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Real-Time Impedimetric Lab-On-A-Chip Based on ISFET Technology for Oncogene Profiling in Pediatric Leukemia

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Abstract

Ion-sensitive field-effect transistors (ISFETs) are microsensors used to detect various clinical markers, showing great promise in gene diagnosis. These devices are particularly attractive to gene diagnosis due to their real-time evaluation capability, high sensitivity, and miniaturization, enabling lab-on-a-chip (LOC) systems. Addressing the need to rapidly determine genetic markers in pediatric acute lymphoblastic leukemia (ALL), this study presents the development and validation of an ISFET-based diagnostic tool for ALL. A four-channel ISFET microchip allowed simultaneous multi-analysis through a miniaturized impedimetric transduction system. The translocations between chromosomes 12 and 21, forming the oncogene ETV6-RUNX1, and between chromosomes 4 and 11, generating the oncogene MLL-AF4, were detected. Isothermal hybridization tests showed changes in impedance spectra, reflected in the quantification of ΔR_{CT} corresponding to increasing sample concentrations. The LOC ISFET system obtained an LOD of 5.19 pg/µL for the ETV6-RUNX1 oncogene and 8.43 pg/µL for the MLL-AF4 oncogene. Clinical sample analysis was conducted using the standard additional method, enabling the quantification obtained by the LOC ISFET to be comparable to conventional techniques. Furthermore, the developed biodetection system demonstrated specificity against negative control samples and reproducibility with RSD (%) values ranging from 1.1% to 3.2%. Thus, the ETV6-RUNX1 and MLL-AF4 oncogenes were successfully detected using the LOC ISFET system, which exhibits a high bioanalytical standard as an innovative tool for leukemia diagnosis.

Keywords: ISFET; Electrochemical impedance spectroscopy; ETV6-RUNX1; MLL-AF4; Pediatric leukemia; Biosensor.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer, accounting for approximately 25% of cancer diagnoses [1, 2]. One of the most recurrent mutations in ALL is the translocation between chromosomes 12 and 21 (t(12;21)), which generates the ETV6-RUNX1 fusion oncogene. Despite its incidence, the ETV6-RUNX1 oncogene is associated with a favorable prognosis and a well-established therapy [3]. However, there are other translocations in ALL that require attention due to their highly unfavorable prognosis, such as t(4,11), which produces the MLL-AF4 fusion oncogene [4]. This mutation leads to rapid and highly aggressive cancer progression and often requires intensive therapy. Furthermore, t(4;11) occurs in approximately 80% of ALL cases in infants under one year of age [4-6]. Accurate determination of the genetic mutations associated with leukemia is crucial for patient prognosis. Current genetic diagnostic methods,

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such as fluorescence *in situ* hybridization (FISH) and reverse transcription-quantitative polymerase chain reaction (RT-qPCR), are effective in detecting leukemia mutations [7, 8]. However, they often require lengthy experimental protocols and a highly controlled laboratory environment [6, 9]. In contrast, biosensors are promising tools in this regard, as they offer rapid detection with accuracy and low cost in a simple format, meeting the criteria for fast, reliable diagnostics. Lab-on-a-chip (LOCs) devices enable real-time detection of molecular targets without requiring a highly controlled environment [10, 11].

Nevertheless, some limitations delay the widespread use of these tools in clinical diagnostics, such as the challenges of miniaturization and portability without compromising bioanalytical performance. Additionally, the scalability and cost of production remain significant barriers, as producing these sensors at a large scale while maintaining affordability is crucial for widespread adoption [12]. To address these challenges, developing sensors for biomarker detection, efficiently combined with microfabrication techniques, is becoming increasingly common [13]. Ion-Sensitive Field-Effect Transistors (ISFETs) are microdevices capable of detecting ions in biochemical environments with nanometric resolution. The main ISFETs are based on the use of silicon nitride transducers (Si_3N_4), silicon oxide (SiO_2), and titanium oxide (TiO_2) as substrates, which provide sensitivity to ionic variations in microenvironments [14, 15]. This structure has been used in various biomolecule detection systems, including immunosensors [16] and genosensors [17]. Furthermore, ISFETs offer low-cost production compatible with industrial processes already established in semiconductor industries [13, 18]. Other advantages include real-time detection, high specificity, compatibility with microfluidic systems, and automation [15, 19].

The attractive features of ISFETs applied to detecting clinical markers of interest are complemented by the need to create simpler systems that provide real-time information [20]. Additionally, ISFETs can be used for multi-analysis in various environments, evaluating complex samples such as blood and serum, demonstrating advantages as LOC systems. However, to ensure the use of microchips in LOCs, it is also necessary for the transduction and signal processing equipment to be portable and miniaturized. This is increasingly feasible since the primary transduction technique applied in ISFETs is potentiometry, which is highly compatible with portable devices [21]. Despite this, electrochemical impedance spectroscopy (EIS) has provided greater analytical sensitivity and label-free detection. Through EIS, parameters widely used in the analysis, such as circuit components and the evaluation of the electric double layer formed by the ion-sensitive surface and solution, can be obtained [22]. However, for the efficiency of these devices, ionic strength and ion dispersion in the electroanalytical solution impact evaluations with long exposure times. Therefore, the thickness of the electric double layer must be carefully controlled so that changes in the dielectric layer interface can be detected [23]. In this context, the present study introduces the development of a LOC ISFET-based system using silicon nitride for genomic detection of the oncogenes ETV6-RUNX1 and MLL-AF4, which have not yet been reported in the literature. Additionally, the developed system features a miniaturized and portable impedimetric transduction system.

