A New Design of Biosensor Integrating Single-Walled Carbon Nanotubes, Metal-Organic Frameworks, and Chitosan for the Detection of Acute Myeloid Leukemia

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Abstract. Acute myeloid leukemia (AML) is a hazardous blood cancer originating from bone marrow. As most contemporary detection methods, such as bone marrow tests and spectroscopic tests, still fail to achieve either high sensitivity or biocompatibility, a novel diagnosis method is needed. Biosensors are devices that detect targeted biomolecules to generate fast, reliable responses in real-time, making them advantageous for blood cancer diagnosis and the detection of minimal residual disease. As only a few biosensors specifically targeting AML have been published recently, here we propose a novel multiplex BioFET sensor, based on nanomaterials like carbon nanotube, copper-zirconium, and iron-based metal-organic framework, by using modified polymer chitosan and applying three aptamers targeting three different biomarkers, to maximize the sensitivity, selectivity, endurance, and biocompatibility of the detection of AML cells in the blood to provide a theoretical yet pragmatic model. Despite that there are some theoretical advantages, such as multiplexity, biocompatibility, and instantaneousness, the biosensor still faces some potential issues, including cross-talk, medical safety concerns, and production costs, which require extensive further research to address and improve these problems fully.

Keywords: Bio-FET Sensor; Acute Myeloid Leukemia; Carbon Nanotube; Metal-Organic Framework; Chitosan; Aptamer.

1. Introduction

According to the Mayo Clinic, Leukemia is a blood cancer of the body's blood-forming tissues, typically located inside the bone marrow and the lymphatic system. Symptoms are not specific and may include fever, fatigue, and weight loss, among others. [1] Leukemia is classified by its speed and location, such as acute lymphocytic leukemia, which spreads rapidly and is typically observed in children. On the other hand, chronic leukemia is primarily observed in older patients. [2]

Standard diagnostic methods include blood tests, bone marrow tests, etc. These methods are mature, cheap, and fast in detection because they have been developed for decades. A bone marrow biopsy can provide a practical examination of a patient's body. However, the insensitivity to the small number of malfunctioning cells, lack of real-time detection, and discomfort may result in the ineffectiveness of this method. [3] Advanced detection approaches contain microfluidics test and surface-enhanced Raman scattering (SERS) spectroscopy. Although these methods address insensitivity, they face environmental contamination or other clinical complications. [4] As a result, it is imperative to develop a new method for detecting leukemia with high sensitivity, low detection limits, and environmentally friendly and disposable features.

Biosensors are analytical devices that detect and react with targeted biomolecules, known as biomarkers, to generate rapid, reliable electrochemical or electronic responses in real-time, thereby enabling the monitoring and diagnosis of a patient's disease. [5] Biosensors generally offer a new approach for fast, sensitive, simple, and low-cost detection of specific targeted biomolecules; by applying biosensor technology to the detection and diagnosis of diseases can develop a more effective and sensitive approach, providing a more affordable, environmentally friendly, and precise resolution for disease diagnosis in the future. Additionally, due to the sensitivity of these biosensors, residual malfunctioning cells can be detected as early as possible, effectively preventing the occurrence of minimal residual disease (MRD) and the subsequent relapse of disease. [6] Electrochemical sensors

are the most commonly used sensors. Biomarkers are bound to the probes of the sensor. Then, the chemical reaction that occurs between the probe and the biomarker transduces electricity, allowing it to detect the targeted biomolecules. They have exceptional sensitivity and specificity. However, it is challenging for electrochemical sensors to detect multiple biomarkers. [7] Atiq Ur Rehman et al devised an electrochemical sensor based on Holey MoS2 nanosheets to detect dopamine and uric acid, achieving remarkable sensitivity and strong anti-interference ability. [8] In addition, Barman et al devised a sensor based on antibody- functionalized MXene nanosheets that is able to detect vitamin D deficiency clinically relevant sensitivity, specificity, and amenability for point-of-care testing. [9] Fluorescent biosensors make great use of lights. When the targeted biomarker binds to the recognition component, a change in fluorescence in the sensor takes place. Fluorescent sensors are fast and in vivo sensors, enabling real-time monitoring for patients. However, the signals of biosensors can be diminished over time. Additionally, the thickness of the body cell and other environmental factors can influence the light intensity and reduce the reliability of the sensor. [10] Saad et al developed a fluorescent biosensor, based on carbon dots and gold nanoparticles, that can detect the presence of Escherichia coli, a type of harmless intestinal bacterial with great effectiveness and sensitivity. [11] Additionally, Chen et al devised a fluorescent biosensor based on gold-nanocluster modified zeolitic-imidazolate-framework nanocomposite that is able to detect pesticide triazophos with ultrasensitivity and selectivity. [12]

Bio-FET sensors consist of a piece of insulator, two electric gates (electrodes), a layer of sensing material (also known as the substrate), and a layer of transfer material, referred to as transducers, which can be either the substrate or the electrode. The insulator is designed to prevent short-circuit and biological contamination when detecting the targeted molecules. When the probe immobilized on the substrate binds to the desired biomarker, the total structure and properties of this combination change; the bound charge alters the surface potential, much like modifying the gate voltage in a MOSFET structure. This potential change modulates the channel conductance (source-drain current), which is measured in real time. Operating in the subthreshold region yields exponential signal amplification for small potential shifts. Recent advanced bio-FET sensors utilize nanomaterials or nanochannels, such as graphene, on the sensor surface to enhance their performance. This not only increases the sensor's sensitivity and lowers its detection limits but also makes it more resistant to being washed away in the sample. The core advantages of bio-FET sensors are that they do not need fluorescent or enzymatic labels, which is ideal for point-of-care testing. Additionally, they can be fabricated using CMOS-compatible processes and integrated into portable microfluidic platforms. [13] In 2022, A. Toral-Lopez et al presented a bioFET sensor based on graphene, which can detect SARS-CoV-2 (COVID-19) virus with potentially great sensitivity for early detection. [14] Additionally, Zeng et al invented a novel aptamer-based BioFET sensor that incorporates a thermally stabilized T-Hg2+-T hairpin structure for the detection of mercury ions. [15] Silva et al also developed a CNT-Based FET on 200mm Si wafers to detect DNA, with extremely high sensitivity and selectivity. [16] Nevertheless, it can be challenging to devise and produce Bio-FET sensors that can detect multiple biomarkers simultaneously. Temperature fluctuations, pH changes, and other environmental factors can affect the binding kinetics of biomolecules to the sensor surface, impacting stability. [17]

