Immobilization of enzyme and antibody on ALD-HfO$_2$-EIS structure by NH$_3$ plasma treatment

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Abstract

Thin hafnium oxide layers deposited by an atomic layer deposition system were investigated as the sensing membrane of the electrolyte-insulator-semiconductor structure. Moreover, a post-remote NH$_3$ plasma treatment was proposed to replace the complicated silanization procedure for enzyme immobilization. Compared to conventional methods using chemical procedures, remote NH$_3$ plasma treatment reduces the processing steps and time. The results exhibited that urea and antigen can be successfully detected, which indicated that the immobilization process is correct.

Keywords: remote plasma, silanization procedure, surface functionalization

Introduction

The variation of human body fluid in tiny concentrations can be critical for clinical diagnosis. Therefore, the detection of chemical and biological species through microelectronic sensor devices has attracted great attention over the past decade. Ion-sensitive field-effect transistors [ISFETs] are one of the silicon-based potential metric sensors with the advantages of compatibility and integration with advanced complementary metal-oxide-semiconductor processes and cost reduction. Until now, plenty of high-$k$ materials have been applied to the sensing membranes of ISFETs, including SiO$_2$, Si$_3$N$_4$ [1,2], Ta$_2$O$_5$ [3-5], Al$_2$O$_3$ [6], TiO$_2$ [7,8], HfO$_2$ [9,10], SnO$_2$ [11], etc. Among numerous proposed high-$k$ materials, hafnium oxide [HfO$_2$], characterized by high pH sensitivity, low drift, low hysteresis, and low body effect, is a promising pH-sensing material [9,12]. In recent years, there are more and more developments on ISFETs such as the chemical field-effect transistor, enzymatic field-effect transistor [EnFET], Bio-FET [13], DNAFET, etc. For the purpose of monitoring the small changes in body fluid during the early stages, an accurate and stable sensor is needed.

As mentioned above, EnFET is one of the sensors for many biomarkers. The earliest report of EnFET was proposed by Caras and Janata in 1980 [14]. Subsequently, many biomarkers have been detected by EnFET, such as penicillin [14], urea [15], glucose [16], creatinine [17], etc. To fabricate the EnFET, a specific enzyme is immobilized on the surface of the sensing membrane of an ISFET. Moreover, to immobilize biomolecules (such as enzymes, antibodies, and probe-DNAs) [18] for monitoring the biomarkers (antigens and target-DNAs), many approaches have been developed, including physical adsorption [19,20], covalent bonding [21], entrapment [22], etc. However, the silanization procedure for producing reactive groups (NH$_2$) on the material is time-consuming and complicated.

In this paper, the pH sensing properties of HfO$_2$ sensing layers with various thicknesses were fabricated by an atomic layer deposition [ALD] system and investigated by an electrolyte-insulator-semiconductor [EIS] structure. The EIS structure is a capacitive sensor in which the changes in surface potential between the electrolyte and the sensing insulator could be measured according to the shift of capacitance-voltage [C-V] curves. Compared to the complex processing of the ISFET, EIS is one of the simplest platforms as an ISFET replacement for the preliminary investigation of the properties of new sensing materials. For the purpose of saving the process time of the bioreactor...
immobilization, HfO$_2$ sensing membranes with post-ammonia [NH$_3$] plasma treatment were used to replace the chemical procedures.

**Experimental process**

The standard buffer solutions from pH 2 to pH 12 for the pH detection were purchased from Merck (Taipei, Taiwan). For the experiment about urea and antigen, all materials were bought from Sigma (St. Louis, MO, USA), including urease, (3-aminopropyl)triethoxysilane, glutaraldehyde [GA], urea, ethanolamine, and bovine serum albumin [BSA]. Anti-BSA is provided from the Biomedical Engineering Center of Chang Gung University. Urea and urease solutions were diluted with a phosphate buffer solution, which has been adjusted to pH 6 and pH 7.4 as a background solution for urea and BSA detection, respectively [15,17].