Material and Methods

Material

Millipore Milli-Q nanopure water (resistivity > 18 MΩ cm) was produced by a Millipore Reagent Water System (Molsheim, France). 11-triethoxysilyl undecanal (TESUD, 90%) was purchased from abcr (Karlsruhe, Germany). Pure ethanol (purity 98.0%), Acetone (99%), ethanolamine (Et-NH2), potassium chloride (KCl), sodium chloride (NaCl), sodium hydroxide (NaOH), hydrochloric acid (HCl), tris-(hydroxymethyl) methylamine, magnesium nitrate ($Mg(NO_3)_2$), sodium bicarbonate ($NaHCO_3$) and phosphate buffer saline (PBS) tablets were purchased from Sigma-Aldrich (Lyon, France). ETV6-RUNX1 and MLL-AF4 DNA probes were obtained from Thermo Fisher (São Paulo, Brazil).

ISFET and cleaning process

The ISFET microchips were developed as described by Ben Halima et al., with four channels consisting of gate, source, and drain [24]. For the evaluation of ionic sensitivity and the subsequent steps of the self-assembly of the genosensitive LOC system, the ISFETs were initially immersed in an acetone/ethanol solution and kept in an ultrasonic bath for 30 minutes at room temperature. Afterward, the electrodes were rinsed with deionized water and carefully dried.

Ionic sensibility characterization

To study the ion-selective behavior of the electrodes, buffer solutions with pH values of 3, 5, 7, 9, and 11 were employed. Initially, a buffer solution was prepared with 5 mM of tris-(hydroxymethyl) methylamine and 0.4 M of magnesium nitrate $Mg(NO_3)_2$, following the method described by Vozgirdaite et al. [25]. The pH was adjusted for different pH solutions using 0.1 M HCl and 0.4 M NaOH, monitored with a pHmeter.

Functionalization and immobilization of DNA probe on ISFET Surface

The steps described in Figures 1 and 3a were followed to functionalize the surface of the ISFETs. After cleaning the ISFETs, the second step involved subjecting them to UV

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radiation using the UV/Ozone ProCleanear for 45 minutes. This process allows the elimination of any organic residues from the surface, as well as the formation of hydroxyl groups on the sensor surface [25]. Next, the electrodes were exposed to the formation of the TESUD layer through a desiccator and a vacuum system for 60 minutes. Afterward, the ISFETs were subjected to 100 ºC for 1 hour. Finally, the electrodes were rinsed with ethanol to remove unbound TESUD from the surface and dried under a nitrogen flow. With the dry electrode surface, 20 µL of DNA probe at 40 Pm were dripped and left for immobilization overnight for 16 hours. The immobilization of the DNA probe on the TESUD layer is possible because ssDNA has an amine group at its end. The covalent binding occurs directly between the aldehyde functional groups (CHO) of TESUD and the NH groups [26, 27].After immobilization, the electrodes were gently washed with PBS, followed by incubation with a 1% ethanolamine (ETA) solution for 30 minutes to block remaining binding sites. Finally, the ISFET system was washed with PBS, dried, and electrochemically characterized.

Potentiometric characterization

For the potentiometric technique measurement of ISFETs, the National Instruments equipment PXI-1031 and LabVIEW software were used. The measurements were performed in an electrochemical cell in the presence of PBS (10 mM, pH 7.4), and an Ag/AgCl reference electrode saturated in 0.4M KCl was used. Electrical measurements were conducted by studying the response of the drain current (I_{DS}) vs. drain-to-source voltage (V_{DS}) with three consecutive readings, varying the V_{DS} from 0V to 3V, increment of 100 mV, and a V_{GS} constant and increment of 0.5V. For the I_{DS} vs. V_{GS} relationship study, a constant V_{DS} equal to 1V was used, with V_{GS} ranging from -1 to 3V and an increment of 100 mV. Fourteen consecutive readings were performed for the estimation of the potential threshold (V_T).

Electrochemical impedance spectroscopy characterization

The EIS characterization was performed in the presence of 10 mM PBS. An Ag/AgCl reference electrode saturated in 0.4M KCl and a platinum wire counter electrode were used. The parameters used were V_{DS} 500 mV, V_{GS} = 0 mV with amplitude 100 mV, and frequency range 10-200000 mHz. Measurements were taken until the response stabilized. For this, a miniaturized system was used, which performs electrochemical impedance spectroscopy characterization simultaneously on the four channels of the ISFET (Fig. 4a). A stable response was considered when there were no significant changes in three consecutive readings.

