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Cell Adhesion Characteristics on Tantalum Pentoxide Gate Insulator for Cultured-Cell-Gate Field-Effect Transistor

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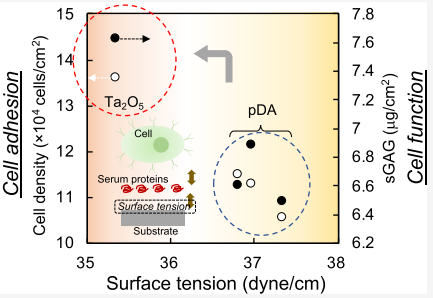
ABSTRACT: Understanding the interaction between living cells and a tantalum pentoxide (Ta_2O_5) gate electrode is important for controlling cell adhesion and functions when developing a cultured-cell-gate field-effect transistor biosensor. In this study, we evaluate the cell adhesion characteristics of the Ta_2O_5 membrane without or with a polydopamine (pDA) coating for chondrocytes, which is expected as a treatment for improving biocompatibility. As a result, the native and pDA-modified Ta_2O_5 membranes are shown to have the appropriate surface tension (35−40 dyn/cm) for the adhesion of chondrocytes owing to the contribution of surface tension to not only the nonspecific adsorption of serum proteins as the scaffold of chondrocytes but also the maintenance of the conformation of serum proteins. In particular, the serum proteins adhere more efficiently to the native Ta_2O_5 membrane than to the pDA-modified ones owing to the relatively smaller surface tension of the native Ta_2O_5 membrane; as a result, the proliferation and production of extracellular matrix (ECM) proteins such as collagen and proteoglycans by chondrocytes are clearly enhanced on the native Ta_2O_5 membrane. Thus, the native Ta_2O_5 membrane shows superior performance for the chondrocyte culture on it compared with the pDA-modified ones.



1. INTRODUCTION

Understanding the interaction between living cells and materials is important for developing biomaterials and biosensors. In general, cells adhere to substrates such as electrodes through the extracellular matrix (ECM) with adhesion proteins (e.g., fibronectin, collagen) in vitro.[1−5] That is, these adhesion proteins, which are mostly included in a culture medium, are first adhered on the substrates and then cells form the attachments between such adhesion proteins and membrane proteins (e.g., integrin), resulting in cell migration. Therefore, the adhesion characteristic of substrate surfaces for the ECM molecules is a key to the induction of cell/substrate interaction.

As one of the surface characteristics of substrates, surface tension is considered, including hydrophobicity and hydrophilicity at the surface depending on the substrate material. In previous studies, it was reported that surface tension of 35−40 dyn/cm is required for the adsorption of the ECM molecules on the substrate with their conformations maintained and for cells to sufficiently adhere to the substrate owing to the specific binding between the ligand molecules at the cell membrane and the ECM molecules.[6−8] Even when the ECM molecules adhered to hydrophobic substrates such as poly(tetra-fluoroethylene) (PTFE), the surface tension of which was lower, cells did not interact with the substrate owing to the change in the conformation and the denaturation of the ECM molecules at the PTFE surface.[8] Therefore, it is important that in order for cells to adhere onto substrates, the conformation of ECM molecules should be maintained by controlling the surface tension of substrates.

The cell/substrate interaction should be considered in the detection of cellular functions and drug effects on cells using biosensors. The solution-gated ion-sensitive field-effect transistor (ISFET) is utilized to continuously monitor cellular respiration in a label-free and noninvasive manner.[9−13] The cells were cultured on gate insulators such as Ta_2O_5 to monitor the changes in the pH caused by carbon dioxide released from cells owing to cellular respiration. This is because oxide membranes such as Ta_2O_5 that have hydroxy groups at the surfaces in an electrolyte solution show an equilibrium reaction with hydrogen ions, that is, the change in potential at the solution/gate insulator interface is electrically output depending on the pH. Such interfacial potential ideally changes according to the Nernstian response (59.2 mV/pH at 25 °C).[14,15] The Nernst equation is strictly reflected by the coefficient $[\beta/(\beta + 1)]$ with parameter β $(= 2q^2N_SK_a^{1/2}/kTC_{EDL})$, where q is the elementary charge, N_S is the site density of hydroxyl groups at the oxide membrane, K_a is the equilibrium constant between hydrogen ions and hydroxyl

