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# High Polarization $HfO_2$ Sensing on $K^+$ for Inflammasome Cell Detection Application

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**Abstract**

In this study, a novel method was proposed to detect the inflammasome activation from extracellular potassium ion concentration. The sensor platform was designed as the electrolyte insulator semiconductor (EIS) structure with fluorinated $HfO_2$ sensing membrane. The potassium ($K^+$) sensitivity of $HfO_2$-EIS structure is 10.28 mV/pK in the concentration between $10^{-5}$ M and 1 M. For the samples with fluorine ($F^{19+}$) ion implantation, the pK sensitivity can be effectively improved to 56.02 mV/pK. The variation of $K^+$ concentration under treatment with the nigericin was 64.65 mV. It takes the advantage of directly monitoring the alteration of $K^+$ as the measurement indicator of inflammasome activation.

**Key words:** inflammasome, extracellular, potassium, $HfO_2$-EIS, fluorine

## Introduction

Inflammasomes are wellknown as the key regulators of the innate immune response triggered by tissue damage or other microbial stimulator, and the activity of these multi-protein complexes has been linked to common autoinflammatory [1]. After the activation, Interleukin-1β (IL-1β), one of the key proinflammatory cytokines, will be processed proteolyticly into bioacitve form and secreted into extracellular spaces [1]. In addition, potassium efflux has been linked to the activation of inflammasomes as shown in Fig. 1 [2]. In the traditional method, to study the relationship between inflammasome activation and potassium efflux is to block the potassium efflux with increased $K^+$ or pharmacologic inhibitors, and then refering to the inhibition of IL-1β processing and secretion. However, this approach is indirect, complicated, time consuming, and high cost for the measuring system.

In recent years, hafnium oxide ($HfO_2$) with high sensitivity, low drift and compatible process with complementary metal oxide semiconductor (CMOS) technology was proposed as the promising sensing material for pH detection [3].However, for multiple ion sensing application in biomedical region, the $HfO_2$ sensing membrane exhibits low capability due to low sensitivity and selectivity for potassium ion detection.

Although the ion-implantation technique was already proposed for $K^+$ ion detection, it seems that the procedure is not suitable for $HfO_2$ membrane which with high potential for advanced CMOS technology integration and sensor applications.In this paper, the potassium ion-sensitive membranes was fabricated by $F^{19+}$ implantation on the $HfO_2$ sensing membrane, which was based on an electrolyte-insulator-semiconductor (EIS) structure [4]. In addition, the alteration of $K^+$ as the measurement indicator will be investigated through the EIS structure under the treatment with NLRP3 inflammasome stimulator (nigericin).

## Experimental

a. Fabrication of EIS device

In order to investigate the sensitivity of fluorinated-$HfO_2$ sensing films using to $K^+$ and $H^+$ ions, EIS structures were fabricated. Fig. 2(a) shows the process flow of the EIS structures with fluorinated-$HfO_2$ sensing membrane. In the former case, p-type (100) silicon wafers with the resistivity of 8-12 Ω • cm were used as a starting substrate after standard RCA cleaning. Then, a 500 Å thick $HfO_2$ sensing membrane was deposited directly on a p-type silicon wafer

using reactive radio frequency (r.f.) sputtering from a pure hafnium target (99.9%). The r.f. sputtering system was initially pumped down to $8\times10^{-6}$ torr and the pressure during processing was maintained at $2\times10^{-2}$ torr with the gas flow rate of $O_2$/Ar mixture as 5/20 sccm. The r.f. power was set 150 Watts. After sputtering with $HfO_2$, the sample was treated with fluorine ($F^{19+}$) implantation with dosages of 1E15/cm$^2$. For concentrating the fluorine atom upon the surface of $HfO_2$ sensing membrane, we set the tilt angle at 60 degree, which is the maximum tilt angle of the implant system. Furthermore, the 3000 Å thick Al film was evaporated on backside of the silicon wafer to form the ohmic contact after native oxide etching. Sensing area of EIS structures were defined by negative-photoresist SU8-2005 (Micro Chem. Inc.). Final, EIS structures were assembled on copper line of printed circuit board (PCB) by silver gel to form conductive line. To package the samples, a hand-made epoxy was used to encapsulate the EIS structure and Cu line of print circuit board.

b. Measurement setup and test solutions

To investigate the potassium ion sensing properties, the 5mM Tris/HCl solution was prepared as buffer electrolyte which the pH value was kept at 8.5. The potassium ion concentrations from $10^{-5}$ M to 1 M in the testing solutions. In order to obtain the stable pK responses, all EIS samples were immersed in the 5mM Tris/HCl for 12 h before measurement. To extract the potassium ion sensing properties, the capacitance-voltage (C-V) curves for EIS structures were measured with gate bias through an Ag/AgCl reference electrode by using HP4284A high precision LCR meter. The ac signal frequency was chosen at 100Hz for all EIS samples. The responsive voltages for all EIS samples were calculated with 0.6 $C_{max}$. All measurement setup were carried out in a Faraday cage at room temperature to keep light and noise interference as shown in Fig. 2(b).