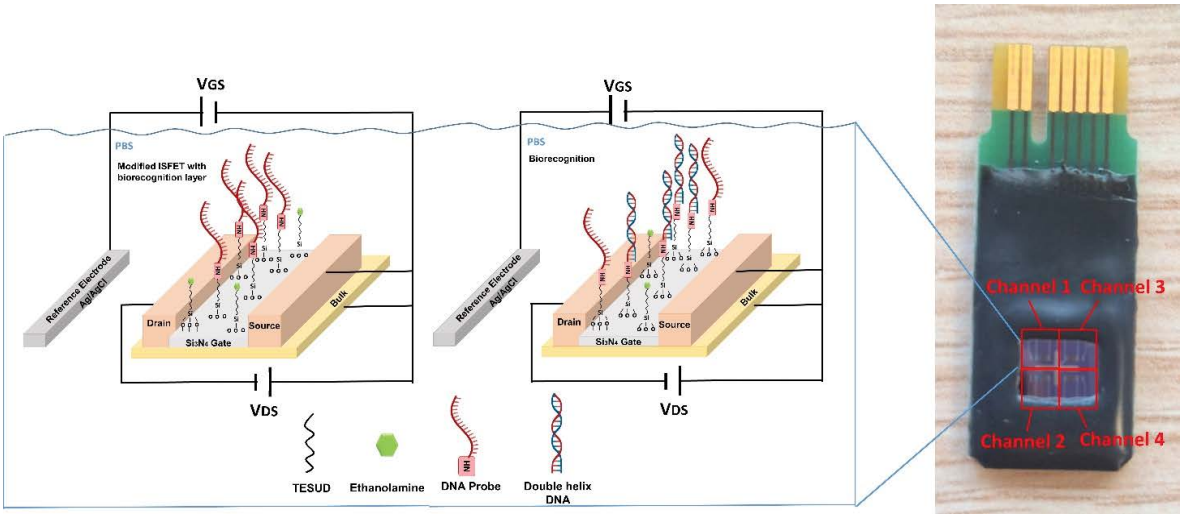

Figure 1: Self-assembly of the LOC ISFET system.

Biorecognition assays

The DNA probes were diluted in 0.01M PBS and used at a concentration of 40 pM. Plasmid samples were obtained using the pCR 2.1 vector with the insertion of the oncogene ETV6-RUNX1 and MLL-AF4 in the sequence. The plasmid samples were diluted in PBS at concentrations of 1, 5, 10, 15, and 20 pg/µL. For the detection tests, the samples were initially subjected to a thermal shock in a thermal bath at ~ 97 ºC for one minute, and quickly, 10 µL were dripped onto the surface of ISFETs modified with the DNA probe. After 20 minutes of isothermal hybridization, the electrodes were rinsed with PBS and dried. Finally, electrochemical characterizations were conducted.

Sample evaluation using the standard addition method

Clinical sample tests were obtained as RT-qPCR products from pediatric patients at the Instituto de Medicina Integral

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Professor Fernando Figueira (Pernambuco, Brazil). For testing by the standard additional method (SAM), patient cDNA samples were diluted into four aliquots, each with a final volume of 80 µL. These aliquots contained 2 µL of clinical sample with an unknown concentration, and 78 µL of PBS for SAM1, and known concentration plasmid samples for the other aliquots (SAM2, SAM3, and SAM4). As a result, the aliquots had plasmid concentrations of 0 pg/µL for SAM1, 5 pg/µL for SAM2, 8.3 pg/µL for SAM3, and 10.7 pg/µL for SAM4. The biorecognition tests followed the same methodology used for plasmid sample evaluation described in section 2.6. The study was approved by the Research Ethics Committee of the Aggeu Magalhães Institute (FIOCRUZ, Pernambuco) with the Certificate of Presentation for Ethical Appreciation (CAAE) n◦ 13296913.3.0000.5190.

Repeatability, reproducibility, and specificity

The repeatability tests were conducted using the percentage value of the standard deviation obtained from three ISFET systems built using the same experimental procedure on different days. The reproducibility calculation was performed considering the relative standard deviation in the biorecognition test at a 15 pg/µL concentration of plasmid samples. The formula used was *RSD (%) = (Standard deviation / Average) × 100%*. Specificity tests were performed through hybridization assays with negative control samples for the target oncogenes of each LOC ISFET. For the ISFET sensitive to t (12;21), translocations 1;19, 4;11, and 17;19 were used at a concentration of 100 pg/µL each. For the ISFET sensitive to t(4;11), translocations 1;19, 12;21, and 17;19 were also used at a concentration of 100 pg/µL.