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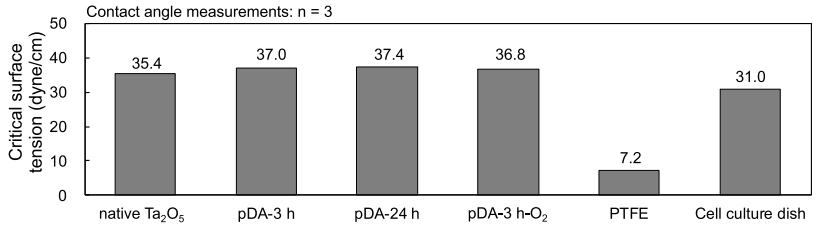

Figure 1. Critical surface tension for different substrates: native Ta_2O_5 membrane, cell culture dish, pDA-3 h membrane, pDA-24 h membrane, pDA-3 h-O_2 membrane, poly(tetra-fluoroethylene) (PTFE) substrate, and cell culture dish. Each critical surface tension at $\cos\theta_c = 1$ was estimated as the surface tension for each substrate from the data shown in Figure S5 according to the Zisman plot. The contact angles were measured at three points ($n = 3$) on each type of substrate.

groups, k is the Boltzmann constant, T is the absolute temperature, and C_{EDL} is the capacitance of the electric double layer.[16,17] The Ta_2O_5-gate ISFET shows better pH sensitivity near the ideal sensitivity owing to the higher density of hydroxy groups at the surface (ca. $10^{15}/cm^2$),[18] which was sufficiently high for $\beta/(\beta + 1)$ to be assumed as 1. The Ta_2O_5 membrane showed no cytotoxicity, which was estimated from the rate of offspring after the transplantation of mouse embryos cultured on the Ta_2O_5 gate insulator to recipient mice.[9] Moreover, the Ta_2O_5 membrane showed long-term stability for cell monitoring for one month with the cellular activities maintained.[13] Thus, the Ta_2O_5 membrane appears to be sufficiently safe as the electrode for the culture of living cells, but the cell adhesion parameters of its surface such as surface tension have not been fully investigated. When investigating them, reference membranes should be used for comparison. As one of the references, polydopamine (pDA), which is easily polymerized by the electrochemical oxidation and self-oxidation of DA monomers,[8,19−22] is an attractive coating material on electrodes. The pDA-coated Au gate FET sensor showed a potentiometric pH responsivity near the ideal Nernstian response because of the equilibrium reaction between catechol and amino groups in the pDA membrane and hydrogen ions depending on the pH.[22] Moreover, the polymerization of pDA on the substrates with low surface tensions such as PTFE, to which cells did not adhere, effectively induced the cell adhesion.[8] Therefore, studying the effect of pDA coating on the Ta_2O_5 surface on cell adhesion may help clarify the cell adhesion characteristics of the Ta_2O_5 membrane.

In this paper, we demonstrate the cell adhesion characteristics of the Ta_2O_5 membrane and compare it with those of the pDA membrane. In particular, the effect of the surface tension of substrates on cell adhesion and migration is discussed as one of the cell adhesion parameters, focusing on whether cell functions are sufficiently expressed on the Ta_2O_5 gate electrode of the ISFET-based biosensor. As a model of living cells, bovine chondrocytes are utilized to estimate the production of ECM proteins that are related to cell functions.

