (CDC) in terms of age, adults older than 65 and children younger than 2 years old are vulnerable to flu and have higher mortality percentages than other possible age groups. (although children younger than 5 are also considered at risk). (33)

Moreover, we have to mention people with diseases or organ complications like: asthma, neurologic and neurodevelopment conditions, blood disorders, chronic lung diseases, endocrine disorders, heart diseases, kidney liver disorder, metabolic disorders.

In addition, obese people with a body mass index higher than forty are at risk, then people with a weakened immune system due to disease or medication, and pregnant woman.

According to these, the main focus will be to introduce the device in high infection places like hospitals, clinical treatment centers or health centers destined to pregnant woman and newborn babies. Lastly, individual use of the device is essential for all people with weakened immune system, complications or even just for self-protection. (33) The device would be primarily targeted at patients older than 65 years, kids and those with health conditions such as asthma, suppressed immunity or pregnancy, among other diseases. (34)

In terms of which geographical areas the product would be destined to, according to World Health Organization (WHO), countries with highest influenza incidence are Central America, North Africa, South Africa and Oceania and Eastern Mediterranean Region. However, due to economic, social and consciousness of safety reasons. Target would be Europe and Asia, especially China, Korea and Japan due to the fact they have more conscience and awareness of virus spread and safety measures. And also Oceania and some eastern Mediterranean countries like Turkey as they have higher influenza incidence and resources are expected to become profitable markets fo the introduction of such a product.

Regardless of the initial targeting plan, the aim is to spread worldwide thanks to its accessibility, low budget device, and to make it reachable for a broader target spectrum.

4. DETECTION METHODS

In medical diagnostics, virus recognition can be performed in laboratories using traditional methods, such as polymerized chain reaction (PCR) amplification and enzyme-linked immunoassays. As mentioned before, although these methods are extremely sensitive and selective, they have important withdraws when coming to fast detection.

Considering the different diagnostic applications, there is an urgent need for effective virus sensors that are small and easy to operate and offer rapid response times as well as high selectivity, cross-sensitivity, and portability.

4.1 BIOSENSOR DEFINITION

According to IUPAC nomenclature, "*biosensors are integrated receptor–transducer devices, which are able to provide selective quantitative or semiquantitative analytical information using a biological recognition element*". (35)

They can be described as compact analytical devices, incorporating biological or biomimetic sensing elements (36). This tools are characterized by being sensitive, accurate, reproducible, high specific toward the target element, non-toxic and cost-effective. They can be divided in several groups depending on the biorreceptor (enzyme, antibodies, DNA, complete cells) or the physicochemical transduction element (optical, electrochemical and mass-dependent). (37)

4.2 ROLE AGAINST PANDEMICS

Rapid diagnostic methods based on biosensors may reduce the spread of illnesses caused by respiratory viral infections. The infected individual may be isolated for a brief amount of time while the virus is treated since quick and early diagnosis of viral infection informs the healthcare professional. By interrupting the cycle of community transfer, this might effectively limit the spread of illness. Better readiness is required for upcoming pandemics, despite the fact that some researchers have learnt important lessons from previous SARS-CoV and MERS epidemics about the possible development of therapeutic interventions against these (and other) viruses.

Wearable biosensors should be integrated with energy harvesters as self-powering sources. Such multipurpose self-powered sensing devices might possibly control health by automatically gathering and sending data, helping considerably to the creation of a society in which people would be able to live without fear of infection by numerous diseases. (38)

However, the present global issues include high transmission rates, continuous altered pathogenic virus strains, a lack of centralized lab facilities, poverty, and misinformation in developing nations. Because of this, there is a critical need for diagnostic tools that are easy to use in scattered laboratories, rapid, user-friendly, inexpensive, and have great sensitivity and specificity.