Metal-organic frameworks (MOFs) are a class of crystalline, porous materials characterized by their periodic network, which is assembled from metal ions or clusters bridged by organic ligands. [18] As hybrid organic-inorganic entities, MOFs feature both the rigidity of metallic materials and the flexibility of organic materials, making them an ideal material for biosensors. Additionally, MOFs have a high surface area and tunable pore size, enabling efficient adsorption and immobilization of biomolecules such as enzymes, antibodies, or DNA. This feature allows for the rigid fixation of the biomarker's position, thereby enhancing the stability of the bond and improving the reliability and sensitivity of the biosensor. The robustness of MOFs enables them to withstand harsh conditions, such as temperature changes and exposure to chemical reactions, making them suitable for various applications and scenes. [19]

Additionally, the biofunctionality of MOFs allows them to integrate antibodies, enzymes, or other biomolecules, thereby enhancing the selectivity and sensitivity of the biosensor. Ibrahim et al invented a biosensor based on Zn-based porphyrin MOF to detect HER2, which is a biomarker for breast cancer. [20] Additionally, a Co-hemin MOF/chitosan biosensor developed by Choi et al can detect lactose with great sensitivity. [21] However, MOFs are not inherently conductive, because their bonds are strong and electrons cannot easily move through these stiff bonds. Hence, a highly conductive material is necessary for electrochemical and bio-FET sensors. [22]

Carbon nanotubes (CNTs) are one-dimensional cylindrical shapes with a nanometer-scale diameter and micrometer-scale length, rolled-up sheets of graphene. [23] The primary merits of CNTs are their high surface area and excellent electrical properties, making them great tools for improving electrochemical sensors. Additionally, CNTs feature high immobilization of biological recognition elements. These merits not only increase the sensitivity and affinity of biosensors but also lower the detection limit of electrochemical biosensors. CNT can also be the platform of different aptamers modifications. [24] Zubkovs et al devised a biosensor, based on single-walled carbon nanotubes, to detect nitric oxide, a common substance released during osteoarthritis, a degenerative inflammatory joint disease. [25] Additionally, Yucer et al developed an electrochemical sensor based on carbon nanotubes to diagnose pancreatic and liver cancer by detecting CA19-9, a cancer biomarker with precision and high sensitivity. [26] Nevertheless, it is essential to realize that carbon nanotubes have potential toxicity. Studies from Kobayashi et al revealed that CNTs may induce inflammation, fibrosis, lung cancer following long-term inhalation, and gene damage in the lung. They may also have a high biopersistence, marking its covert damage to the human body. The potential environmental contamination may hinder the widespread commercialization and cause safety concerns. [27] Therefore, reducing the toxicity and increasing the biocompatibility of the carbon nanotube become the pivotal issue for biosensor engineering.

To address the toxicity issue, several methods have been introduced to reduce the toxicity of carbon nanotubes. One of the most significant ways is surface modification, in which polymers or other nanomaterials are applied to carbon nanotubes to increase their stability and biocompatibility, as well as reduce the damage to the human body. It has been found by K.S. Wisdom et al. that Chitosan (CS), a natural polymer derived from chitin, which is found in the exoskeletons of crustaceans such as shrimp and crabs, exhibits good chemical stability, biocompatibility, and biodegradability when incorporated into carbon nanotubes. Consequently, they developed a nanohybrid of carboxylate-functionalized single-walled carbon nanotubes conjugated with CS (COOH-SWCNT-CS) through the ionotropic gelation process, using tripolyphosphate (TPP) as a cross-linker, with slight modifications. Their assays show no or minor DNA damage, indicating that living cell damage resulted from COOH-SWCNT-CS, which highlights its safety and biocompatibility. Chitosan has great mechanical strength. Han et al discovered that mixing chitosan with graphene oxide can enhance the mechanical strength of the nanocomposite without damaging the material itself. [28] Pok et al also discovered that the conductivity of carbon nanotube and chitosan nanocomposite was not influenced at all but enhanced by chitosan. Additionally, this nanocomposite has numerous binding sites for diverse macro- or micro-molecules; thus, the functionality of SWCNT remains intact, creating a safer and environmentally friendlier strategy for applying carbon nanotubes in biosensors. [29] As early as 2006, Qian et al had already devised a new amperometric biosensor combining cross-linking horseradish peroxidase (HRP) by glutaraldehyde with multiwall carbon nanotubes/chitosan (MWNTs/chitosan) composite film coated on a glassy carbon electrode. The biosensor had good repeatability and stability for the determination of H2O2. [30] Additionally, Lin et al invented a biosensor based on silver nanoparticles, carbon nanotubes, and chitosan to detect glucose in the human body, offering sensitive amperometric responses to glucose. [31]

Aptamers are probes that capture biomarkers, such as oligonucleotides (DNA or RNA), antibodies, or antigens, that interact with biomarkers and change the surface charge. [32] Biomarkers like PSA, HER2 and CEA are antigens that identify the presence of cancer cells. PSA is common for prostate cancer [33], HER2 for breast cancer [34], and CEA for colorectal cancer [35]. The technique for

finding aptamers that bind specifically to molecules, especially proteins, is called SELEX. [36] Gene mutations lead to the development of leukemia, such as NPM1 [37], which causes acute myeloid leukemia. Additionally, binding biomarkers relating to or expressing such a change in selected molecules on the surface of the biosensor can generate signals that can be transmitted in different ways. Most of the biosensors this article aforementioned are mostly using these mechanisms, to provide a strong selective and sensitive solution.

In our design, sensing layers (SWCNTs, CuZr-MOFs, and Fe-MOFs) are placed atop the sensor by applying multiplex patterning for different aptamers (S30-T1, ATW0081, and CTApt-796) that target corresponding biomarkers. (CD33, CD45 and HLA-DR) [38] The protection layer of Chitosan is applied to SWCNT by conjugating it with CS through carboxylate functionalization to form COOH-SWCNT-CS, thereby preventing the biotoxicity and damage to the patient caused by SWCNT.

Hence, our biosensors are highly sensitive, biocompatible and able to withstand harsh conditions for an extended period. Biosensors are capable of accurately detecting leukemia cells and producing sensitive, specific results. Their small size, user-friendly operation, and minimal sample requirements make them ideal for point-of-care applications, offering the potential for quick and convenient diagnosis in various settings. After the reaction of each biomolecule to each receptor, the changes of saturation and gate-induced-drain-leakage (GIDL) currents are monitored in the FET sensor. It is confirmed that two different biomolecules are independently detectable by the changes in the saturation and the GIDL currents in the FET sensor. As a result, a multiplex Bio-FET sensor is viable to manufacture for enhanced performance and improved application in future generations of leukemia detection.