The EIS structures with an ALD-HfO$_2$ sensing membrane were used in this study (hereafter, the sample is called ALD-HfO$_2$-EIS). After standard RCA clean, thin HfO$_2$ layers with different thicknesses were deposited on p-type Si wafers by an ALD system at 200°C using tetrakis(ethylmethylamino)hafnium as the precursor. H$_2$O vapor served as the oxygen source, and Ar gas was supplied as the purge and carrier gas. The thicknesses of ALD-HfO$_2$ films are 3.5, 5, 7.5, and 10 nm. The ALD system was initially pumped down to $1 \times 10^{-7}$ Torr, and the working pressure was maintained at $5 \times 10^{-1}$ Torr with purified Ar flow of 200 sccm. Next, a 300-nm-thick aluminum (Al) film as the back-side contact was evaporated on the wafer after removing the native oxide. Hereafter, to define the sensing area, a negative photore sist SUB-2005 (MicroChem Corporation, Newton, MA, USA) was used in a standard photolithography process. Finally, the EIS structures were assembled on printed circuit boards with a silver paste (TED PELLA, Inc., Redding, CA, USA) and then encapsulated with epoxy.

In order to avoid the instability from the leakage current, a 50-nm-thick buffered SiO$_2$ layer was thermally grown by dry oxidation before the deposition of HfO$_2$ layers. The remote NH$_3$ plasma was also performed in the ALD system without breaking vacuum. The treatment was produced in Ar (25 sccm) and NH$_3$ (100 sccm) ambient at 200 W for 6 min as shown in Figure 1a.

In order to compare with the samples with plasma treatment, the ALD-HfO$_2$-EIS structures with conventional covalent bonding were used on the HfO$_2$ layer without NH$_3$ plasma treatment as the control samples as shown in Figure 1b[23]. On one hand, for urea detection, the urease powder was mixed with the phosphate buffer in a concentration of 1.5 μg/mL, and the urease was dripped on the open window of EIS before storing the sample at 4°C (in the fridge) overnight. On the other hand, for BSA detection, anti-BSA was immobilized after the sample was immersed in GA. Afterwards, ethanolamine was dripped for blocking. After rinsing the non-immobilized biomolecular by phosphate buffer, the EISs were ready for measurement.

**Results and discussion**

At first, the C-V curves of EIS structures were measured in various standard pH buffer solutions ranging from pH 2 to pH 12. The real pH value was determined using a commercial pH electrode (S120C, Sensorex, Garden Grove, CA, USA) and a pH meter (HTC-201U, HOTEC, Newton, MA, USA) before measurements. The pH sensitivity was calculated from the slope of output voltage, which is obtained at the 0.6 Cmax of the normalized C-V curves. The dependences of the calculated pH sensitivity and linearity of the ALD-HfO$_2$-EIS structures with different thicknesses of HfO$_2$ layers are exhibited in Figure 2a. For the thickness lower than 10 nm, the pH sensitivity is around 40 to 45 mV/pH, and for the 3.5-nm-thick ALD-HfO$_2$-EIS structure, the available pH range is only from pH 4 to pH 12. Figure 2b shows the normalized C-V curves of 3.5-nm-thick ALD-HfO$_2$-EIS structures, which were measured at pH 2 to pH 12. In this case, the C-V curve measured at pH 2 represents an unstable response in the accumulation region. It could be the result of the leakage current due to its flimsy thickness. The pH sensitivity is high enough (54 mV/pH) and stable when the thickness of HfO$_2$ layers is higher than 15 nm. As compared to our previous study, the drift coefficient of the ALD-HfO$_2$-EIS is stable and quite low (< 0.2 mV/h) when the thickness of the ALD-HfO$_2$ film decreases. However, for the sputtered HfO$_2$-EIS, the drift coefficient increases when the thickness of the sputtered HfO$_2$ film decreases [24]. It could be that the thin HfO$_2$ film prepared by ALD was much denser than that deposited by sputtering [25].

