The passivation/modification of AlGaAs/GaAs surfaces by amorphous TiO$_2$ for the bio-sensing use in electrolytes

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Abstract

In order to apply two-dimensional electron-gas field effect transistors (2DEG-FETs) for bio-sensing devices operating in electrolytes, the chemical/electrical properties of TiO$_2$ thin film is investigated as a material for the gate oxide of FETs. TiO$_2$ films of thickness 13–15 nm are prepared with sol–gel technique on the gate surfaces of AlGaAs/GaAs 2DEG-FETs, followed by heat treatment at 450°C. The TiO$_2$ surface has nanometer-scale roughness with a peak-to-peak height difference of approximately 10 nm. The TiO$_2$/2DEG-FETs exhibit a good performance for operation in electrolytes in the dark, with a transconductance of 0.20 mA/V. Large and continuous threshold shifts to the negative side of $V_{gs}$ are resulted when the TiO$_2$/2DEG-FETs are operated under light at negative gate biases ($V_{gs} < 0$ V). This reveals that photoexcited holes are accumulated at the interface of TiO$_2$/GaAs by the negative gate biases. The sensitivity for pH and H$_2$O$_2$ is examined for the TiO$_2$/2DEG-FETs, where a sensitivity of 71 mV/pH and a finite response for H$_2$O$_2$ are obtained. Cell culture on TiO$_2$ surfaces is successfully demonstrated, showing a high potential of the TiO$_2$/2DEG-FETs for cell activity sensors.

Keywords: TiO$_2$; Sol–gel technique; GaAs; FETs; Bio-sensors; Cell culture

1. Introduction

Quantitative measurements of living cell activities such as cell adhesion or cell–cell interaction are increasingly important for the developments of tissue/cell engineering [1,2]. Fluorescence dyes are widely used as markers to measure protein concentrations, cell activities, and gene expressions. However, the fluorescence of dyes is excitation-dependent, less quantitative, and decreasing in time course of hours. A long-term sustainable measurement of cell activities is therefore difficult to achieve with the fluorescence markers. Semiconductor-based sensing devices including ion-selective (IS) FETs [3–10] seems to be advantageous for stable and quantitative measurements of the cell activities as well as for the integration to micro-total-analysis system (μ-TAS). Si FETs with rather thicker SiO$_2$ and SiN$_x$ films (100 nm or more) are usually used for the semiconductor chemical-sensing devices. For instance, Lehmann et al. have reported the measurement of cellular respiration and acidification with ISFET based sensors [6]. They measured the pH and oxygen partial pressure of the cell culture media with a single ISFET placed near the cultured cells [6]. Compared with these Si ISFETs with thicker SiO$_2$ or SiN$_x$ gate films, better S/N ratios and higher sensitivities for cell activities can be expected by employing 2DEG-FETs with thinner gate oxide films. Furthermore, cell culture directly on the gate oxide films is desirable for cell activity sensing with quick response and high sensitivity. Thin films of TiO$_2$ are attractive and promising for the gate oxide since they exhibit higher
bio-compatibility [11]. Moreover, artificial peptides that specifically bind to TiO$_2$ surface have been developed recently with phage display method [12,13], which is highly potential as an interface medium between cells and TiO$_2$/FETs with sensing functions built in the peptides. Our long-term aims are to demonstrate the bio-sensors based on TiO$_2$/2DEG-FETs that enable us the long-term, quantitative, and highly sensitive measurement of the activities of cells cultured directly on TiO$_2$ surfaces.

In this report, we present the chemical/electrical properties of TiO$_2$ thin film prepared on the gate surface of AlGaAs/GaAs 2DEG-FETs with sol–gel technique [14,15]. TiO$_2$ films of thickness 13–15 nm are formed on the surface by the spin-coating of titanium $n$-butoxide and heat treatment at 450 °C. The TiO$_2$/2DEG-FETs show a good performance in $V_{gs}$-$I_{ds}$ characteristics in electrolytes (pH 6.9) in the dark. A large threshold shift is found when the devices are operated under light at negative gate biases, revealing that photoinduced carriers are accumulated in the TiO$_2$ film by the negative biases. The sensitivity for pH and H$_2$O$_2$ is examined for the TiO$_2$/2DEG-FETs, and a sensitivity of 71 mV/pH and a finite response for H$_2$O$_2$ are obtained. Cell culture on the TiO$_2$ surfaces is successfully demonstrated. Considerably better cell adhesion is obtained on TiO$_2$ surfaces compared to GaAs surfaces.