As is shown in figure 1, some recommendations towards biosensors and their job in pandemics outbreaks include their intonations with the internet of Things (IoT), ease the compatibility with regulation bodies such as Food and Drug Administration, create collaborative networks and a global investment in pandemic preparedness what would include the invest on biosensors and large-scale population screening. (39)

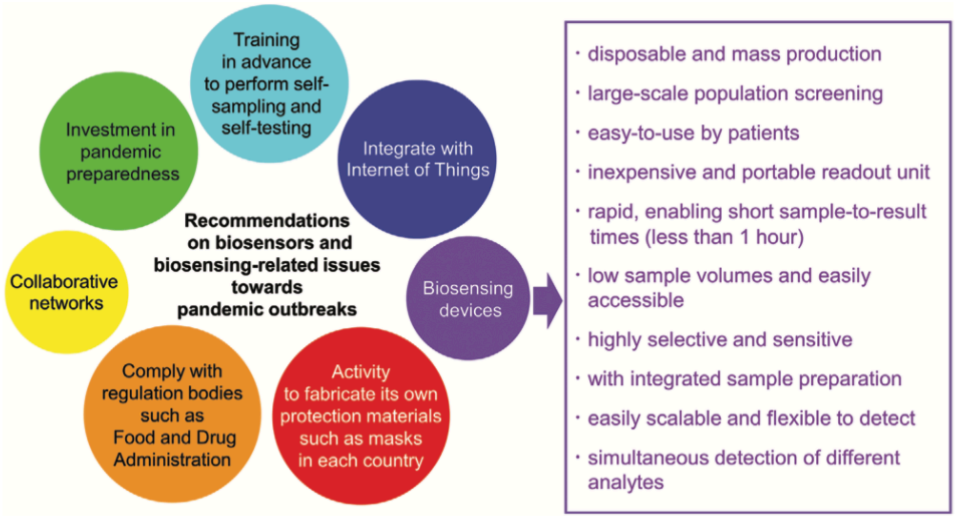

Figure 1. Main recommendations and requirements for the bio sensing community dejecting infectious diseases (39)

4.3 TYPES OF BIOSENSORS

4.3.1 PIEZOELECTRIC BIOSENSORS

Piezoelectric materials have become important biomaterials that can be interfaced with biological tissues and used in miniaturized bioelectronic and biochemical devices (40). That is why piezoelectric sensors are already being used for the detection of human diseases such as papilloma, ebola, influenza A, human immunodeficiency, and hepatitis B.

The basis of this method is the measurement of changes in the resonance frequency. Under an alternating current (AC) voltage, a piezoelectric material shows a mechanical oscillation that results in an oscillating electric field. Because of the interactions between molecules, as a mass m rises, the frequency f governed by the AC voltage falls. Figure 2 provides a schematic representation of the piezoelectric biosensor's workings.

The upper electrode surface of the piezoelectric material is coated with probe antibodies, as illustrated in Figure 2a. The upper and lower electrodes drive the resonation of the piezoelectric material, while target antigen binds with the probe antibodies.