2. Design

The sensing layer is made of SWCNT, CuZr-MOFs, and Fe-MOFs. CuZr-MOF is synthesized through the sonication and heating of Zr-MOF, prepared from ZrCl4 and DMF solutions, and then combining it with CuCl2 · 2H2O through sonication and centrifugation to form a CuZr-MOF solution. Fe-MOFs are obtained through the self-assembly of metal clusters and organic linkers, achieved by carefully controlling synthetic parameters such as time and temperature to yield crystalline materials.

SWCNT can be manufactured through the sonication and centrifugation of purchased SWNT and SDS solutions, by removing the metal crystal and impurities to obtain a pure SWCNT solution. SWCNT is then modified by a carboxylate solution to bond CuZr-MOFs together to form a hybrid SWCNT using a discharging method. The mixture is then connected by Fe-MOFs at the edge of the hybrid material using covalent bonds through a hydrothermal reaction, where it is mixed with metal salt precursors and organic linkers, facilitated by dispersion and crystallization. The mixture is then applied to the surface of the FET sensor using the dispersion method, connecting to the source and drain, which are made of gold, to maximize the transit efficiency. The insulator is made of silicate dioxide, serving as a gap and a substrate. A multi-fingered back-gated design is used to enhance signal amplification, making it suitable for detecting low concentrations of biomarkers. Figure 1 presents a simple representation picture of the bioFET sensor, and figure 2 represents the simple process of such three materials.

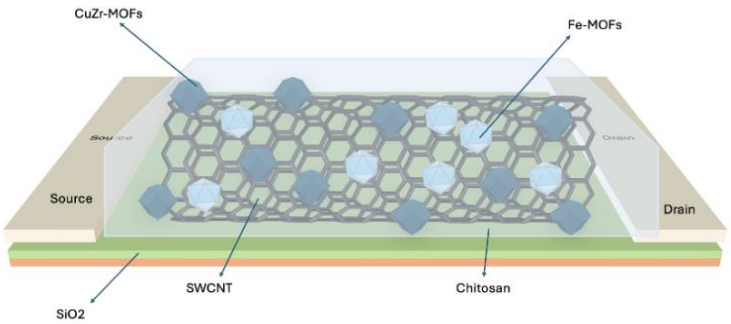

Fig. 1 Basic Representation of the FET Sensor

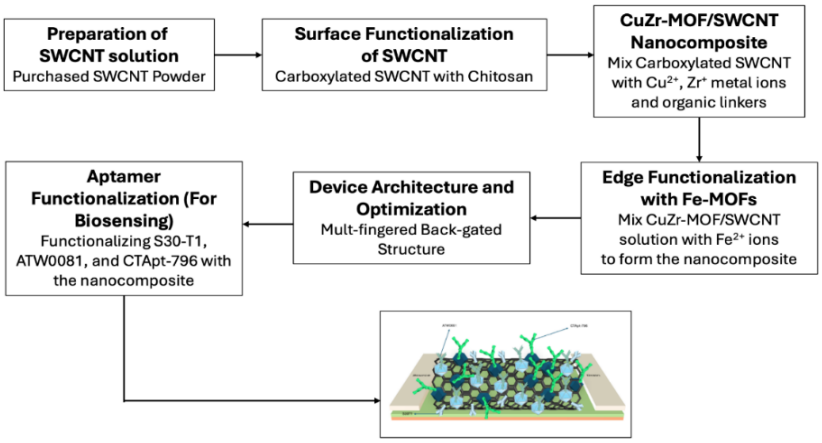

Fig. 2 Basic Representation of the FET Sensor

2.1. Aptamers and Biomarkers

The biomarkers used in this communication are CD33, CD45, and HLA-DR. The corresponding aptamers are S30-T1, ATW0081, and CTApt-796. S30-T1 is an aptamer that can specifically inhibit CD33-positive acute myeloid leukemia HL-60 cell proliferation by arresting the cell cycle at the G2 phase. [38] This aptamer can be synthesized through SELEX – a process that involves the collection and selection of unbound gene sequences, elution and amplification of the selected ssDNA sequence, trimming to its core binding region, optimizing the sequence to the highest quality, and then using a solid-phase chemical method to mass-produce such an aptamer. CTApt-796 and ATW0081 are commercially available and able to be mass-produced using cell SELEX as well.

Aptamers are linked to various materials to conduct electricity in response to different current changes. S30-T1 is connected to the carbon nanotube through immersing into PBASE solution with non-covalent interactions for minimal disruption of the CNT's electrical properties by π-stacking and Van Der Waals force. CTApt-796 is connected to CuZr-MOF using a covalent interaction through its nitrate bonding immobilization. ATW0081 is connected to Fe-MOFs through covalent interactions, utilizing the connection between the nitric-iron bond as well. Before binding to biomarkers, aptamers undergo a significant shape change to bind specific targeted biomolecules. After binding with the biomarker, the aptamer's physical properties, such as its mass and conductivity, will change, resulting in a change in the current, especially for delocalized electrons. Going through it. As a result, the current change can be recorded through the gate and drain and transmitted to the computer, where it can be interpreted as the detection of a particular biomolecule, in this scenario, biomarkers that indicate the presence of AML cells, which is illustrated in Fig. 3.

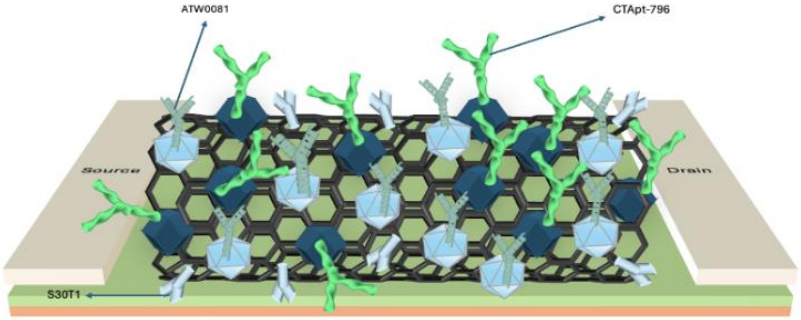

Fig. 3 Basic Representation of the FET Sensor

2.2. Protection Layers

The protection layer of the biosensor is Chitosan, for its biocompatibility and physical strength, making it an ideal layer for protection. Chitosan is the last modification to the carbon nanotube, the primary conductive layer of the FET sensor. The synthesis is almost the same as in the literature: the

polymer chitosan is dissolved in a 1% acetic acid solution, filtered through glass wool to remove insoluble substances, and then sodium hydroxide solutions are added. After removing the sodium hydroxide solutions, the remaining filtrates are washed with water to adjust the pH to 7.0, thereby obtaining the purest form of Chitosan. Degradation through natural bacterial protease and water bath is used to modify the polymer. 2% acetic acid is then added and constantly stirred with the previously functionalized carbon nanotubes to obtain conjugated COOH-SWCNT-CS nanocomposites. [40] Figure 4 shows the basic chemical structure of the chitosan-modified single-walled carbon nanotube.