2. RESULTS AND DISCUSSION

2.1. Surface Characteristics of Ta_2O_5 and pDA Membranes. As a reference membrane, the pDA membrane polymerized by self-oxidation on the Ta_2O_5 membrane (Cr/Au/Cr/glass substrate) was prepared in this study. In particular, the self-oxidation time was varied to control the pDA membrane thickness, and the surface roughness of the pDA membrane was also controlled under the O_2 bubbling condition, where the aggregation of DA monomers in a solvent was suppressed; that is, DA monomers were consecutively polymerized on the substrate surface.[23] First, pDA membranes were formed in the solvent for 3 and 24 h under atmospheric conditions, the thicknesses of which were measured to be approximately 21 and 64 nm by atomic force microscopy (AFM), respectively (Supporting Information, Figure S1 and Table S1). The surface roughness based on arithmetical mean height (Sa) was found to increase with the increasing self-oxidation time. Moreover, Sa was improved from approximately 10 to 5 nm by the O_2 bubbling with the same self-oxidation time (4 h), although the thickness was not changed by the O_2 bubbling (ca. 21 nm). Also, the coating of the Ta_2O_5 membrane with pDA was confirmed by X-ray photoelectron spectroscopy (XPS) (Figure S2); the peak intensities for Ta ($4f_{7/2}$) and O (1s) based on Ta_2O_5 largely decreased after the pDA modification, whereas that for C (1s) increased on the basis of pDA components. In particular, the sharp increase in the peak intensity for N (1s) was observed at around 400 eV after the modification, depending on R−NH−R and R−NH_2 of pDA, which was in good agreement with that shown in a previous work.[24] For the native Ta_2O_5 and each pDA-modified Ta_2O_5 electrode, the pH sensitivities were also analyzed via the extended-gate ISFET sensors (Supporting Information, Figure S3 and Table S3). The interfacial potential between the phosphate buffer solution and each membrane was measured with the change in pH from about 6 to 8 using a source−follower circuit (Figure S4). The pH sensitivity of each substrate was within 52−55 mV/pH near the ideal Nernstian response, which exhibited sufficient performance as a pH sensor. Thus, the functional groups at the surface in contact with the solution (hydroxy groups at Ta_2O_5, catechol and amino groups at pDA) showed the equilibrium reaction with hydrogen ions, resulting in the change in the density of surface charges, which depends on the pH. Such functional groups with charges may affect the surface tension, which is related to cell adhesion characteristics. Figure 1 shows the critical surface tensions of the native Ta_2O_5 membrane, pDA-modified Ta_2O_5 membranes, PTFE, and conventional cell culture dish (polystyrene with tissue culture treatment) based on the Zisman plot obtained from the static contact angle (θ) measurement (Supporting Information, Figure S5).[25] The critical surface tension ($\cos\theta_c = 1$) of each type of substrate was evaluated and regarded as the surface tension for each type.[26] Six solvents, namely, water (72.8 dyn/cm), glycerol (64 dyn/cm), ethyleneglycol (47.7 dyn/cm), dimethyl sulfoxide (42.1 dyn/cm), pyridine (38 dyn/cm), and ethanol (22.1 dyn/cm), which were expected to show different surface tensions,[8] were dropped for the measurement at three points ($n = 3$) on each type of substrate. Results showed that PTFE was the most hydrophobic; its critical surface tension was the smallest (7.2 dyn/cm). On the other hand, the other substrates showed

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similar critical surface tensions within 30−40 dyn/cm, which indicated their usefulness for the adsorption of ECM molecules with their conformations maintained.[6−8] In particular, the critical surface tension was slightly smaller for the native Ta_2O_5 membrane than that for the pDA-coated Ta_2O_5 membranes, regardless of the thickness and surface roughness of the pDA membranes, within 35−40 dyn/cm.[8] Considering the surface tensions of these membranes, the adsorption of serum proteins [fetal bovine serum (FBS)], which were included in the culture medium, with fluorescein isothiocyanate (FITC) onto the substrates was investigated by fluorescence measurement (Figure 2). In other words, the nonspecific adsorption of