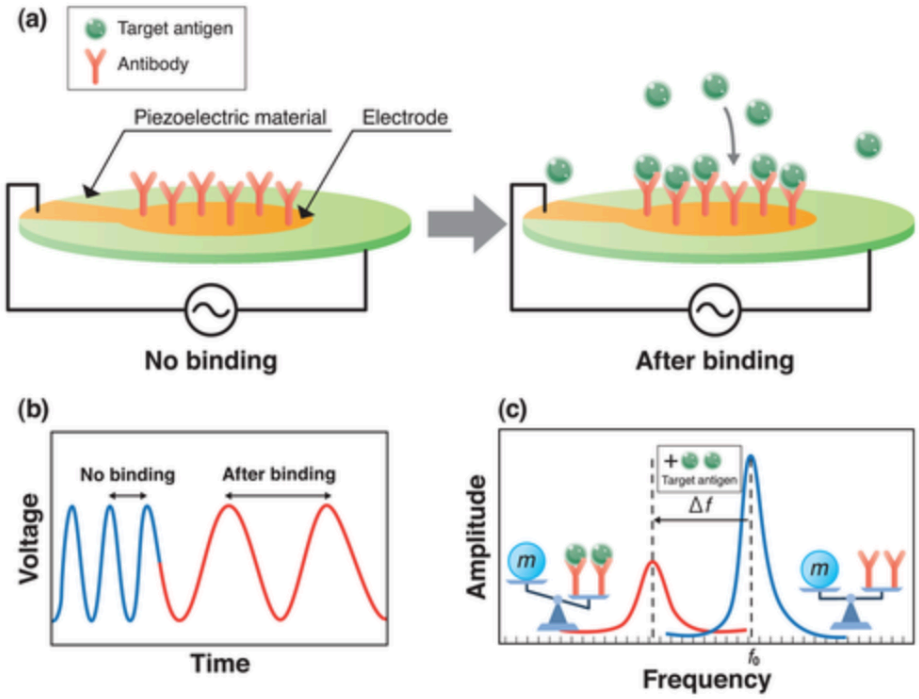

Figure 2. Schematic representation of the piezoelectric biosensor's principle: (a) basis of a piezoelectric biosensor's operation (b) interaction between voltage and the sensor's mass (c) When detecting viruses, there is a link between amplitude and frequency.

The frequency shift caused by the mass change Δm on the electrode surface is represented by the frequency change Δf (Figure 2c) of the material in the oscillation circuit. (41) The mainly anisotropic materials (having a physical property which has a different value when measured in different directions) proposed for this type of sensors are aluminum nitride (AlN), zinc oxide (ZnO), barium titanate (BaTiO3), lead titanate (PbTiO3), quartz (SiO2), and poly(vinylidene fluoride) (PVDF).

Table 3 lists the Young's modulus E, shear modulus μ, Poisson's ratio v, mass density ρ, longitudinal piezoelectric coefficient d33, and transverse piezoelectric coefficient d31 of each of these materials. (42)

	E [GPa]	μ [GPa]	v	ρ[gcm-3]	d33[pCN-1]	d31[pCN-1]
AlN	308.3	130.8	179	3.26	6.72	-2.71
ZnO	112.2	42.2	336	5.53	12.3	-5.12
BaTiO3	112	43	0.35	5.4	140	-60
PbTiO3	213.7	84.3	0.26	7.52	79.1	-23.1

	E [GPa]	μ [GPa]	v	ρ[gcm-3]	d33[pCN-1]	d31[pCN-1]
SiO2	72.52	30.97	166	2.204	2.3	-0.67
PVDF	2	752	0.33	1.8	-22	23

Table 3. Engineering constants of piezoelectric materials (42)

The mass sensitivity, which defines the change in resonance frequency f_0 due to viral particle adsorption, and the mechanical quality factor (Q value), which characterizes the sharpness of the resonance peak, are used to describe the sensor's performance.

This Q value is approximated by equation 1:

$$Q = \frac{f_0}{\Delta s}$$

Equation 1. Mechanical quality factor (Q value) is equal to the change in resonance frequency f0 divided by *Δs*. Δs is defined as the signal trace's spread at a distance from the baseline equal to the magnitude divided by 2

A sharper resonance peak is indicative of a higher Q value, which also translates into greater accuracy in detecting resonance frequency and a smaller minimum observable frequency shift. The size and form of the sensor electrode may be adjusted to maximize the Q value. (43)

4.3.2 MAGNETOSTRICTIVE BIOSENSORS (MSMC)[2]

Magnetostrictive components, which are used to create sensors and actuators, can transform magnetic energy into mechanical energy or the opposite. The Tohoku University research group has reported studies on magnetostrictive alloys and composites during the past ten year (44). They works nicely in both liquid and air and are treble to detect proteins, viruses, and bacterial spores.

They are based on the resonance frequency change Δf. Their main difference with the piezoelectric transducers mentioned before is that they utilize magnetostrictive properties if the materials to convert magnetic's field energy into mechanical energy (instead of converting the electrical energy directly into mechanical) (45)

[2] MSMC: magnetrostrictive biosensor

Magnetostrictive effect caused by application of an AC magnetic field (magnetic field generated because of the effect of a alternating current passing through a coil) using a driving coil induces an oscillation of the magnetostrictive microcantilever (MSMC, high-performance transducer).