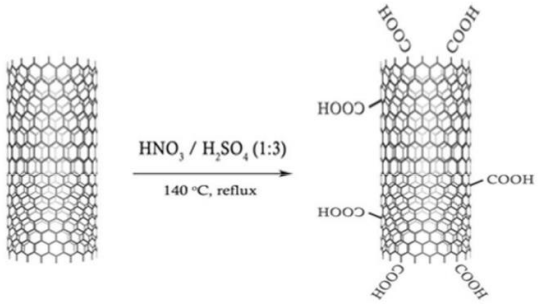

Fig. 4 Hongwu International Ltd's Picture of COOH-SWCNT-CS [39]

2.3. Transduction Principles

This biosensor aims to achieve real-time monitoring of leukemia cells in the patients' bodies, improving upon conventional diagnosis by enabling direct electric readout and extremely sensitive cancer detection. When the aptamer captures the targeted biomolecule, the electrical conductance of these materials changes, measured as ΔG/G0, due to the conductivity difference; thus, the detection of different biomarkers can be measured without difficulty in identification. Thus, the data is recorded in potential differences against current. By interpreting the significant gap between any smooth points, the presence of biomarkers can be confirmed.

The Bio-FET operates based on field-effect modulation of charge carriers within the semiconductor channel. When no target molecules are present, the current between the source and drain electrodes follows a baseline characteristic. Upon the introduction of the target analyte, specific binding occurs at the functionalized gate interface, resulting in a local change in surface charge density. This variation alters the effective gate potential, leading to a measurable shift in the drain current (I_D) or threshold voltage (V_t). The magnitude of this change correlates with the concentration of the target molecule, enabling quantitative detection. All electrical responses were monitored under controlled temperature and pH conditions to ensure signal stability and reproducibility.

As S. Chen et al. once mentioned in their essay, the electrostatic gating effect describes the biomolecule-induced charges that dope the sensing material, changing its electrical properties and resulting in a detectable response signal. [41] Studies of FET biosensing have shown that the negative charges on the phosphate groups of DNAS contribute to P doping of graphene through the gating effect, thereby changing the Fermi level and modulating the carrier density, resulting in a significant signal change that can be identified as a detection. The equation for the total geometrical capacitance is calculated as:

$$C = \left(\frac{1}{C_{G1}} + \frac{1}{C_{G2}} + \frac{1}{C_{G3}} + \frac{1}{C_Q}\right)^{-1} \quad (1)$$

Where CG1, CG2, and CG3 measure the geometrical capacitance between the substrate and the solution, and CQ measures the targeted molecule (mDNA, in this case) density. For the sensor bound with charged molecules, except for gate voltage, n is also modulated by negatively charged molecules (V molecules), as shown in the equation:

$$n = \frac{C_g}{q}(V_G + V_0) + \frac{C_g}{q}V_{molecules} \quad (2)$$

Where Cg is the gate capacitance per unit area. Vg is the gate voltage, V0 is the natural voltage equivalent to the carrier inherent in the sensing material. So, the modulation of the carrier density (Δn) by negatively charged molecules is related to the change of V molecules (ΔV molecules), as shown in the equation.

$$\Delta n = \frac{C_g}{q}\Delta V_{molecules} \tag{3}$$

The relationship between the readable output and changed DNA density (ΔQ, which is Δn in the previous equations) is shown by the equation:

$$\Delta V_{cup} = \frac{\Delta Q}{C} = \frac{N_e \rho S}{C} \tag{4}$$

Where N is the number of bases in the added DNA, and S is the sensing area.

The charges interact and transfer between biomolecules and sensing materials. In FET biosensing, the transfer of charge causes an increase or decrease in the carrier density of the sensing material, resulting in detectable response signals.

Sometimes, charged molecules on the sensor's surface may hinder carrier transport through the local carrier scattering effect. This phenomenon occasionally causes a negative current response. The scattering centers formed by the absorption of charged biomolecules onto the substrate significantly decrease the mobility of the substrate carrier, thereby generating the response signal.

2.4. Fabrication Process

The FET sensor is manufactured through semiconductor-processing technology. The fabrication process of this FET sensor includes the module base, photolithography of the circuit, production of the substrate (as mentioned in the sensing layers), and finally, the complete combination and connection to the computer. The module base of the sensor is heavily doped silicon dioxide, a standard industrial-level material for FET sensor base and photolithography. The silicon dioxide is first put with photoresist into the photolithographer to produce an undeveloped circuit base through laser modification. The base is then developed in positive photoresist developers, such as metal-ion-free tetramethylammonium hydroxide (TMAH). Spin dries the wafer and clean all the remaining photoresistors to avoid further contamination. Subsequently, the base is transferred negatively for channels. The gate and drain are made of gold ions, and an adhesive protection layer of chromium is applied using the evaporated gold film method. The components are then assembled on top of the base. For back-gate FET sensors, the gate and drain are put on the same side of the surface. The diagram of the basic circuit arrangement is demonstrated in Figure 5.