c. Cell culture & drug

THP-1 cells, a human acute monocytic leukemia cell line, (obtained from American Type Culture Collection, ATCC) were cultured in RPMI 1640 complete medium (Invitrogen) at 37$^o$C with 5% $CO_2$ in a humidified incubator. THP-1 derived macrophages, differentiated by Phorbol 12-myristate 13-acetate (Sigma) are replaced RPMI 1640 medium of 2CaNa saline solution (normal physiological salt solution) and treated with the inflammasome stimulator, nigericin (Sigma) at 1 μM. In order to investigate the IL-1β processing and secretion, THP-1 derived macrophages were pretreated with lipopolysaccharide (1 μg/ml) for 1 hour. Analysis of immunoblotting and the DuoSet ELISA development system kit (R&D Systems) for human IL-1β was used to determine IL-1β levels in the culture media of the inflammasome-activated cells.

## Results and Disscusion

At first, Fig. 3 shows the output response of $HfO_2$ EIS structures without and with fluorine implantation with the $K^+$ concentration from $10^{-5}$ M to 1 M. For the EIS structure with fluorinated-$HfO_2$ sensing membrane, the sensitivitiy is 56.02 mV/pK. Without fluorinated-$HfO_2$ sensing membrane, the sensitivity is 10.28 mV/pK. It suggests that the increases of pK sensitivity according to incorporate F ion on $HfO_2$ surface.

To determine which concentration of nigericin could induce caspase-1 activation and IL-1β secretion, THP-1 macrophages were treated with nigericin at various concentrations for 2 hours. Caspase-1 cleavage and IL-1β releasing, the biomarkers of inflammasome activation, were analyzed by ELISA and immunoblotting.

Fig. 4(a) and 4(b) indicated that the treatment with nigericin at least 0.5 μM could activates caspase-1 and promotes IL-1β release. Afterwards, the cell culture media was obtained and dropped into the sensing window of EIS structure. The normalized C-V curves and the output response for the sample without and with the nigericin at 1 μM in Fig. 5(a). The output voltage contributed from $K^+$ variation is 64.65 mV in Fig. 5(b), which shows the high potential to apply for the detection of inflammasome activation.

## Conclusion

In this article, a pK-sensor was successfully developed based on $HfO_2$ membrane with F implantation. Based on the fluorine incorporation, the potassium sensitivity of $HfO_2$ EIS structure increased from 10.28 mV/pK to 56.02 mV/pK. In addition, the sensor was used on monitoring the inflammasome activation, the output voltage for the nigericin at 1 μM is 64.65 mV.

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## References

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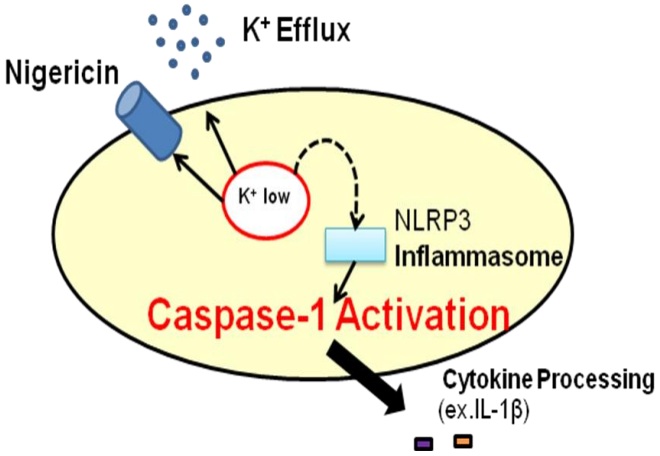



*Fig. 1. Model of NLRP3 inflammasome activation.*

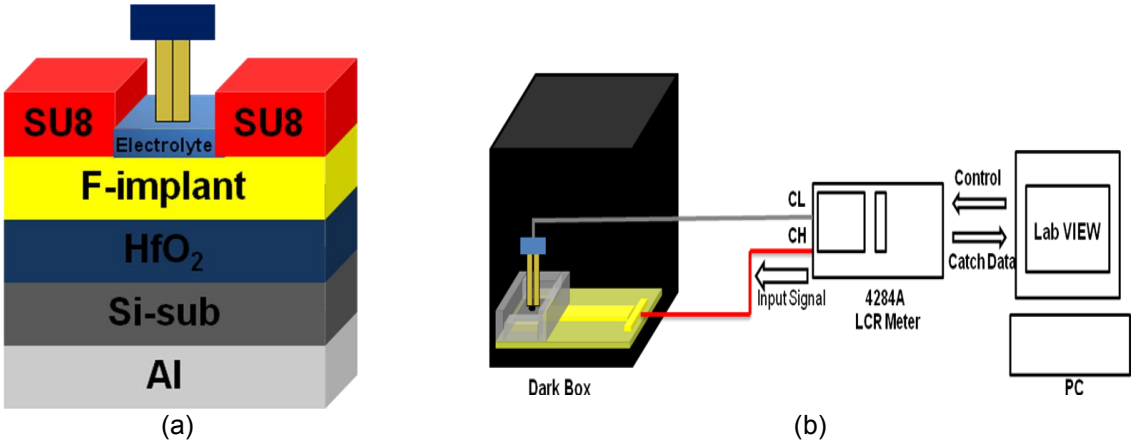



*Fig. 2. (a) Cross-sectional view and process flow of EIS structure. (b) Setup of EIS measurement.*