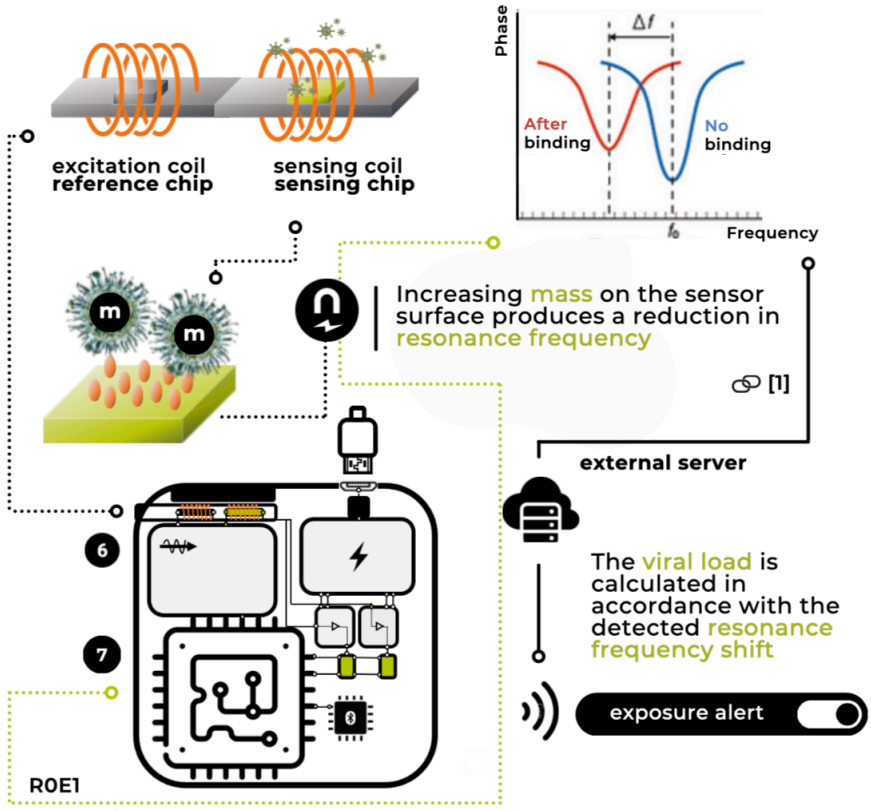

Figure 3. Mechanism of magnetostrictive biosensor. Biosensor shown in lower left corner with the two coils, excitation and sensing. The change of mass because of viral particle joining in sensing coil would case a reduction in resonance frequency (upper right corner) that will be interpreted as a viral load.

This oscillation results in an emission of a magnetic flux and changes in the amplitude and phase signal of the oscillation lead to a magnetic flux change that can be detected using a pick-up coil. The attachment of a mass load Δm such as an antigen to the sensor surface with probe antibodies lowers the resonance frequency.

Resonance frequency is linearly reduced when mass is added to the sensor surface. When the mass of a virus particle is well characterized and known, the change in frequency can be used to determine the number or type of the virus (39)

The main advantage of this type of sensor is that no direct physical connections to the sensor are needed, nor is any internal power source (i.e., battery) needed for sensor operation. Sensitivity ($\Delta f / \Delta m$) is proportional to the resonance frequency $f0$ and inversely proportional to the magnetostrictive biosensor mass.

The materials used for this type of sensor are metglas amorphous alloy, Fe-Co allow and cobalt ferrite ceramics.

Sang et al. (46) recently developed a compact, affordable, stable metglas 2826 MB*biosensor with a detection limit as low as 0.039 g/mL to quickly and precisely detect human serum albumin (HSA). For that they used specific antibodies, anti-HSA Immunoglobulin, immobilized on the surface of the magnetostrictive sensor to selectively capture HSA. As expected, this sensor surface state change resulted in a resonance frequency shift that could be used to monitor in real time qualitative and quantitative data of human serum albumin. Using various HSA solution concentrations, the performance of the portable device was confirmed to be equivalent to that of the Vector Network Analyzer (VNA) AV3620.

Anti-HSA Immunoglobulin G (IgG) was immobilized on the surface of the ME sensor to selectively capture HSA. The rapid conjugation between the antibody and antigen changed the sensor surface states and thus induced resonance frequency shifts (RFS), which were monitored in real time for the qualitative and quantitative analysis of HSA. The performance of the portable device was validated to be comparable to that of the Vector Network Analyzer (VNA) AV3620 using different concentrations of HSA solution.

This biosensor, which served as the inspiration for the development of the VAir biosensor, has straightforward hardware, low power consumption, good real-time detection, low detection limits, and a small system structure. It is suited for quick medical diagnosis.