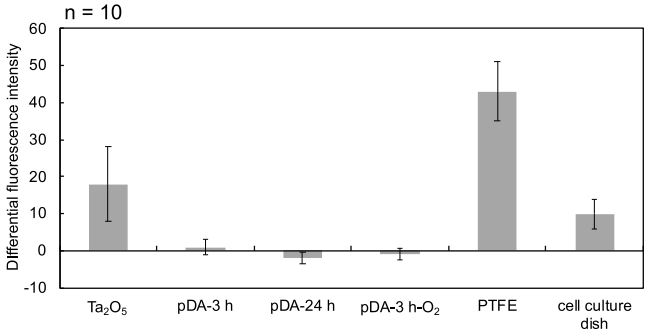

Figure 2. Differential fluorescence intensity of serum proteins with FITC on different substrates: native Ta_2O_5 membrane, cell culture dish, pDA-3 h membrane, pDA-24 h membrane, pDA-3 h-O_2 membrane, poly(tetra-fluoroethylene) (PTFE) substrate, and cell culture dish. The fluorescence intensities of the pDA membranes with the serum treatments were originally as low as those without serum treatments; therefore, the negative differences (pDA-24 h and pDA-3 h-O_2) may be regarded as almost zero.

ECM proteins such as fibronectin onto these substrates was qualitatively investigated. In this experiment, each substrate was immersed in the culture medium with the serum proteins (serum treatment) and then washed with phosphate buffer. After that, the FITC molecules dissolved in the mixed solution [phosphate buffer (95 v/v %) and ethanol (5 v/v %)] were allowed to interact with the serum-treated substrates. As the control substrate, only the FITC treatment was carried out for each substrate without the serum treatment. The difference in the fluorescence intensity between the substrates with and without the serum treatments is shown in Figure 2. The most hydrophobic substrate, PTFE, showed significant nonspecific adsorption of serum proteins as expected, but the conformational change of serum proteins at the surface prevented cells from adhering to it because of its smaller surface tension. Indeed, the cells could not be cultured on the PTFE substrate, as shown in a previous study.[8] On the other hand, the native Ta_2O_5 membrane showed the nonspecific adsorption of serum proteins to a greater extent than the pDA-coated membranes, which appeared to reflect the surface tension. That is, such nonspecific adsorption hardly occurred in the pDA-coated membranes as shown by the fluorescence measurement, even if their critical surface tensions were not so markedly different from that of the native Ta_2O_5 membrane. Thus, even a slight difference in the surface tensions would suppress the nonspecific adsorption of serum proteins on the pDA-modified Ta_2O_5 membrane. In addition, the difference in the electrostatic interaction between the serum proteins and each substrate was also assumed because the pDA membranes had different functional groups from those of the Ta_2O_5 membrane. This is because serum for treatment was included in the culture medium of pH 7−8, resulting in the difference in the density of surface charges between the unmodified and modified Ta_2O_5 membranes. Considering the surface charges, some ligands such as sugar chains and proteins at the cell membrane may electrostatically and directly interact with such functional groups at the pDA-modified membrane, resulting in the cell attachment. However, the subsequent cell migration and function appeared to be enhanced by receptors such as the serum proteins at the substrates, depending on the surface tension (see Section 2.2).

2.2. Cell Attachment/Migration and Function on Native and Modified Ta_2O_5 Membranes. Autologous chondrocytes are implanted or transplanted to promote cartilage recovery in the knee by filling cartilage defects with repair tissue.[27] In this case, a patient's own cartilage cells are taken from another part of the body and cultured. When chondrocytes are cultured in vitro, their quality and durability can be evaluated on the basis of the amounts of ECM proteins they produced, such as collagen with hydroxyproline as its major component and proteoglycans containing sulfated glycosaminoglycans (sGAGs).[28,29] Therefore, the amounts of hydroxyproline and sGAGs produced on each substrate were evaluated as the indicators of cell functions. Before the evaluation of ECM products, we determined the density of bovine chondrocytes that adhered to the native and modified Ta_2O_5 membranes for the culture period, as shown in Figure 3.