Results and Discussion

Ionic sensibility assay

Potentiometry: The ionic sensitivity evaluation of the ISFET systems was performed by evaluating the drain current (I_{DS}) vs. drain-to-source voltage (V_{DS}). A decrease in the plateau current was observed with increasing pH (Fig. 2a). This occurs because the Si_3N_4 surface has the ability to interact amphoterically with Si-OH sites and interact with basic sites via Si-NH_2 groups. At acidic pH, protonation of Si-OH to Si-OH_2^+ and Si-NH_2 to Si-NH_3^+ occurs due to the increase in positive charge on the gate surface caused by the high concentration of H^+ in the medium. In basic pH regions, deprotonation of Si-OH groups to Si-O^- occurs, while basic sites are neutralized and remain as Si-NH_2. This combination results in a decrease in drain potential, reducing the current density at the gate [19, 25, 28]. This process is also reflected in the evaluation of I_{DS} vs V_{GS}, where a shift to more positive potentials in I_{DS} and transconductance (G_M) (Fig. 2b) occurred with increasing pH. The signal detection sensitivity was evaluated by obtaining the variation of the threshold voltage (V_T) versus pH variation, according to the equation: S = ΔVT / ΔpH. The different ISFETs used showed an average sensitivity of 68.1 ± 3.4 mV/pH. This response reflects the voltage variation for each unit of change in ionic concentration [24, 29]. Thus, the evaluated systems demonstrated excellent electrical performance as field-effect transistors.

Electrochemical impedance spectroscopy: The EIS characterization of the analytical sensitivity of the ISFET

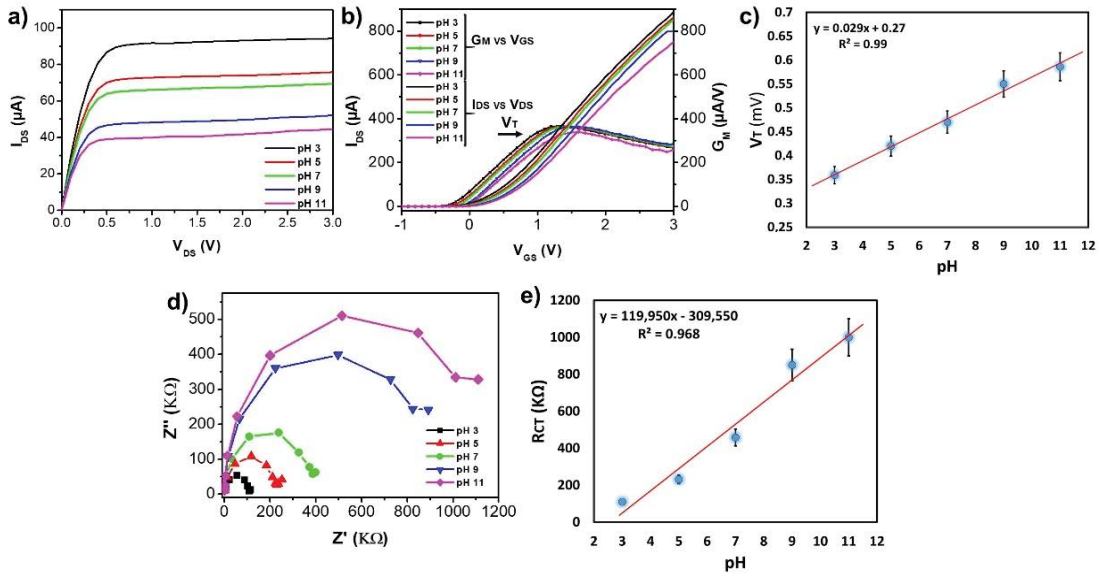

Figure 2: Validation of the ionic sensitivity of the ISFET system in response to solutions with pH 3, 5, 7, and 11. (a) Potentiometric I_{DS} vs. V_{DS} assay, (b) Potentiometric assay for evaluating V_T: I_{DS} vs. V_{GS} and G_M vs. V_{GS}, (c) Linearity study of the response between V_T and pH, (d) Impedimetric assay of the ISFET in solutions with different pH values and (e) Linearity study of the response between R_{CT} and pH.

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was conducted using the same buffers at pH 3, 5, 7, 9, and 11, as in the previous potentiometric assays. An increase in the semicircle on the Nyquist plot was observed with the elevation of pH (Fig. 2d), indicating a change in the system's impedance. This observation is consistent with the expected behavior of ISFETs, where the charge distribution at the gate surface is affected by pH. The equivalent circuit used to model the electrochemical impedance of the ISFET system is shown in Figure 4e, which illustrates the components responsible for the observed changes in resistance. Where the circuit components are defined as follows: R_s: solution resistance, R_{CT}: charge transfer resistance, and CPE: electrical double-layer capacitance. The elevation of the charge transfer resistance (R_{CT}) with increasing pH (R_{CT} = 110.1 ± 4.01 kΩ at pH 3, R_{CT} = 231.5 ± 6.78 kΩ at pH 5, R_{CT} = 388.2 ± 5.72 kΩ at pH 7, R_{CT} = 895.7 ± 9.48 kΩ at pH 9, and R_{CT} = 1000 ± 18.5 kΩ at pH 11) further supports the ISFET's ion sensitivity. As pH increases, occur the deprotonation of Si-OH and the neutralization of Si-NH_2 groups, leading to an increase in R_{CT}. This increase in resistance indicates a reduced flow of charge carriers at the interface, reflecting the change in the ion concentration and the electrical double layer at the sensor surface. This behavior aligns with the results obtained from potentiometric assays. However, the EIS technique demonstrated higher analytical sensitivity as the signal variation across different pH values [19, 20].