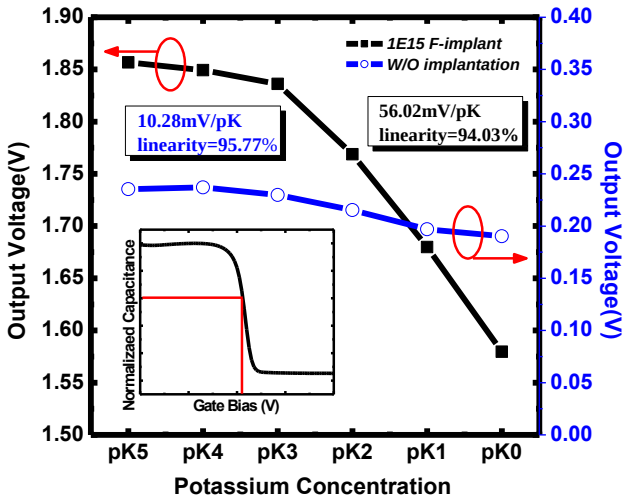
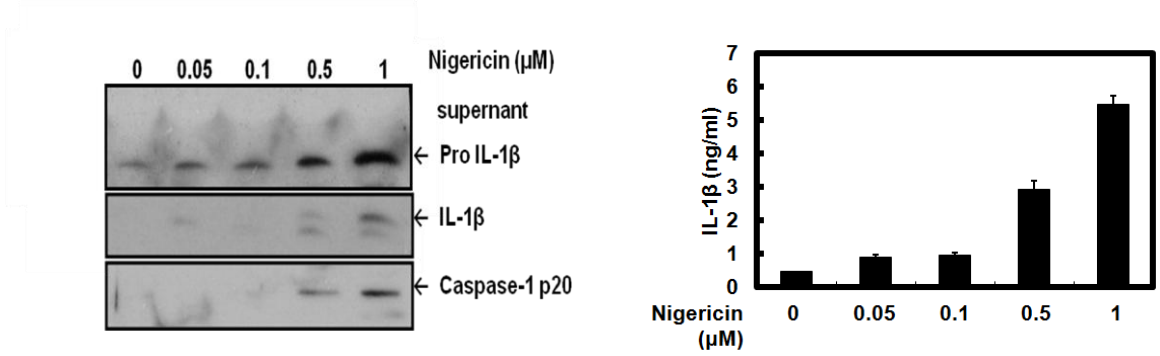



*Fig. 3. pK-sensing responses of $HfO_2$ EIS structure with and without fluorine implantation.*




(a) (b)

*Fig. 4. Nigericin, infalmmasome stimulator, activates caspase-1 and induces IL-1β secretion in macrophages. (a) Ca activation and IL-1β production were observed by immunoblotting. (b) ELISA.*

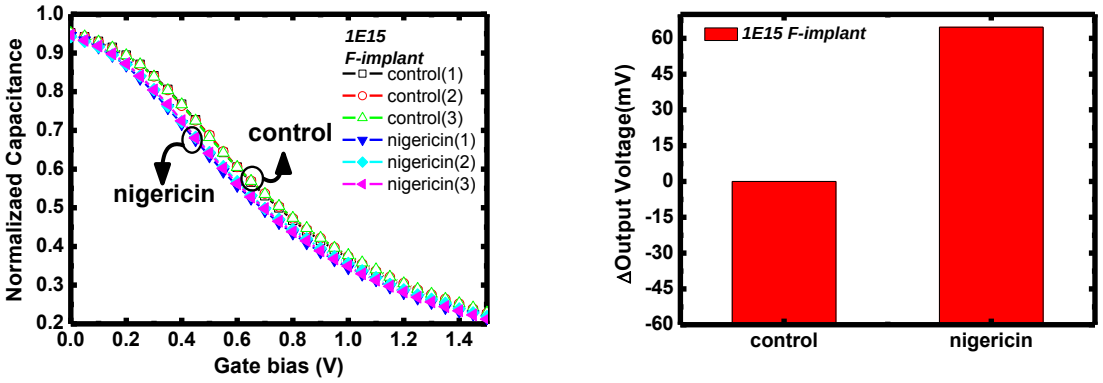



*Fig. 5. Normalized C-V curves for the sample without and with the nigericin. (b) The variation of $K^+$ concentration under treatment with the nigericin was 64.65 mV.*