2. Experiments

TiO$_2$ thin films were prepared with the sol–gel technique on the surface of AlGaAs/GaAs FETs, by spin-coating of 3 wt% of titanium $n$-butoxide Ti(O(CH$_2$)$_3$CH$_3$)$_4$ in ethanol with 5000 rpm × 20 s. The thickness of as-prepared TiO$_2$ films was approximately 26–30 nm estimated using a spectroscopic ellipsometer (J.A. Woollam Co. Inc., M-2000U1). The films were then baked at 450 °C in air for 30 s for chemical stability. The thickness of the TiO$_2$ film after the bake was approximately 13–15 nm. A change of refractive index was also estimated by the ellipsometric measurements, which revealed that the film density was considerably increased by the heat treatment. No further reduction of film thickness was observed by longer heat treatments more than 30 s. Atomic force microscope (AFM) observation (PNI Corp., Nano-I2) was performed to evaluate the surface structure of the TiO$_2$ films.

AlGaAs/GaAs 2DEG-FETs were fabricated from molecular beam epitaxy (MBE) wafers supplied by Sumitomo Electric Industries Ltd. The epitaxial layers grown on undoped GaAs (001) substrates were 800 nm undoped GaAs layer, 15 nm undoped AlGaAs layer, 40 nm Si-doped AlGaAs layer (Si = 2.0 × 10$^{18}$ cm$^{-3}$), 5 nm undoped AlGaAs layer, and 5 nm Si-doped GaAs top layer (Si = 2.0 × 10$^{18}$ cm$^{-3}$). The sheet carrier density of 6.9 × 10$^{11}$ cm$^{-2}$ and electron mobility of 6400 cm$^{-2}$V$^{-1}$s$^{-1}$ were obtained at room temperature in air. The size of the 2DEG-FETs used in this study was 3 × 11 mm, cut out from the above MBE wafers. The patterning of FET devices was carried out with the conventional photolithography technique using a maskless-exposure system (Sanyo Electric Co. Ltd., MMP-100S) [16] and wet etching. After forming ohmic contacts for drain and source electrodes on the patterned devices, TiO$_2$ thin film was prepared on the entire surface of the devices with the process described above (including the heat treatment). Then the surface of TiO$_2$ film was coated with a photo-resist film (Tokyo Ohka Kogyo Co. Ltd., OFPR-800LB) for water protection. The resist film was partly removed for the gate area with the photolithography in order to contact the TiO$_2$ surface of the gate area to electrolytes for measurements (solution gate [17,18]). Typical dimension of gate width and length were 460 μm and 260 μm, respectively. Relatively large gate area was for cell culture on the gate surface.

For the measurements of TiO$_2$/2DEG-FET performances in electrolytes, standard pH solutions were used with dilution; phthalate solution (50 mM C$_6$H$_4$(COOK)(-COOH), pH 4.0) and phosphate equimolal solution (25 mM KH$_2$PO$_4$ + 25 mM Na$_2$HPO$_4$, pH 6.9). The gate voltage $V_{gs}$ was applied to a reference electrode (Ag/AgCl(KCl), BAS Inc., RE-1C) in the solution. Two source meters (Keithley, 2400) and a personal computer (PC) were employed to supply/measure the voltages ($V_{ds}$ and $V_{gs}$) and currents ($I_{ds}$ and $I_{gs}$) independently. A constant $V_{ds} = 1.0$ V was used in this study, which results in approximately 150–200 μA as the saturation current of $I_{ds}$. Basically the operation of TiO$_2$/2DEG-FETs was conducted in the dark. To see the effect of light, visible (VIS) white room-light of approximately 60 μW/cm$^2$ was used.