Functionalization and immobilization of ISFET Surface

Each functionalization step with TESUD and immobilization of the ETV6-RUNX1 probe on the ISFET surface was characterized by impedance (Fig. S1). It was observed that after cleaning, the ISFET showed a semicircle diameter with an equivalent R_{CT} of 153.3 ± 18.5 MΩ. Upon surface functionalization with a TESUD layer, the R_{CT} increased to 590.9 ± 85.2 MΩ, indicating proper formation [22]. In the third step, with the covalent immobilization of the DNA probe and blocking of remaining sites with 1% ETA, a significant decrease in the impedance response occurred, with R_{CT} values of 513 ± 29.4 MΩ for the MLL-AF4 probe and 397.6 ± 11.1 MΩ for the ETV6-RUNX1 probe. This behavior is related to the anionic profile of DNA, with a globally negative charge, and to the functional –COOH groups of the ethylamine. A difference in amplitude between the ISFETs modified with the probes was observed [30]. This may be related to the size and nucleotide sequence of the probes. The ETV6-RUNX1 probe has 20 nucleotides and 45% guanine-cytosine (GC) content, while the MLL-AF4 probe has 26 nucleotides and 57.7% GC content. This also occurs because DNA sequences with higher CG content have a more compact conformation [31].

AFM and contact angle characterization

After the electrochemical verification of the self-assembly steps on the ISFET system surface, evaluations were performed using Atomic Force Microscopy (AFM) and Contact Angle Measurement (CAM). According to the self-assembled layers described in Figure 3a, a topographic scan of the silicon nitride surface was obtained, with a maximum surface peak of 6.6 nm (Fig. 3b I). After UV/Ozone activation and coating the Si_3N_4 surface with TESUD molecules, a 3.4 nm increase in the surface peak was observed (Fig. 3b II). This slight elevation indicates the formation of a thin, organized TESUD layer [27, 32]. In the third construction step, DNA probe immobilization was performed, resulting in a surface peak of 14 nm, with a more homogeneous topographic profile. In the final modification step, ETA was used to block the remaining sites, which reflected in a 2 nm increase in the surface peak (Fig. 3b III and IV). This slight modification in the surface peak occurs because ETA only intervenes at the sites where TESUD did not form covalent bonds with the DNA probe [33]. The effectiveness of the functionalization strategy was also evaluated through CAM of each self-assembled layer. This technique analyzes the contact angle between the surface and water, allowing the evaluation of the substrate's ability to retain or repel water depending on the surface modification [34]. In Figure 3c I, the contact angle of the bare Si_3N_4 surface (42.18 ± 0.13°) is observed, showing a slightly hydrophobic characteristic due to its silicon composition [20]. After UV/Ozone oxidation, a marked increase in the surface hydrophilicity occurred, making it impossible to obtain a contact angle (Fig. 3c II). The oxidation process generated by UV rays provides hydroxyl groups on the surface, responsible for the high hydrophilicity, resulting in water spreading [27]. In the subsequent TESUD-functionalized layer, the hydrophobic profile of the surface returned, with a contact angle of 88.44 ± 3.54°, due to the hydrocarbon chains oriented on the surface (Fig. 3c III) [19, 20]. After DNA probe immobilization, a slight decrease in the contact angle (37.72 ± 1.29°) was observed (Fig. 3c IV). This can be related to the polyanionic property of DNA, which facilitates interaction with water [31]. With ethanolamine, the CAM was 45.25 ± 0.96°, and this slight increase can be attributed to the functional groups of ETA and the blocking of available surface sites (Fig. 3c V). Thus, the AFM and CAM studies confirm that the functionalization and DNA probe immobilization steps were successful.

Biorecognition Tests

After the process of functionalization and immobilization of the DNA probe onto the ISFET surface, impedimetric characterization of hybridization assays was performed. In the EIS characterization, a decrease in the semicircle diameter was observed with increased plasmid concentration, indicating an inverse relationship between ΔR_{CT} and biorecognition (Figs. 4a and b). This may occur because the impedance assessment process is closely related to forming