Although optical sensors have the benefit of providing a quick qualitative evaluation of the analyte, electrochemical biosensors offer more precise quantitative data. Moreover, the combination of mass-based biosensors with microfluidic technology looks promising for the creation of a multiplexed (simultaneous detection of more than one biomarker) integrated platform for the evaluation of illness state and the potential to stop current and future pandemics. (47)

After showing both types of mass-based transducers biosensor, piezoelectric and magnetostrictive and further analysis on other type of transducers (annex II), the magnetostrictive alloy is the selected one to the development of VAir.

4.4 BIOLOGICAL PART: ANTI-HA SINGLE DOMAIN ANTIBODIES

DNA probes, antibodies, enzymes, and cell receptors that participate in analyte-related interactions can all be used as biological sensing components. (48)

The performance of an antibody is strongly influenced by its stability (i.e. its specificity and affinity). Due to the growing usage of antibodies in treatments, diagnostics, and quick analytical systems, stability is a significant problem. (49)

Monoclonal antibodies are the most widely used reagents for specific detection and quantification of proteins. However, classic antibodies may lack robustness or may not be appropriate to use in VAir system due to their supramolecular structure.

A different type of antibody is the heavy chain antibody (hcAb), where the variable domain only consists of the heavy chain (VHH). These hcAbs have been identified in llama, camelids and sharks, from which the VHH were cloned and expressed.

By only cloning the VHH, single-domain antibodies (sdAbs) were obtained, which were shown to be more stable than conventional antibodies and have the ability to reversibly refold to their native structure after being denatured by heating.

Similar to the case of VHH, sdAbs can also be obtained from the VH and VL of regular antibodies. Due to their smaller size, sdAbs have advantages with respect to production, solubility, and delivery to their targets and are shaping up to become promising experimental and therapeutic tools. (50)

For practical applications of sdAbs such as antibody drugs and biosensors, it is important to understand factors that affect their thermal stability.

To sum up, *VHH* antibody, or nanobody, is the heavy chain of camelid antibodies with a size of around 15 kDa. As nanobodies are only composed of a single domain, they are more stable and robust than conventional antibodies, allowing great heat-resistance up to 90 degrees and stability towards solvents and detergents as well as proteolysis compounds. (51)

Thus, bacterially-produced nanobodies have been chosen as recognition elements for *Influenza A* at room temperature.

First, the sequence of the heavy chain of camelid antibodies is isolated and inserted into a vector by gene cloning to allow bacterial production in *E. coli*. The engineered recombinant nanobodies can then be immobilised at high density on a gold chip to form stable and well-oriented nanobody monolayers.

Thus, a great strength of the suggested technology would be its adaptability. To quickly create new nanobodies, we would simply need to immunise camels with the corresponding viral proteins or search among the existing libraries by phage-display. This would make it possible to detect different viruses according to the user's needs.

The use of nanobodies have shown several advantages but in contrast, they get saturated. In order to solve this problem, it is expected to achieve the reusability by treating the immunochip with a high salt concentration as for most antibody-protein interaction a low pH (1-4) effectively undo the interaction. (52,53) Damage to bioreceptors (antibody or aptamer) in particular might impair their capacity to identify target biomarkers, leading to a reduction in sensitivity and selectivity (54). Robust biosensors are needed when using cleaning methods in order to prevent biosensor surface damage during the regeneration process, meaning further testing should be done in order to test the efficacy and security.

5. DESCRIPTION OF BIOSENSOR'S FEATURES

Taking into account the previous discussion the features of the device described would aim for the following description.

The air would enter the device through a dust filter, so that large particles do not reach the chips made of a magnetostrictive alloy. It would be able to reversely convert magnetic energy into mechanical one, and therefore is a promising energy-harvesting material for IoT-devices. An alternating current (AC) excitation signal acts on the air-cored solenoids, which drive chips to oscillate.

Increasing mass on the sensor surface due to the viral particle binding produces a reduction in the resonance frequency, and the viral load is calculated in accordance with the detected resonance frequency shift. This signal would be detected and processed resulting in a notification of exposure that would be sent to the patient, and the data transferred to healthcare services.