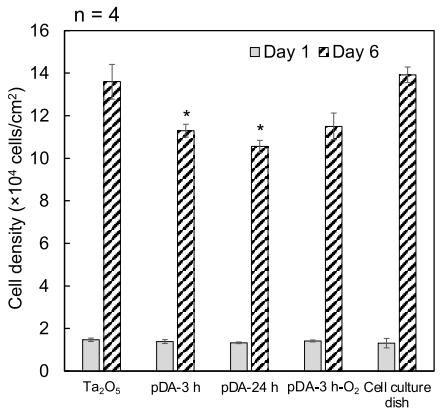

Figure 3. Cell density on different substrates: native Ta_2O_5 membrane, pDA-3 h membrane, pDA-24 h membrane, pDA-3 h-O_2 membrane, and cell culture dish. Four samples for each type of substrate were evaluated with standard error. The asterisk * shows $p < 0.05$ (t-test) for the cell density on the native Ta_2O_5 membrane.

The cell density was almost constant for each substrate on day 1, whereas the cell density for the native Ta_2O_5 membrane was significantly higher than that for the pDA-modified Ta_2O_5 membranes and also the same as that for the conventional cell culture dish on day 6. This may be because chondrocytes proliferated more effectively on the native Ta_2O_5 membrane and the cell culture dish than on the pDA-modified Ta_2O_5 membranes after day 1. That is, cell attachment similarly occurred on each substrate, but subsequently, these cells migrated more smoothly on the native Ta_2O_5 membrane with larger amounts of serum proteins and then proliferated with higher activities. This may also be due to the fact that the pDA membranes had a thin hydrated layer on the relatively hydrophilic surfaces and then the cell migration was slightly

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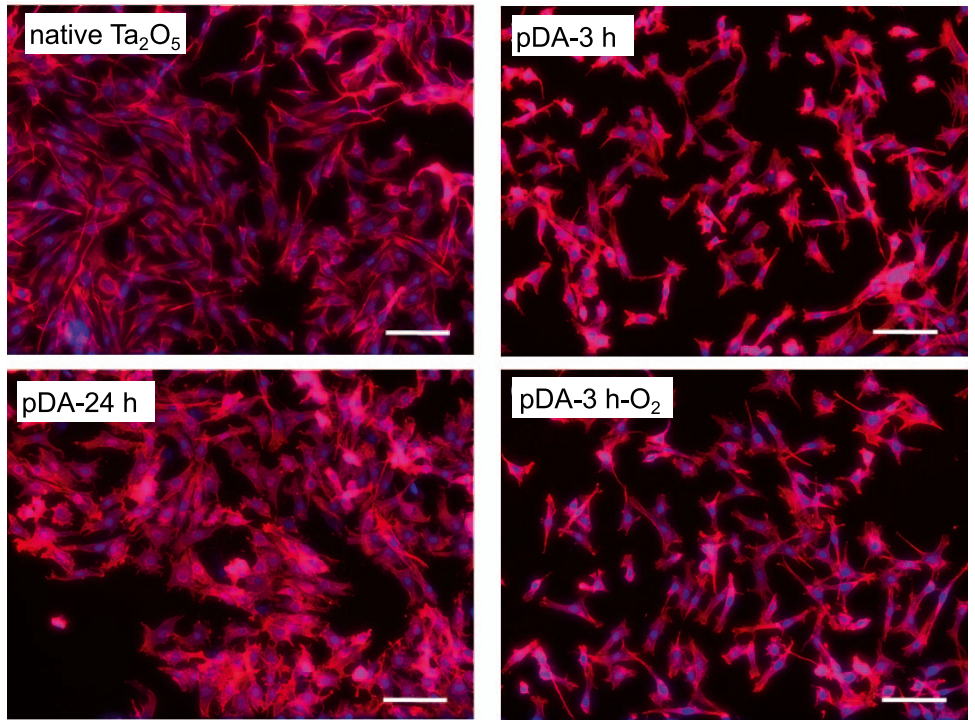