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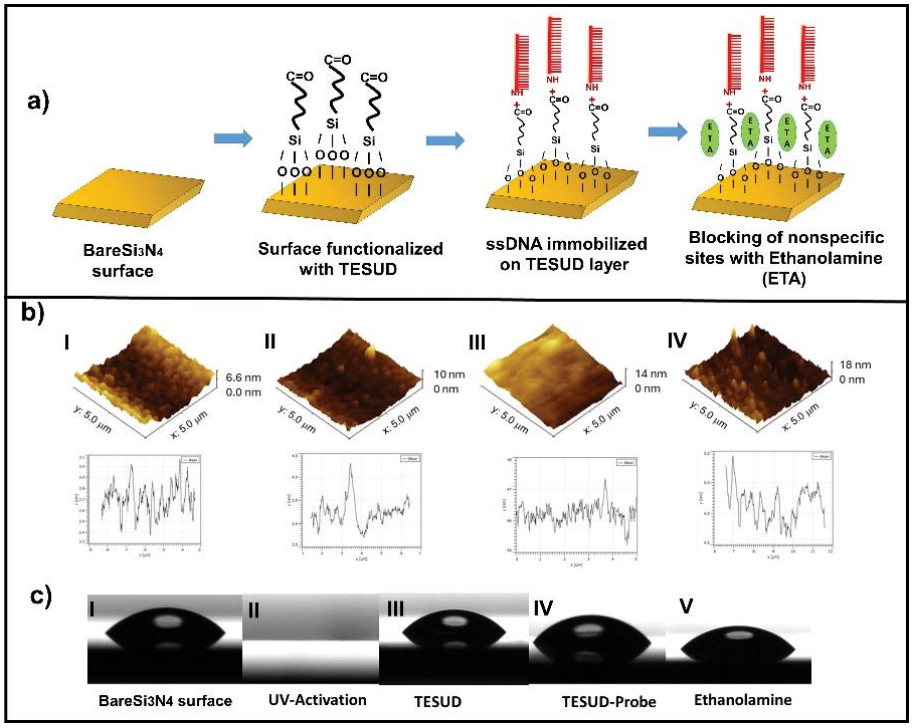

Figure 3: Self-assembly steps of the LOC ISFET system (a), Atomic force microscopy evaluations (b), and contact angle measurements (c) for each construction stage.

the electric double layer at the sensor-liquid interface. During hybridization, H+ protons that are closely bound to the surface can be released. This release occurs when hydrogen bonds form between the hydrogen atoms in the amino and carbonyl groups of the nitrogenous bases of DNA (Ogawa et al., 2019). This process causes an ionic redistribution of charges, and the H+ ions contribute to the protonation of the Si-OH and Si-NH_2 groups. However, this process occurs at a lower detection level than a pH change, which is observed in the lower response amplitude compared to ionic sensitivity evaluation. When evaluated by potentiometry, hybridization detection showed less response distinction due to the lower sensitivity of the technique for such subtle ionic variation processes (Fig. 4f).

Calibration curves for each translocation were obtained (Fig. S2), with y = 0.01 x + 0.082 and R^2 = 0.94 for t (4;11) and y = 0.005x + 0.123 and R^2 = 0.94 for t (12;21). The ISFET LOC for the oncogene MLL-AF4 showed a LOD of 0.367 pg/µL and LOQ of 1.11 pg/µL, while for the oncogene ETV6-RUNX1, a LOD of 0.166 pg/µL and LOQ of 0.5 pg/µL were achieved. In this way, it demonstrates high analytical sensitivity with label-free and isothermal detection within 30 minutes of incubation.

DNA quantification by standard addition method (SAM)

To evaluate the bioanalytical performance of the gene-sensitive ISFET LOC system, samples from patients with ALL (Acute Lymphoblastic Leukemia) positive for the ETV6-RUNX1 and MLL-AF4 oncogenes were analyzed. First, 2 µL of clinical cDNA sample was diluted in 78 µL of solution to reach a final volume of 80 µL. Next, four aliquots (SAM1, SAM2, SAM3, and SAM4) were prepared, each containing a portion of the clinical cDNA sample (of unknown concentration) and a portion of plasmid with final known concentrations of 0, 5, 8.3, and 10.7 pg/µL. During the biorecognition tests, a marked decrease in the semicircle diameter was observed in the impedance spectra for the ETV6-RUNX1 (Fig. 5a) and MLL-AF4 (Fig. 5c) oncogenes, indicating significant biorecognition as in previous tests. As a result, a good linear relationship was achieved between ΔR_{CT} and the SAM sample concentration, reflecting the linear equation y = 0.014 x + 0.588 with R^2 = 0.928 for the ETV6-RUNX1 oncogene (Fig. 5b) and y = 0.007 x + 0.27 with R^2 = 0.995 for the MLL-AF4 oncogene (Fig. 5d). In the SAM method, x = |-b / a| when y = 0, resulting in x = 42.25 pg/µL and x = 38.57 pg/µL. These values reflect the expected concentration for aliquot SAM1. By multiplying these values by the dilution factor, we estimated the actual patient sample concentration at 1.69 ng/µL for the ETV6-RUNX1 oncogene and 1.54 ng/µL for the MLL-AF4 oncogene. To verify this analysis, the C0 samples were pre-quantified using a NanoDrop Thermo Scientific, yielding concentrations of 1.59 ng/µL for t(12;21) and 1.7 ng/µL for t(4;11).