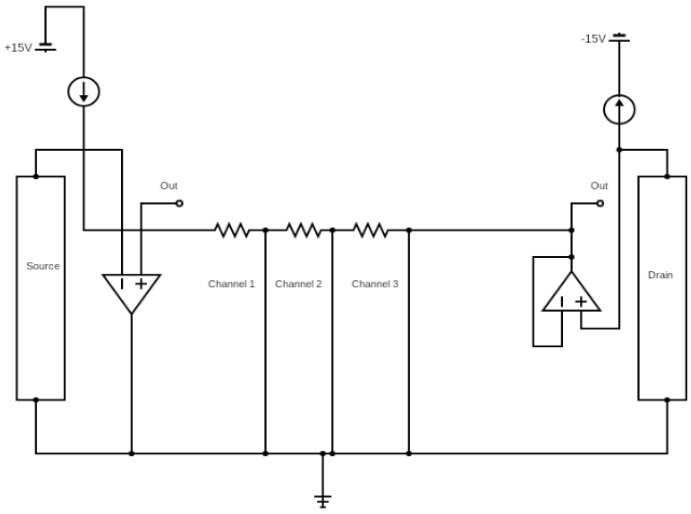

Fig. 5 Schematic Diagram for Measuring Circuit Arrangement

The next step is to produce the substrate, which has been mentioned before, by combining sensing layers, protection layers, and aptamers into a single 3D substrate, separated by multiple channels. Polydimethylsiloxane (PDMS) microfluidic channels are created and integrated on the sensor surface to deliver samples and facilitate reactions with the functionalized probe. The nanocomposite is transferred to the module base using the Ar plasma cleaning method, which employs air plasma to clean the nanocomposite. The nanocomposite and the base are combined and immobilized through a double plate under high pressure and temperature. This can clean the carbon nanotube surface injury-free, but it is more complicated than the PMMA-assisted transfer method. Finally, the electrical wires are connected to the exterior instruments, such as the Source-Meter instrument and other measuring devices, which measure the current change of the source and drain. The whole setup is hence prepared for experimentation and further research purposes. [41]

3. Discussion

Here, we propose a new design that integrates CuZr-MOFs, SWCNTs, and chitosans to combine their benefits and overcome the intrinsic drawbacks and issues of each material. The biosensor will be designed for acute myeloid leukemia (AML), as it is the most severe type of leukemia, and there are relatively fewer AML biosensors compared to other leukemia biosensors. The primary reason for AML's occurrence is cellular genetic mutation that occurs during mitotic cell division, which typically involves. Hence, the primary biomarker for this type of cancer can be found on the cell surface or inside the cell. Based on this theory, two primary methods are devised: immunophenotyping and genetic analysis. The former detects the presence of specific proteins on the cell surface, such as antigens, whereas the latter analyzes DNA or RNA for mutations or other genetic abnormalities.

Here, we do not consider DNA analysis as the best plan for aptamers and biomarkers. The first reason is that there are few or little effective or verified aptamers designed or manufactured for DNA or mRNA that are specific and prolific in AML cells. Hence, the latter method is not available in biosensor design. Possible reasons could be that most of the particular and practical DNA or mRNA is located inside the cell, rather than on its surface. It is challenging for biosensors to enter the cell to detect DNA and mRNA, as well. Secondly, mRNA detection is typically done using complementary probes, not aptamers. However, this probe hybridization results in low affinity, thus reducing the effectiveness of the detection.

Selecting materials for different aptamers is imperative. Combining MOF and CNT has been proven effective, and the CNT can be modified to improve its biocompatibility by introducing COOH groups, such as COOH-SWCNT-CS. The central issue in selecting materials lies in choosing MOFs for different aptamers. MOFs are advantageous because of their flexible frameworks, ease of functionalization, and narrow pore sizes. Due to their high surface area and strong binding feature, Fe-MOFs have achieved a significantly low detection limit and a wide detection range, with a reported biosensor that is said to reach 0.33 fg/mL for the detection limit and 1 fg/mL to 1 ng/mL for the detection range. [44] The CuZr-MOFs also possess a high surface area, large pore size, and a strong affinity for aptamer strands, making them suitable for immobilizing aptamers on the electrode surface. Bimetallic CuZr-MOFs were reportedly obtained through the MOF-on-MOF technique and utilized as a scaffold for immobilizing an aptamer on the electrode surface to form an electrochemical aptasensor for detecting miR-21, a biomarker for cancer diagnosis. The fabricated aptasensor exhibited excellent sensing performance with an ultra-high sensitivity for the detection of miR-21, yielding a significantly low detection limit of 0.45 zM, as determined by square-wave voltammetry, within a wide detection range of 1 zM to 1 pM. The aptasensor also exhibited excellent selectivity, stability, and reproducibility, and accurate target detection in human serum. Hence, it is entirely possible to transfer such materials from electrochemical sensors to Bio-FET sensors while still performing high selectivity, sensitivity, and affinity. [45]

According to Maria Basharat et al., the most commonly expressed antigens for Acute Myeloid Leukemia are CD13, CD33, CD45, and HLA-DR. Among all subtypes of AML, the mean positivity

rates for CD13 and CD33 are 57% and 67%, respectively. Almost all blasts expressed CD45 with no remarkable difference among the subtypes of AML. For corresponding antibodies, preclinical studies demonstrate that anti-CD33 aptamers bind effectively to CD33+ AML cells and internalize similarly to their antibody counterparts. [42] A commercial CD45-binding DNA aptamer exists, reported by Cambio: A 32-mer DNA aptamer selected against recombinant human CD45. The affinity Kd is 9.7 nM, and it is customizable with 3′ modifications, which are amine, biotin, fluor, and thiol. No aptamer specific for CD-13 exists, however. A commercial anti-HLA-DRA DNA aptamer selection service is available from Creative Biolabs. It targets the extracellular alpha chain of HLA-DR, and it has an excellent affinity from 1 nM to 1 μM. Apart from its availability and effectiveness, it has been proven that immunophenotype biomarkers and aptamers can easily compose multiplex biosensors with multiple panels, significantly improving biosensor reliability and efficiency. This is because more immunophenotypes can reduce the possibility of false positivity or false negativity. Hence, it is entirely possible to use these biomarkers and aptamers to design a new biosensor for leukemia detection.

The binding of such aptamers can be achieved by covalent binding or non-covalent Binding (Coordination Bonding). [43] For covalent binding, it involves creating chemical bonds between the aptamer and the MOF, often using cross-linkers or functional groups on the aptamer and the MOF's organic linkers. The advantage is that it provides a durable and stable attachment, minimizing aptamer detachment. However, it may require more complex synthesis and functionalization steps. For coordination binding, aptamers, particularly those with thiol or other coordinating groups, can directly bind to the exposed metal sites of the MOF. It can be simpler to implement than covalent methods, but it may result in less stable attachment compared to covalent methods. Here, we use covalent binding to achieve a more stable outcome for aptamers. Since the biosensor is in vivo, located inside the patient's body, scattering acute myeloid leukemia cells can be captured by aptamers as they flow through the body or a specific part of the organ, due to their high sensitivity and specificity, which is highly effective for detecting minimal residual disease.