Considering the application on biomedical sensors, the stacked structure of 15-nm-thick HfO$_2$/50-nm-thick SiO$_2$/Si EIS was used. After the urease was immobilized on the surface of HfO$_2$ layers with NH$_3$ plasma post-treatment or the conventional silanization method, the HfO$_2$-EIS structures were immersed into the PB solutions with different concentrations of urea. As shown in Figure 3, the output voltage of the HfO$_2$-EIS structure with plasma treatment is similar to the response of the samples with chemical procedures, where the urea sensitivity are 105 ± 15 and 117 ± 9 mV/pUrea, respectively. The sensitivity value was the average value of five results. The C-V curves and the voltage shift in a linear range of these two methods are almost the same. The linearity of the calibration curves for both output
voltages are very high and very suitable for physiological detection [15]. Based on these results, the chemical silanization method for urease immobilization is successfully replaced by remote NH3 plasma treatment, which has advantages of improving process safety, reducing environmental pollution, and lessening the process time. In addition, comparing the two methods, processing time can be reduced by almost 24 h by remote NH3 plasma treatment.

Moreover, the replacement of silanization procedure using NH3 plasma was also performed on the immobilization process of anti-BSA. Figure 4a shows that the response signal of the EIS membrane without any modification is 6.4 mV. The detection responses of chemical silanization and NH3 plasma treatment are 16.8 and 19.9 mV, respectively. The result indicates that the response of remote NH3 plasma is much higher than that of without plasma treatment. The results show that the NH3 plasma treatment is suitable and attractive for bio-sensing application.

**Conclusions**

In this work, we investigated the effect of thickness on the pH sensitivity of thin HfO2 films formed by ALD based on the EIS structure. Moreover, a simple remote NH3 plasma treatment developed on an ALD-HfO2 membrane to replace the complicated silanization procedure for biomolecular immobilization in a covalent bonding method was proposed. Promising results in urea and antigen detections were obtained. They indicated that the remote NH3 plasma treatment is an attractive method to form the NH2 group on the
Figure 2 pH Sensitivity and linearity characteristics and normalized C-V curves. (a) pH sensitivity and linearity characteristics of the ALD-HfO₂-EIS devices with various HfO₂ thicknesses. (b) Normalized C-V curves for the ALD-HfO₂ and 3.5-nm-thick ALD-HfO₂-EIS structure measured at pH 2 to pH 12.

Figure 3 The urea detection of ALD-HfO₂-EIS structure with chemical silanization and NH₃ plasma treatment.
membrane surface, suggesting an excellent potential on bio-sensing application.