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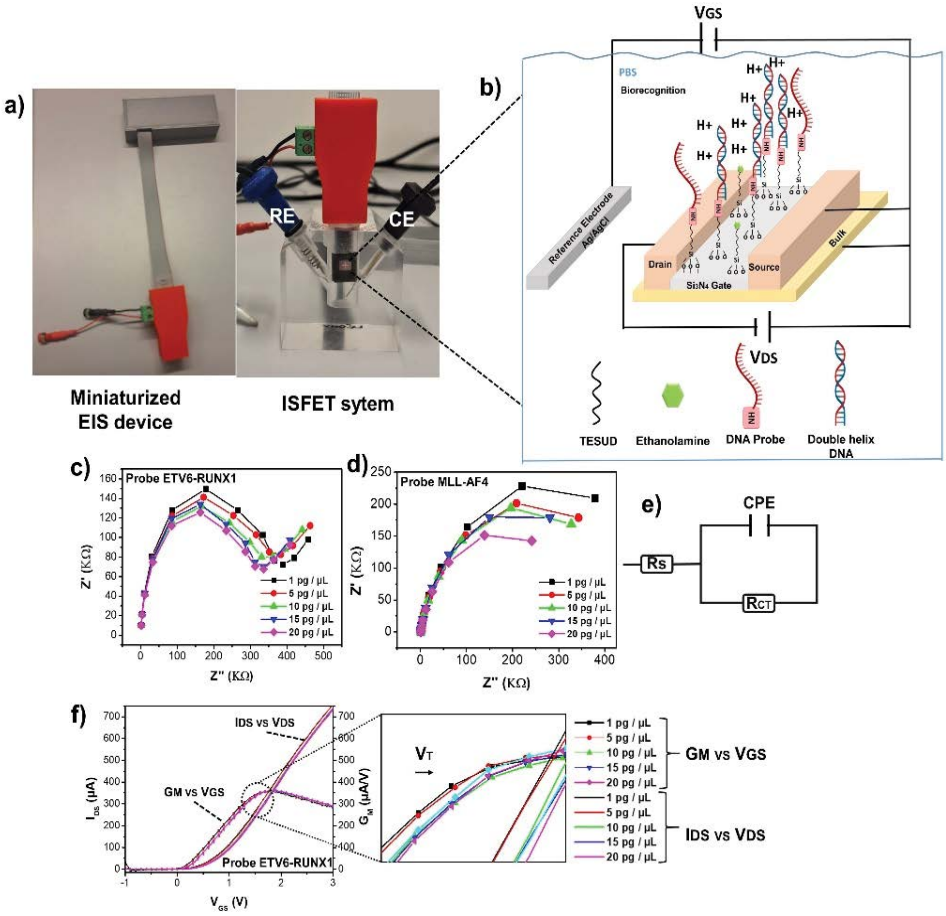

Figure 4: Evaluation of the biorecognition of the gene-sensitive ISFET system. (a) Miniaturized impedimetric transduction system consisting of an electrochemical cell, reference electrode (RE), counter electrode (CE), and ISFET. (b) Representation of the bioelectrochemical detection process through hybridization between the DNA probe and complementary sample. (c) Impedimetric biorecognition assay using plasmid samples with t(12;21) and (d) t (4;11) at concentrations of 1–20 pg/µL. (e) Equivalent circui. (f) Potentiometric evaluation of biorecognition tests with plasmid samples, I_{DS} vs. V_{GS}.

Quantification by the ISFET-LOC showed variations of ± 5.6% and ± 9.4% for the respective translocations compared to the NanoDrop quantification. Thus, the genesensitive LOC developed demonstrated excellent performance in detecting and quantifying the studied oncogenes.

Reproducibility, repeatability and specificity

To evaluate the specificity of biorecognition, the LOC ISFET for each evaluated oncogene was exposed to a solution containing positive plasmid samples for different types of translocations related to ALL. Following the impedimetric characterization, no significant changes in biorecognition were observed in the ΔR_{CT} evaluation (Fig. 6). Moreover, as a general overview of the genosensitive ISFET, a clear distinction was achieved between the ΔR_{CT} impedimetric responses for the negative control, positive plasmid samples, and clinical samples (Fig. 6). This indicates an excellent specificity of the developed LOCs.

Li et al. introduced an ISFET system for monitoring the amplification of the oncogene BCR-ABL1, which is present in cases of chronic myeloid leukemia (CML). Despite its high sensitivity, some disadvantages include lengthy execution time and the multiple required steps [35]. In contrast, the present strategy offers an isothermal oncogene detection system with 30-minute hybridization and ultra-sensitive detection using markers not previously reported in other studies. Furthermore, all EIS measurements were conducted using a fully miniaturized and portable system with reproducibility and repeatability of 1.1% and 5% for the ETV6-RUNX1 LOC ISFET, and 3.2% and 0.8% for the MLL-AF4 LOC ISFET. For RSD evaluations, a value of 5% or lower is considered excellent for high-precision applications [36].