Potential advantages of this BioFET sensor include its multiplexity, biocompatibility, and instantaneous response. The first advantage is the multiplexity, which enables the sensor to comprehensively examine the presence of AML cells, as these genes cover almost all known AML cells. These aptamers are bound explicitly to their biomarkers, demonstrating strong selectivity. The false detection of normal white blood cells is so low that it is almost impossible to occur. As a result, the detection rate will be extremely high and accurate. With these features, this AML biosensor will be one of the most sensitive and selective biosensors of its kind. The second advantage is its biocompatibility. As mentioned earlier, carbon nanotubes pose a risk to the human body due to their toxicity and hazardous properties. The modification of Chitosan can effectively address this issue by creating carbon nanotubes that are significantly more biocompatible, thereby reducing the potential for damage to the body, particularly the lungs, which are the most susceptible to the effects of carbon nanotubes. It is utmostly important to know that the biocompatibility of chitosan-protected carbon nanotubes is nearly harmless to the human body, as aforementioned, making the sensor more advantageous than other carbon nanotube-based biosensors to perform in vivo detection in the patient's blood. The presence of Chitosan also provides a protective layer that empowers the substrate with exceptional mechanical strength, making it suitable to withstand flowing blood; hence, an in vivo FET sensor is possible. The third advantage of this biosensor over other types is that, as an in vivo sensor, it can monitor the concentration of AML cells in both the blood and the bone marrow, making it a powerful tool for assessing a patient's health condition. It also enables doctors to examine the AML cells at a more thorough level, allowing them to provide specialized treatment as early as possible, as they can identify the condition promptly. The performance can be inferred through the substrate material carbon nanotube and the secondary material CuZr-MOFs and Fe-MOFs, which all perform exceptionally well in other biosensor assays, indicating that the detection rate can be as low as 10 fM and the detection boundary can be from ~10 fM to ~100 pM (this is an estimation), which makes it extremely sensitive, better than many bioFET sensors recently published. The selectivity of

this bioFET sensor is guaranteed because it uses aptamers that only bind to their extremely specific corresponding biomarkers. As a result, a false signal is unlikely to happen.

However, there are some potential drawbacks, focusing on the cost of production, safety issues, and. First, the multiplex BioFET sensor can be challenging to manufacture because the reproducibility of MOF growth and CNT alignment varies across different experimental conditions and materials. Additionally, controlling MOF crystallization and CNT network uniformity from wafer to wafer is also a significant challenge. This not only makes it hard to repeat the experiment and produce the same products but also obstructs mass production and scaling. Second, even though biocompatibility is no longer an issue, there may be some peculiar negative outcome to the patient when using this measuring device, since the design is a theoretical model that has not been proven by experiment. Hence, before any clinical use or experiment, it must be tested on other individuals and conducted with multiple health examinations to ensure the safety of the volunteers. Third, the cross-talk in multiplex design can potentially be a significant factor that reduces the selectivity and sensitivity of the biosensor, because in a back-gated layout, one gate may control all channels simultaneously, if there is a leakage current or parasitic coupling, it can mix signals to make it impossible to identify whether the detection is based on a specific gene or a mixture of different genes. However, this issue is unlikely to occur, as blood is a fast-moving liquid, making it difficult for unrelated biomolecules or objects to adhere to the sensor surface. Assuming it is cross-linked by some unknown molecules, the protection layer chitosan will prevent it staying for too long as its great mechanical strength only allows molecules that can bind into aptamers to stick with the bioFET sensor, which are only the biomarkers we want to capture. Nevertheless, even if these potential issues do not occur, further research is still needed to comprehensively examine the sensor and gain a complete understanding of its advantages and disadvantages, as well as potential resolutions to address these issues.

4. Conclusion

To conclude, this science communication article describes a novel design of a BioFET sensor – based on carbon nanotube, CuZr-MOFs, and Fe-MOFs as sensing materials, chitosans as protection layers, SiO2 as module base, S30-T1, ATW0081, and CTApt-796 as aptamers and CD33, CD45, and HLA-DR as corresponding biomarkers – attempting to design a multiplex back-gated in vivo FET sensor that highlights very high selectivity, sensitivity, biocompatibility, and instantaneousness to provide an exceptional resolution and augment for contemporary acute myeloid leukemia detection and diagnosis, as there are few biosensors for this type of leukemia and this type of leukemia is one of the most dangerous blood cancers. This communication article discusses the design and principles of the biosensor, as well as the selection of materials, aptamers, and biomarkers. In addition to these, potential advantages and disadvantages are also discussed. This novel design has the potential to make rapid leukemia screening more affordable, precise, and sensitive. As this design is still a theoretical model, further research and a comprehensive assay must be conducted to ensure the effectiveness of this biosensor. Additional research can also focus on improving stability in real biological fluids and integrating the system into portable diagnostic platforms, paving the way for specific and point-of-care leukemia diagnosis systems.

References

[1] Mayo Clinic. Leukemia. Mayo Clinic. https://www.mayoclinic.org/diseases-conditions/leukemia/symptoms-causes/syc-20374373.

[2] Cleveland Clinic. Leukemia Symptoms, Signs & Treatment Options. Cleveland Clinic. https://my.clevelandclinic.org/health/diseases/4365-leukemia.

[3] Mayo Clinic. Leukemia - Diagnosis and treatment - Mayo Clinic. Mayoclinic.org. https://www.mayoclinic.org/diseases-conditions/leukemia/diagnosis-treatment/drc-20374378.

[4] Workman, J. New SERS-Microfluidic Platform Classifies Leukemia Using Machine Learning. Spectroscopy Online. https://www.spectroscopyonline.com/view/new-sers-microfluidic-platform-classifies-leukemia-using-machine-learning (accessed 2025-10-20).

[5] Mehrotra, P. Biosensors and Their Applications – a Review. Journal of Oral Biology and Craniofacial Research 2016, 6 (2), 153–159. https://doi.org/10.1016/j.jobcr.2015.12.002.

[6] Ahmed, A.; Rushworth, J. V.; Hirst, N. A.; Millner, P. A. Biosensors for Whole-Cell Bacterial Detection. Clinical Microbiology Reviews 2014, 27 (3), 631–646. https://doi.org/10.1128/cmr.00120-13.

[7] Grieshaber, D.; MacKenzie, R.; Vörös, J.; Reimhult, E. Electrochemical Biosensors - Sensor Principles and Architectures. Sensors 2008, 8 (3), 1400–1458. https://doi.org/10.3390/s80314000.

[8] Rehman, A. U.; Anwar, M.; Khan, A.; Kalhoro, K. A.; Zhang, C.; Zhang, Y.; Shokouhimehr, M.; Liu, Z. Perforated MoS2 Nanosheets Adorned with Ni Nanoparticles: An Electrochemical Sensor for Concurrent Detection of Dopamine and Uric Acid. Microchemical Journal 2025, 209 (209), 112821. https://doi.org/10.1016/j.microc.2025.112821.