3. Results and discussion

The surface image of TiO$_2$/GaAs(001) taken with AFM is shown in Fig. 1. The image represents the surface after the heat treatment, but no structural difference was found before/after the heat treatment. Nanometer-sized structures of 30–60 nm wide and 5–10 nm high were observed (RMS = 1.5 nm). The relatively larger height roughness compared with the thickness of TiO$_2$ (16.7 nm in this case)

![Fig. 1. AFM topographic image of TiO$_2$/GaAs surface after heat treatment. RMS in this image is 1.5 nm. Scan area is 1.0 × 1.0 μm and height 10 nm, as indicated in the figure.](image)
indicates that the TiO$_2$ films prepared were rather porous and not uniform in the scale of nanometer. The nanometer-scale roughness may contribute to higher sensitivities for chemicals since effective area for chemical adsorption is larger than smooth surfaces. Furthermore, cell adhesion will be improved by nanometer-scale roughness since cells can grasp the surface tightly. On the contrary, there is a negative possibility that pinholes may exist on the surface where underlying GaAs may be exposed to ambient air/electrolytes. The crystallinity of the TiO$_2$ films should be amorphous since the films were prepared with the sol–gel technique, but has not been elucidated further.

A good FET performance was obtained for $V_{gs}$–$I_{ds}$ characteristics in electrolytes in the dark. Fig. 2 shows the typical $V_{gs}$–$I_{ds}$ characteristics of the TiO$_2$/2DEG-FETs measured in the pH 6.9 solution in the dark, or under VIS-light (60 $\mu$W/cm$^2$). The curves in Fig. 2 were obtained by cyclic $V_{gs}$ sweep with a speed of 50 mV/s to increase/decrease the $I_{ds}$ between 2.0 and 175 $\mu$A in the dark, or 2.0 and 185 $\mu$A under VIS-light. A constant voltage of 1.0 V was applied for $V_{ds}$. The $I_{gs}$ was less than 50 nA in the dark and 100–300 nA under VIS-light. The $V_{gs}$–$I_{ds}$ characteristic was stable in the dark, although a slight hysteresis probably due to the special movements of ions in the TiO$_2$ gate film was observed. Transconductance ($g_m = \Delta I_{ds}/\Delta V_{gs}$) in the dark was approximately 0.20 mA/V. Under VIS-light, however, a large shift of $V_{gs}$–$I_{ds}$ curve to negative $V_{gs}$ side was resulted (negative threshold shift). This negative threshold shift cannot be attributed to the ion movements in the TiO$_2$ gate film since the shift is too large (the shift continued down to $-20$ V at least) and not hysteresis (does not go back to original position). Ten times higher speed of the negative threshold shift was observed for the constant $V_{gs} < -5$ V (230 mV/min) than that for $V_{gs} = 0$ V (21 mV/min), indicating that the negative $V_{gs}$ enhances the threshold shift considerably. Based on these results, we consider that the negative threshold shift should be attributed to the capture of photoexcited holes at the interface trap states at TiO$_2$/GaAs, i.e., the holes photoexcited in GaAs drift to the interface of TiO$_2$/GaAs due to an electric field generated by applied $V_{gs}$ between the GaAs and TiO$_2$ and are captured at the interface trap levels. Another possibility is that the photoexcited holes in GaAs are injected into the TiO$_2$ film by the electric field. The negatively shifted threshold cannot be recovered even after being kept in the dark for one night in air. This suggests that the capture or injection of photoexcited holes is persistent and not reversible. Similar threshold shift was observed for SiO$_2$ gate film (28 nm) prepared with the sol–gel technique with commercially available source (Tokyo Ohka Kogyo Co., LTD, OCD T-2 Si-59000-SG) and for TiO$_2$ gate film (27 nm) prepared with atomic layer deposition at 150 $^\circ$C using titanium tetraisopropoxide (tetraisopropylorthotitanate, Ti(OPri)$_4$) and pure water [19,20]. The independence of the shift on the oxide film variation indicates that the capture of photoexcited holes at the interface trap levels between the gate oxide film and 2DEG-FET is the probable mechanism for the negative threshold shifts rather than the injection of holes into the gate oxide films.