The device may have three possible configurations: a wearable, like a fitness band; a portable, the gadget can be placed in different places of a room or space; or an interior, introduced into the ventilation system of public spaces.

The app would offer several features which we expect to be advantageous for the users. These include a map of partners offering service for the device, push-notifications on the exposure to the viral hazard, storage of the health data and the instant contact with the support team and health specialists.

6. REGULATION

Due to the extremely sensitive nature of working with health data, there are many regulations dealing with medical devices. The most notable of these is GDPR (EU General Data Protection Regulation) (56), which covers all data protection in the

European Union, inspiring other similar laws. It has to be followed by any company that collects, processes or stores the personal data of citizens in the EU. (57)

In addition to this, the HIPAA (Health Insurance Portability and Accountability Act of 1996) (58) covers healthcare information in the USA other laws and agencies existing for regulating medical devices should be consider such as EU Regulation 2017/745 on medical devices for human use, and the French agency ANSM. (47)

However, with appropriate user agreements for personal use, terms for public use and both private and secure storage of data, there should not be any legal problems with these data protection laws that could restrict the usage of VAir, neither as an individual, nor as a collective detection system.

7. PERSPECTIVES

To meet the requirements of high reliability, portability and rapid response times, it is crucial to consider alternatives for the communication and signal processing of the existing respiratory virus detection systems, and to improve the power efficiency with no significant increase in cost.

The following steps would be manufacturing the prototype of a magnetostrictive device and testing antibodies reliability for the detection of *Influenza A*, in particular, their specificity, thermal stability and immobilisation capacity. Then, appropriate electronic components and fabricated immunochip would be used to construct a System-on-Chip. Finally, to integrate the sensing device into an IoT-network, a data processing software, a communication interface and a mobile application have to be developed.

The resources that may be needed are:

- human resources: (1) software engineers to develop and maintain data processing and communication software (2) a mobile app developer and a UX/UI designer to create a user-friendly platform for patients (3) a microelectronic engineer to integrate the components into a working sensing device (4) biochemists and molecular biologists to develop a sustainable immunochip (5) a marketing specialist.
- materials required: (1) microelectronic components: a direct digital synthesis (DDS) unit, a 32-bit ARM-Cortex-M4 microcontroller unit (MCU), a Bluetooth Low Energy (BLE) transceiver, a Li-Po Battery, 2 RMS-to-DC converters, amplifiers, a micro-USB port (2) a microporous filtration membrane (3) magnetostrictive immunochip, covered with immobilised anti-IAV hemagglutinin (HA) fragment antigen-binding antibody (FI6V3). Recombinant antibodies could either be produced in *E. coli* by the research group, or bought from a partner manufacturer, for example, CreativeBiolabs (60).
- financial resources are to be determined during further project development.

We are in the early days of Artificial Intelligence (AI), but it seems as if data-assisted diagnosis has the potential to transform the healthcare sector. The last few years have seen an explosion in Internet of Things (IoT) devices and connected products such as wireless sensors, smart meters, home automation systems and wearables. The lowered cost of components such as sensors and processors along with increasing wireless connectivity has resulted in many products being made smart.(55) Sensor node selection is largely a factor of the function and purpose designers are trying to fulfill. For a simple end sensor node that senses and transmits data a few times a day, an 8-bit MCU might be the correct answer.

The sensing biosensor will transform into a constant supply of health data when incorporated into Internet of Things (IoT) intellectual frameworks, offering useful data on the exposure to infectious dangers and contact tracking.. By measuring prognostic biomarkers and combining this knowledge with clinical observations and risk factors, patients can be stratified according to disease severity, which is regarded as a key player in the concept of personalized medicine.

Biosensor-based point-of-care methods not only can be used as home diagnostic tools but can also be exploited for environmental monitoring in public places such as airports, railway stations, and hospitals. Further, sewage and wastewater analysis can also be done using sensors. Moreover, overall monitoring of the viral threat and implementation of preventive measures holds promise to curb the current pandemic situation. (61)

Thus, considering a variety of diagnostic applications, there is a burning need behind the ambitious aim to develop an effective respiratory virus sensor that would be easy to operate and offer rapid response times.

8. BIBLIOGRAPHY

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