[9] Barman, S. C.; Jin, Y.; El-Demellawi, J. K.; Thomas, S.; Wehbe, N.; Lei, Y.; Hota, M. K.; Xu, X.; Hasan, E. A.; Mohammed, O. F.; Bakr, O. M.; Alsulaiman, D.; Alshareef, H. N. Antibody-Functionalized MXene-Based Electrochemical Biosensor for Point-of-Care Detection of Vitamin D Deficiency. Communications Materials 2025, 6 (1). https://doi.org/10.1038/s43246-025-00756-9.

[10] Frei, M. S.; Mehta, S.; Zhang, J. Next-Generation Genetically Encoded Fluorescent Biosensors Illuminate Cell Signaling and Metabolism. Annual Review of Biophysics 2024, 53 (1), 275–297. https://doi.org/10.1146/annurev-biophys-030722-021359.

[11] Saad, S. M.; Abdullah, J.; Abd Rashid, S.; Fen, Y. W.; Salam, F.; Yih, L. H. A Carbon Dots Based Fluorescence Sensing for the Determination of Escherichia Coli O157:H7. Measurement 2020, 160, 107845. https://doi.org/10.1016/j.measurement.2020.107845.

[12] Chen, J.; Wang, Y.; Shen, R.; Li, W.; Gao, S.; Xiao, Z.; Lv, Q.; Song, X.; Xu, J.; Xu, G.; Cui, H.; Li, Z. Accurately Tunable AuNC-ZIF Content Architecture Based on Coordination-Dissociation Mechanism Enables Highly Brightness Dual-Site Fluorescent Biosensor. Advanced Science 2024, 12 (4). https://doi.org/10.1002/advs.202408400.

[13] Sung, D.; Koo, J. A Review of BioFET's Basic Principles and Materials for Biomedical Applications. Biomedical Engineering Letters 2021, 11 (2), 85–96. https://doi.org/10.1007/s13534-021-00187-8.

[14] Toral-Lopez, A.; Kokh, D. B.; Marin, E. G.; Wade, R. C.; Godoy, A. Graphene BioFET Sensors for SARS-CoV-2 Detection: A Multiscale Simulation Approach. Nanoscale Advances 2022, 4 (14), 3065–3072. https://doi.org/10.1039/d2na00357k.

[15] Zeng, G.-C.; Wang, Y.-L. Development and Validation of a Thermally Stable Aptamer-Based BioFET Sensor for Sensitive Mercury Ion Detection. 2025 23rd International Conference on Solid-State Sensors, Actuators and Microsystems (Transducers) 2025, 2036–2039. https://doi.org/10.1109/transducers61432.2025.11110738.

[16] da Silva, L. A.; Hartmann, M.; Böttger, S.; Hermann, S. High-Performance CNT-Based FETs on 200 Mm Si Wafers with Low Drift and AM-Level Biosensing Sensitivity. IEEE Sensors Letters 2025, 9 (10), 1–4. https://doi.org/10.1109/lsens.2025.3612488.

[17] Pandey, M.; Bhaiyya, M.; Rewatkar, P.; Zalke, J. B.; Narkhede, N. P.; Haick, H. Advanced Materials for Biological Field-Effect Transistors (Bio-FETs) in Precision Healthcare and Biosensing. Advanced Healthcare Materials 2025, 14 (13). https://doi.org/10.1002/adhm.202500400.

[18] Science Direct. Metal-Organic Framework - an overview | ScienceDirect Topics. www.sciencedirect.com. https://www.sciencedirect.com/topics/materials-science/metal-organic-framework.

[19] Salinas Domínguez, R. A.; Águila Rosas, J.; Martínez Tolibia, S. E.; Lima, E.; Dutt, A. Opportunities in Functionalized Metal-Organic Frameworks (MOFs) with Open Metal Sites for Optical Biosensor Application. Advances in Colloid and Interface Science 2025, 344, 103598. https://doi.org/10.1016/j.cis.2025.103598.

[20] Ibrahim, M. R.; Shaikha Alneyadi; Truong, K.-N.; Hesham El- Maghraby; Abdellah, M.; El-Zohry, A. M.; Wuttke, S.; Yaser Greish. Design of a Zn-Based Porphyrin MOF Biosensor for Fluorometric Detection of HER2 as a Breast Cancer Biomarker. RSC Advances 2025, 15 (27), 21479–21492. https://doi.org/10.1039/d5ra01942g.

[21] Choi, H. S.; Yang, X.; Liu, G.; Kim, D. S.; Yang, J. H.; Lee, J. H.; Han, S. O.; Lee, J.; Kim, S. W. Development of Co-Hemin MOF/Chitosan Composite Based Biosensor for Rapid Detection of Lactose. Journal of the Taiwan Institute of Chemical Engineers 2020, 113, 1–7. https://doi.org/10.1016/j.jtice.2020.07.021.

[22] Maryland Energy Innovation Institute. Added Molecules Allow Metal-Organic Frameworks to Conduct Electricity. Umd.edu. https://energy.umd.edu/news/story/added-molecules-allow-metalorganic-frameworks-to-conduct-electricity (accessed 2025-10-21).

[23] Mohammadreza Kolahdouz; Xu, B.; Aryanaz Faghih Nasiri; Fathollahzadeh, M.; Manian, M.; Hossein Aghababa; Wu, Y.; Radamson, H. H. Carbon-Related Materials: Graphene and Carbon Nanotubes in Semiconductor Applications and Design. Micromachines 2022, 13 (8), 1257–1257. https://doi.org/10.3390/mi13081257.

[24] Sengupta, J.; Hussain, C. M. Carbon Nanotube-Based Field-Effect Transistor Biosensors for Biomedical Applications: Decadal Developments and Advancements (2016–2025). Biosensors 2025, 15 (5), 296–296. https://doi.org/10.3390/bios15050296.

[25] Zubkovs, V.; Belcastro, L.; Sajjadi, S. H.; Rabbani, Y.; Ristaniemi, A.; Mdingi, V.; Peez, C.; Tognato, R.; Serra, T.; Cattaneo, S.; Grad, S.; Boghossian, A. A.; Basoli, V. Single-Walled Carbon Nanotube Biosensor for Real-Time Monitoring of Nitric Oxide in Inflammatory Responses. Biosensors and Bioelectronics 2025, 118092. https://doi.org/10.1016/j.bios.2025.118092.

[26] Seydanur Yücer; Sarac, B.; Fatih Ciftci. Electrochemical Biosensors Based on Carbon Nanotubes (CNTs) Used to Diagnosis Pancreatic and Liver Cancer. Microchemical Journal 2025, 215, 114289–114289. https://doi.org/10.1016/j.microc.2025.114289.