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Authors’ contributions
I-SW and T-FL executed the experiments, participated in the data analysis, and drafted and wrote the manuscript. C-EL and C-HH participated in the data analysis and executed the antibody immobilization. DGP provided the method of immobilization. C-MY participated in the data analysis. J-CW, J-SY, Y-SC, CC, and the corresponding author C-SL conceived and guided this study, integrated the analysis, and supplied the materials and instruments in this experiment. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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References
1. Matsuo T, Wise KD: An integrated field-effect electrode for biopotential recording. IEEE Trans Biomed Eng 1974, 21:485.
2. Bousse L, Mostarshed S, van der Schoot B, de Rooij NF: Comparison of the hysteresis of Ta2O5 and Si3N4 pH-sensing insulators. Sens Actuat B 1994, 17:157.
3. Bobrov PV, Tarantov YA, Krause S, Moritz W: Chemical sensitivity of an ISFET with Ta2O5 membrane in strong acid and alkaline solutions. Sens Actuat B 1991, 27:5.
4. Iwon DH, Cho BW, Kim CS, Sohn BK: Effects of heat treatment on Ta2O5 sensing membrane for low drift and high sensitivity pH-ISFET. Sens Actuat B 1996, 34:641.
5. Ito Y: Long-term drift mechanism of Ta2O5 gate pH-ISFETs. Sens Actuat B 2000, 64:152.
6. Chou JC, Weng CY: Sensitivity and hysteresis effect in Al2O3 gate pH-ISFET. Mater Chem Phys 2001, 71:120.
7. Shin PK: The pH-sensing and light-induced drift properties of titanium dioxide thin films deposited by MOCVD. Appl Surf Sci 2003, 214:214.
8. Chou JC, Liao LP: Study on pH at the point of zero charge of TiO2 pH ion-sensitive field effect transistor made by the sputtering method. Thin Solid Film 2005, 476:157.
9. Lai CS, Yang CM, Lu TF: pH sensitivity improvement on 8 nm thick hafnium oxide by post deposition annealing. Electrochem Solid-State Lett 2006, 9:G90.
10. van der Wal PD, Brand D, Mondin G, Jenny S, Jeannest S, Millon C, Roussel H, Dubourdieu C, de Rooij NF: High-k dielectrics for use as ISFET gate oxides. Proceedings of IEEE Sensors 2004: October 24-27 2004; Vienna IEEE, 2004, 677.
11. Chou JC, Wang YF: Preparation and study on the drift and hysteresis properties of the tin oxide gate ISFET by the sol-gel method. Sens Actuat B 2002, 86:58.
12. Lai CS, Lu TF, Yang CM, Lin YC, Pijanowska DG, Jaroszewicz B: Body effect minimization using single layer structure for pH-ISFET applications. Sens Actuat B 2010, 143:494.
13. Shrivani MW, Deen MJ, Selvaganapathy PR: Finite-element modelling of biotransistors. Nanoscale Res Lett 2010, 5:494.
14. Caras S, Janata J: Field effect transistor sensitive to penicillin. Anal Chem 1980, 52:1935.
15. Pijanowska DG, Torbizc W: pH-ISFET based urea biosensor. Sens Actuat B 1997, 52:570.
16. Dzyadevich SV, Korpan YI, Akhipova VN: Application of enzyme field-effect transistors for determination of glucose concentrations in blood serum. Biosens Bioelectron 1999, 14:283.
17. Soldatkin AP, Montorion J, Sant W, Mantelet C, Jaffrezic-Renault N: Creatinine sensitive biosensor based on ISFETs and creatinine deiminase immobilised in BSA membrane. Talanta 2002, 58:351.
18. Ruixin D, Xunling Y, Ke L, Ge B, Menghong W, Shouen C, Bing Y: Effects of metal ions on conductivity and structure of single DNA molecule in different environmental conditions. Nanoscale Res Lett 2010, 5:1431.
19. Temple-Boyer P, Benyahia A, Sant W, Pourciel-Gouzy ML, Launay J, Martinez A: Modeling of urea-ENFETs for haemodialysis applications. Sens Actuat B-Chem 2008, 131:525.
20. Sun LP, Zhang Z, Wang S, Zhang J, Li H, Ren L, Weng J, Zhang QQ: Effect of pH on the interaction of gold nanoparticles with DNA and application in the detection of human p53 gene mutation. Nanoscale Res Lett 2009, 4:216.
21. Dhawan G, Sumana G, Malhotra BD: Recent developments in urea biosensors. Biochem Eng J 2009, 44:42.
22. Prakash O, Puliga S, Upadhyay LSB: Immobilization of watermelon (Citrullus vulgaris) urease in agarose gel for urea estimation. Biotechnol Bioproc E 2007, 12:131.
23. Lue CE, Yu TC, Yang CM, Pijanowska DG, Lai CS: Optimization of urea-EnFET based on Ta2O5 layer with post annealing. Sensors 2011, 11:4571.
24. Lai CS, Yang CM, Lu TF: Thickness effects on pH response of HfO2 sensing dielectric improved by rapid thermal annealing. Jpn J Appl Phys 2006, 45:3807.
25. Lu TF: ISFET with programmable structures and high-k sensing membranes for bio-applications. PhD thesis Chang Gung University, Electronic Engineering Department; 2011.

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