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DEVELOPMENT OF AN ISFET-BASED SYSTEM FOR GENETIC DETECTION OF LEUKEMIA ONCOGENE

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Summary:

A Point-of-care device was developed using Ion-selective field-effect transistors (ISFETs) for real-time detection of molecular targets, eliminating the requirement for a highly controlled environment. This device successfully detected the translocation between chromosomes 12 and 21 (t(12;21)), which is one of the most common mutations associated with childhood acute lymphoblastic leukemia (ALL).

Keywords: ISFET, leukemia, point-of-care, potenciometry, genosensor.

Title

Development of an isfet-based system for genetic detection of leukemia oncogene.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer, accounting for approximately 25% of cancer diagnoses. Accurate determination of the genetic mutations associated with leukemia is crucial for patient prognosis. One of the most recurrent mutations in ALL is the translocation between chromosomes 12 and 21 (t (12;21)) [1]. Current genetic diagnostic methods, such as fluorescence in situ hybridization (FISH) and reverse transcription quantitative polymerase chain reaction (RT-qPCR), are efficient in detecting these mutations. However, they often require lengthy experimental protocols and a highly controlled laboratory environment [2]. In contrast, point-of-care devices enable real-time detection of molecular targets without the need for a highly controlled environment. Ion-selective field-effect transistors (ISFETs) are miniaturized devices that can be utilized for genetic analysis through the hybridization of complementary DNA sequences [3].

Objective

In this study, we developed an ISFET-based system for the detection of t (12;21) in the pediatric population.

Methods

Therefore, the ISFET system was used for the detection of genetic markers in childhood ALL that had never been studied before in biosensors field (Fig.1a). For this The ISFET was initially characterized using different pH buffers to assess its ionic selectivity. Subsequently, the ISFET was coated with a layer of TESUD, and a specific DNA probe for t (12;21) was immobilized on its surface. The biorecognition process was evaluated through hybridization tests with plasmid samples at various concentrations. Potentiometry was employed to characterize all steps of the process.

Results and Discussion

The ISFET exhibited a decrease in drain-to-source current (I_{DS}) plateau with an increase in

pH (Fig.1b). In addition, the threshold potential (V_T) increased with higher pH levels, indicating the system's sensitivity to ion concentration (Fig.1c) [3].

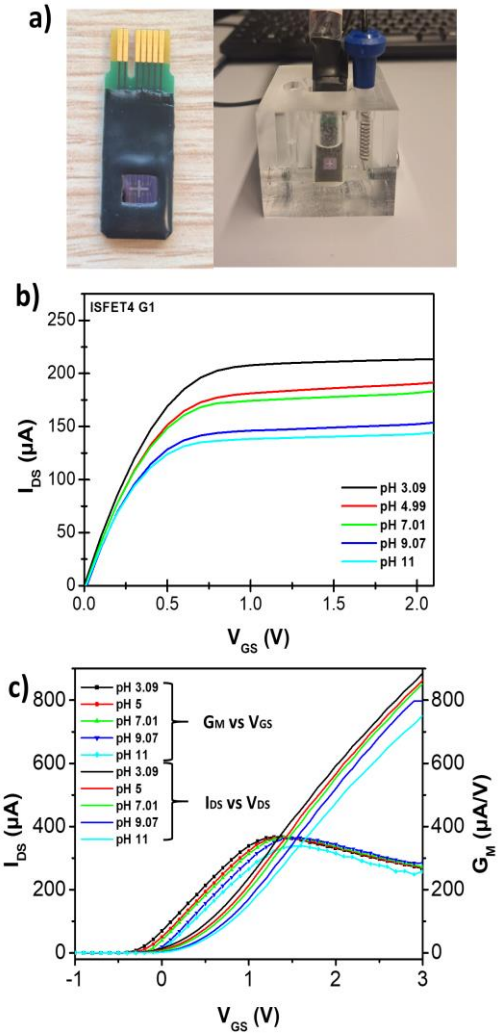

Fig. 1. ISFET system (a) and influence of pH on I_{DS} (b) and V_T (c) ISFETpH 3.09, 4.99, 7.01, 9.07 and 11.

In the hybridization tests, an increase in V_T and were observed with increasing sample concentrations in the same pH buffer, suggesting successful biorecognition through the hybridization process. This occurs because DNA is negatively charged due to phosphate groups in its structure. Thus, the hybridization process of the onto the ISFET increases the fixation of negative charge on the surface, leading to a change in the gate potential [4] In addition to surface charge alteration, the DNA hybridization process can also cause ionic redistribution in the medium [4]. Thus, a good linearity of response was achieved by correlating ΔV_T x Plasmid Concentration, indicating the system's detection sensitivity (Fig.2b).

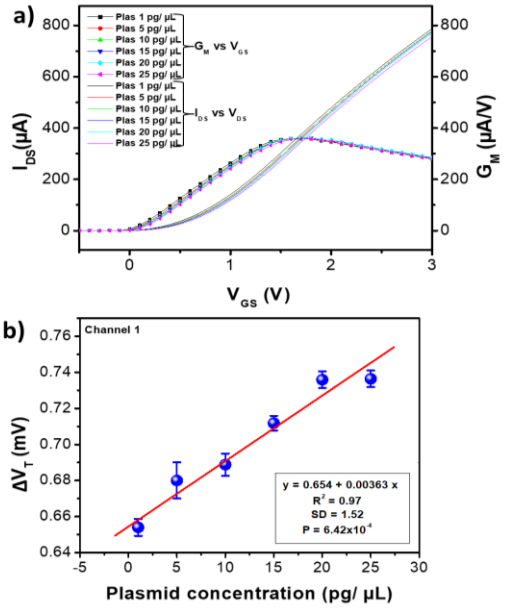

Fig. 2. Influence of hybridization on ISFET (a) and linear plot assays of V_T variation of ISFET (b).

Conclusion

The evaluated ISFET-based systems demonstrated promising performance for genetic detection of leukemic oncogenes, offering potential applications in clinical settings.

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