Conclusion

Developing a lab-on-a-chip system based on an ISFET device enabled sensitive detection of target oncogenes in both plasmid and patient samples. Additionally, the hybridization

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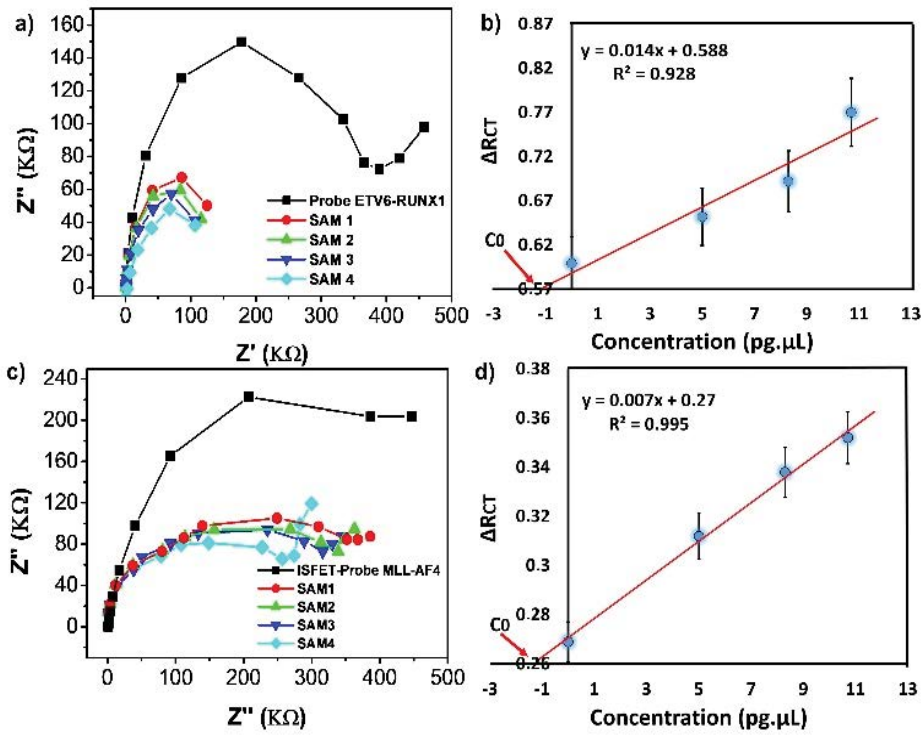

Figure 5: Biorecognition assays of cDNA samples using the standard additional method (SAM): (a) Impedimetric characterization of ETV6-RUNX1, (b) Linear assays of SAM for ETV6-RUNX1, (c) Impedimetric characterization of MLL-AF4, (d) Linear assays of SAM for MLL-AF4.

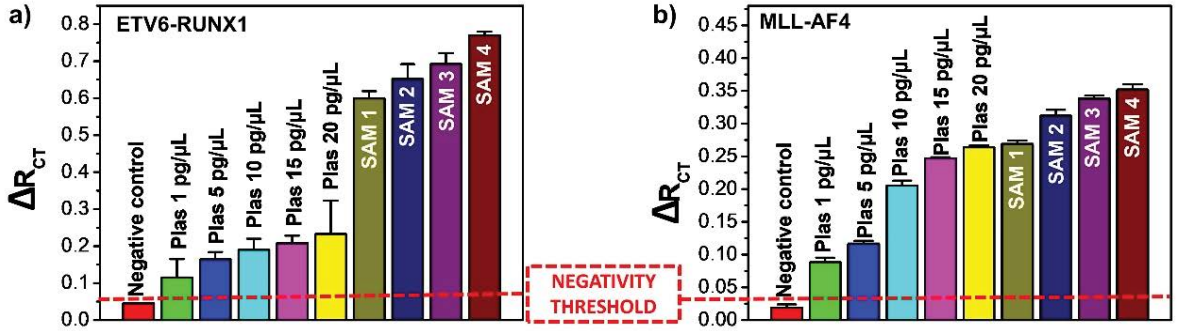

Figure 6: Overview of the ISFET system's biorecognition performance for the ETV6-RUNX1 (a) and MLL-AF4 (b) oncogenes.

process was advantageous, occurring label-free with only 30 minutes of incubation, demonstrating ease of use. Through the ISFET microchip combined with miniaturized impedimetric transduction, simultaneous and real-time multi-analysis of the studied samples was achieved, ensuring satisfactory reproducibility and repeatability. Using EIS as a validation technique for biorecognition studies provided enhanced detection sensitivity. Furthermore, the developed ISFET LOC exhibited excellent DNA quantification capability, comparable to established conventional methods. Thus, it proves to be a promising genetic diagnostic tool that can be used for leukemic oncogene detection in a miniaturized system. To the best of our knowledge, up until the publication of this work, no other biosensors using ISFETs have identified the oncogenes ETV6-RUNX1 and MLL-AF4. This LOC, therefore, introduces a unique and innovative application to the fields of biosensors, ISFETs, and leukemia diagnosis.

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