The TiO$_2$/2DEG-FETs exhibit pH sensitivities as well as the conventional ISFETs. Fig. 3 shows the temporal $V_{gs}$ change corresponding to the pH change (4.17–6.19) of buffer solution. The pH of the solution was changed stepwise by injecting 400 $\mu$L NaOH solution (44 mM) into 14 ml phthalate solution (diluted to 5.0 mM, continuously stirred) for five times with 4.0 min intervals. The resulted steps of pH were 4.17, 4.69, 5.05, 5.35, 5.68, and 6.17. A constant voltage $V_{ds} = 1.0$ V was applied to the TiO$_2$/2DEG-FET, whereas the $V_{gs}$ was adjusted automatically with PC-control to keep $I_{ds}$ constant (100 $\mu$A). Stepwise stable increases in $V_{gs}$ were obtained for the 1st to 3rd injections, but gradual increase in $V_{gs}$ was observed after the stepwise increase for 4th and 5th injections. The gradual $V_{gs}$ increase becomes larger and continuous after 5th injection accompanying a small increase in $I_{gs}$ (2–4 nA). The pH sensitivity of ISFETs is based on the proton adsorption/desorption.

![Fig. 2. $V_{gs}$ – $I_{ds}$ characteristics of TiO$_2$/2DEG-FETs obtained in pH 6.9 solution in the dark and under VIS-light (60 $\mu$W/cm$^2$). Transconductance in the dark is approximately 0.20 mA/V.](image1)

![Fig. 3. Change of $V_{gs}$ caused by stepwise increase in pH. The pH of the solution was changed stepwise by adding NaOH solution in phthalate solution for five times with 4 min interval (indicated by arrows). $V_{ds}$ and $I_{ds}$ were kept constant, 1.0 V and 100 $\mu$A, respectively. The inset shows the pH dependence of $V_{gs}$.](image2)
from the oxide/nitride surface [21–24]. We consider that the gradual increase in $V_{gs}$ is caused by the release of proton from the inside of the TiO$_2$ film since many dangling bonds are included in the sol–gel prepared oxide films, which can work as proton trapping sites. The dependence of $V_{gs}$ on pH was plotted in the inset in Fig. 3. The $V_{gs}$ depends linearly on pH for 4.17–5.35 with the pH sensitivity of 71 mV/pH. The pH sensitivity is comparable to that of the conventional ISFETs with SiO$_2$, SiN$_x$, or Ta$_2$O$_5$ gate oxides (50–70 mV/pH).

The sensitivity for H$_2$O$_2$ is also important for the cell activity sensing since many enzymes such as glucose-oxidase and glutamate-oxidase produce H$_2$O$_2$ as byproducts. By the similar scheme employed in the pH measurements, we examined the H$_2$O$_2$ sensitivity of TiO$_2$/2DEG-FETs. The H$_2$O$_2$ concentration of pH buffer solution (2.5 mM diluted phosphate equimolar solution, 14 mL, continuously stirred) was changed stepwise from 0 to 30 mM by injecting 100 µl H$_2$O$_2$ solution (3.0%). Temporal changes of $V_{gs}$ and $I_{gs}$ are shown in Fig. 4. The stepwise increases in $V_{gs}$ can be seen in Fig. 4, but it became less and less as injection proceeded. This is reasonable since the response (i.e., membrane potential) should depend logarithmically on the concentration of H$_2$O$_2$. The gradual increase in $V_{gs}$ was observed for the 1st to 3rd H$_2$O$_2$ injections, but it turned to decrease for the 4th and 5th. Small changes less than 5 nA were observed in $I_{gs}$. The H$_2$O$_2$ sensitivity calculated for the stepwise increase of $V_{gs}$ by the 2nd H$_2$O$_2$ injection in Fig. 4 was 17 mV/mM. More examinations are required to elucidate the origin of the H$_2$O$_2$ sensitivity as well as that of the gradual increase/decrease in $V_{gs}$, but this result indicates that the TiO$_2$/2DEG-FETs can be applied for protein sensors when H$_2$O$_2$-producing enzyme is placed on the TiO$_2$ surface. Additionally, it should be noted that no obvious stepwise response was resulted in the similar H$_2$O$_2$ experiment performed with conventional ISFET (BAS Inc., ISFET electrode 001314), but $V_{gs}$ started decreasing after the 1st H$_2$O$_2$ injection with 3.1 mV/min. The sensitivity for H$_2$O$_2$ is thus one of the advantages of TiO$_2$/2DEG-FETs superior to the conventional ISFETs.