[27] Kobayashi, N.; Izumi, H.; Morimoto, Y. Review of Toxicity Studies of Carbon Nanotubes. Journal of Occupational Health 2017, 59 (5), 394–407. https://doi.org/10.1539/joh.17-0089-ra.

[28] Wisdom, K. S.; Bhat, I. A.; Chanu, T. I.; Kumar, P.; Pathakota, G.-B.; Nayak, S. K.; Walke, P.; Sharma, R. Chitosan Grafting onto Single-Walled Carbon Nanotubes Increased Their Stability and Reduced the Toxicity in Vivo (Catfish) Model. International Journal of Biological Macromolecules 2020, 155 (volume 155), 697–707. https://doi.org/10.1016/j.ijbiomac.2020.03.189.

[29] Pok, S.; Vitale, F.; Eichmann, S. L.; Benavides, O. M.; Pasquali, M.; Jacot, J. G. Biocompatible Carbon Nanotube–Chitosan Scaffold Matching the Electrical Conductivity of the Heart. ACS Nano 2014, 8 (10), 9822–9832. https://doi.org/10.1021/nn503693h.

[30] Qian, L.; Yang, X. Composite Film of Carbon Nanotubes and Chitosan for Preparation of Amperometric Hydrogen Peroxide Biosensor. Talanta 2005, 68 (3), 721–727. https://doi.org/10.1016/j.talanta.2005.05.030.

[31] Lin, J.; He, C.; Zhao, Y.; Zhang, S. One-Step Synthesis of Silver Nanoparticles/Carbon Nanotubes/Chitosan Film and Its Application in Glucose Biosensor. Sensors and Actuators B: Chemical 2009, 137 (2), 768–773. https://doi.org/10.1016/j.snb.2009.01.033.

[32] Mayol, B.; I. Zeina Qubbaj; Nava-Granados, J.; Vasquez, K.; Keene, S. T.; Sempionatto, J. R. Aptamer and Oligonucleotide-Based Biosensors for Health Applications. Biosensors 2025, 15 (5), 277–277. https://doi.org/10.3390/bios15050277.

[33] Ilic, D.; Djulbegovic, M.; Jung, J. H.; Hwang, E. C.; Zhou, Q.; Cleves, A.; Agoritsas, T.; Dahm, P. Prostate Cancer Screening with Prostate-Specific Antigen (PSA) Test: A Systematic Review and Meta-Analysis. BMJ (Clinical research ed.) 2018, 362 (8168), k3519. https://doi.org/10.1136/bmj.k3519.

[34] Loibl, S.; Gianni, L. HER2-Positive Breast Cancer. The Lancet 2017, 389 (10087), 2415–2429. https://doi.org/10.1016/s0140-6736(16)32417-5.

[35] Duffy, M. J. Carcinoembryonic Antigen as a Marker for Colorectal Cancer: Is It Clinically Useful? Clinical Chemistry 2001, 47 (4), 624–630. https://doi.org/10.1093/clinchem/47.4.624.

[36] Stoltenburg, R.; Reinemann, C.; Strehlitz, B. SELEX—a (R)Evolutionary Method to Generate High-Affinity Nucleic Acid Ligands. Biomolecular Engineering 2007, 24 (4), 381–403. https://doi.org/10.1016/j.bioeng.2007.06.001.

[37] Verhaak, R. G. W. Mutations in Nucleophosmin (NPM1) in Acute Myeloid Leukemia (AML): Association with Other Gene Abnormalities and Previously Established Gene Expression Signatures and Their Favorable Prognostic Significance. Blood 2005, 106 (12), 3747–3754. https://doi.org/10.1182/blood-2005-05-2168.

[38] Yang, C.; Wang, Y.; Ming Hua Ge; Yu Jie Fu; Hao, R.; Islam, K.; Huang, P.; Chen, F.; Sun, J.; De Fei Hong; Hua Naranmandura. Rapid Identification of Specific DNA Aptamers Precisely Targeting CD33 Positive Leukemia Cells through a Paired Cell-Based Approach. Biomaterials Science 2019, 7 (3), 938–950. https://doi.org/10.1039/c8bm01393d.

[39] Hongwu International Ltd's Picture. high quality -OH and -COOH functionalized single walled carbon Nanotubes SWCNT. Hwnanomaterial.com. https://www.hwnanomaterial.com/high-quality-oh-and-cooh-functionalized-single-walled-carbon-nanotubes-swcnt_p388.html (accessed 2025-10-21).

[40] Carson, L.; Kelly-Brown, C.; Stewart, M.; Oki, A.; Regisford, G.; Luo, Z.; Bakhmutov, V. I. Synthesis and Characterization of Chitosan–Carbon Nanotube Composites. Materials Letters 2009, 63 (6-7), 617–620. https://doi.org/10.1016/j.matlet.2008.11.060.

[41] Chen, S.; Bashir, R. Advances in Field-Effect Biosensors towards Point-of-Use: Ultrasensitive, Parallelized, and Reusable. Nanotechnology 2023, 34 (49), 492002–492002. https://doi.org/10.1088/1361-6528/acf3f0.

[42] Basharat, M.; Khan, S. A.; Ud din, N.; Ahmed, D. Immunophenotypic Characterisation of Morphologically Diagnosed Cases of Acute Myeloid Leukaemia (AML). Pakistan Journal of Medical Sciences 2019, 35 (2). https://doi.org/10.12669/pjms.35.2.614.

[43] Chen, Z.; Luo, H.; Amu Gubu; Yu, S.; Zhang, H.; Dai, H.; Zhang, Y.; Zhang, B.; Ma, Y.; Lu, A.; Zhang, G. Chemically Modified Aptamers for Improving Binding Affinity to the Target Proteins via Enhanced Non-Covalent Bonding. Frontiers in Cell and Developmental Biology 2023, 11. https://doi.org/10.3389/fcell.2023.1091809.

[44] Kidanemariam, A.; Cho, S. Metal–Organic-Framework-Based Optical Biosensors: Recent Advances in Pathogen Detection and Environmental Monitoring. Sensors 2025, 25 (16), 5081. https://doi.org/10.3390/s25165081.

[45] Gorgani, L.; Mohammadi, M.; Darzi, G. N.; Raoof, J. B. Electrochemical Aptasensor Based on Bimetallic CuZr-MOF for Ultrasensitive Detection of MiR-21. Sensors and Actuators B: Chemical 2022, 378, 133194. https://doi.org/10.1016/j.snb.2022.133194.