Higher sensitivities for cell activities can be expected by placing the target cells directly on the gate surface of the FETs rather than at the side of the gate or outside the FETs. Therefore, the bio-compatibility of TiO$_2$ surfaces is important to achieve higher sensitivities by direct cell culture on the gate oxide surface of the FETs. The cell culture on TiO$_2$ surfaces was examined on TiO$_2$/undope-GaAs(001) samples (8 × 12 mm), prepared with the sol–gel technique and the heat treatment as described previously. Alveolar type-2 epithelial cells of rats immortalized by SV40-large T antigen (T2 cells) [25–27] were used for the examination. The T2 cells were seeded at the density of 1.9 × 10$^5$ cm$^{-2}$ on TiO$_2$/GaAs surfaces and GaAs surfaces (control), without fibronectin (FN) treatment. Dulbecco’s modified Eagle’s medium (DMEM) was employed for the cell culture medium. After the seeding, the cell culture was carried out in 625-µl DMEM without serum (in the 24-well tissue culture plate with a bottom area 2.0 cm$^2$ for each well) in a CO$_2$ incubator at 37 °C. The optical phase-contrast images of TiO$_2$/GaAs and GaAs surfaces with T2 cells after one day incubation are shown in Fig. 5. A considerably higher concentration of living cells was obtained for TiO$_2$/GaAs surfaces compared to that for GaAs surfaces without TiO$_2$ films. The cell density was 22,000 cells/cm$^2$ on TiO$_2$/GaAs surfaces, whereas it was 4300 cells/cm$^2$ on GaAs surfaces. This indicates that the porous surface of TiO$_2$/GaAs is preferable for the adhesion of T2 cells. The T2 cells live on the TiO$_2$/GaAs surface without obvious damages in their shapes and spreadings. The result shows that the direct cell culture on the gate surface of TiO$_2$/2DEG-FETs will be promising for higher sensitivities for cell activity sensing.

4. Conclusion

The chemical/electrical properties of TiO$_2$ thin film prepared on the gate surface of AlGaAs/GaAs 2DEG-FETs with the sol–gel technique have been investigated from the viewpoints of surface roughness, $V_{gs}$–$I_{ds}$ characteristics in electrolytes, pH and H$_2$O$_2$ sensitivity, and cell affinity. The TiO$_2$/2DEG-FETs show a good performance in $V_{gs}$–$I_{ds}$ characteristics in electrolytes (pH 6.9) in the dark with transconductance $g_m = 0.20$ mA/V, although a large threshold shift was found when the devices were operated under light at negative gate biases. The mechanism of the negative threshold shifts was attributed to the accumulation of photoinduced carriers at the interface of TiO$_2$/GaAs. The sensitivities of 71 mV/pH and 17 mV/mM were obtained for pH and H$_2$O$_2$, respectively. Direct cell culture on the TiO$_2$ surfaces was successfully demonstrated, which showed a considerably better cell adhesion compared to GaAs surfaces. The results obtained in this study indicate that the TiO$_2$ films prepared with the sol–gel technique is highly potential as the gate material of 2DEG-FETs for the use of cell-activity sensing.

Fig. 4. Change of $V_{gs}$ and $I_{gs}$ caused by the stepwise injection of H$_2$O$_2$ solution into phosphate equimolar solution (pH 6.9) for five times with 4 min interval (indicated by arrows). $V_{ds}$ and $I_{ds}$ were kept constant, 1.0 V and 100 µA, respectively.
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References

[2] See, also recent issues of Tissue Engineering.

Fig. 5. Phase-contrast images of T2 cells cultured for one day on (a) GaAs and (b) TiO$_2$/GaAs. Scale bar shows 100 µm.