Figure 4. Fluorescence imaging of chondrocytes cultured for 3 days on different substrates: native Ta_2O_5 membrane, pDA-3 h membrane, pDA-24 h membrane, and pDA-3 h-O_2 membrane. The nucleus and actin filament were stained with 4′,6-diamidino-2-phenylindole (DAPI) (blue) and phalloidin (red), respectively, and then observed by fluorescence microscopy. Scale bar shows 100 μm.

interrupted owing to less adsorption of serum proteins. In fact, a larger number of chondrocytes migrated and spread on the native Ta_2O_5 membrane than on the pDA membranes (Figure 4). On the basis of the images shown in Figure 4, the major axis of chondrocytes was analyzed assuming their elliptical shape, as shown in Figure 5. Chondrocytes cultured on the native Ta_2O_5 membrane clearly migrated while extending through the serum proteins as their scaffold, compared with those cultured on the pDA-modified membranes regardless of their thickness and roughness. Thus, the effect of the surface tension on cell migration and proliferation was clearly observed rather than the cell attachment when seeding cells. The native Ta_2O_5 membrane should be a better electrode for cell functional analyses.

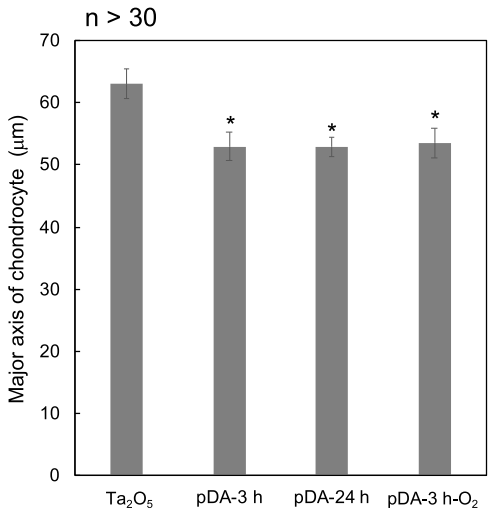

Figure 5. Major axis of chondrocytes cultured for three days on different substrates: native Ta_2O_5 membrane, pDA-3 h membrane, pDA-24 h membrane, and pDA-3 h-O_2 membrane. More than 30 cells for each type of substrate were evaluated with standard error. The asterisk * shows $p < 0.01$ (t-test) for the major axis of chondrocytes on the native Ta_2O_5 membrane.

In addition, the amounts of hydroxyproline and sGAGs produced on the native and modified Ta_2O_5 membranes were investigated in relation to the functions of cultured chondrocytes, as shown in Figure 6. On the whole, relatively larger amounts of both ECM components were produced on the native Ta_2O_5 membrane than on the pDA-modified Ta_2O_5 membranes, although the difference was not statistically significant. This is because a larger number of chondrocytes proliferated and were activated on the native Ta_2O_5 membrane owing to the appropriate surface tension, considering the cell density (Figure 3) and cell migration (Figures 4 and 5). The change in the amount of hydroxyproline means that collagen was produced as one of the components of ECM. That is, a larger amount of collagen had already been produced on the native Ta_2O_5 membrane than on the pDA-modified Ta_2O_5 membrane on day 7, by the activated chondrocytes, whereas the effect of the surface tension on the amount of sGAGs was observed from day 14. This means that sGAGs were gradually maintained inside ECM with collagen produced on the substrates, as reported previously.[13] Thus, the control of surface tension as one of the cell adhesion parameters should contribute to not only the promotion of cell attachment but also the induction of cell proliferation and the subsequent enhancement of cell functions.

2.3. Cell Adhesion Characteristics for Surface Tension. By focusing on the effect of the pDA modification on the Ta_2O_5 membrane, we evaluated the cell adhesion characteristics in terms of the change in the (critical) surface

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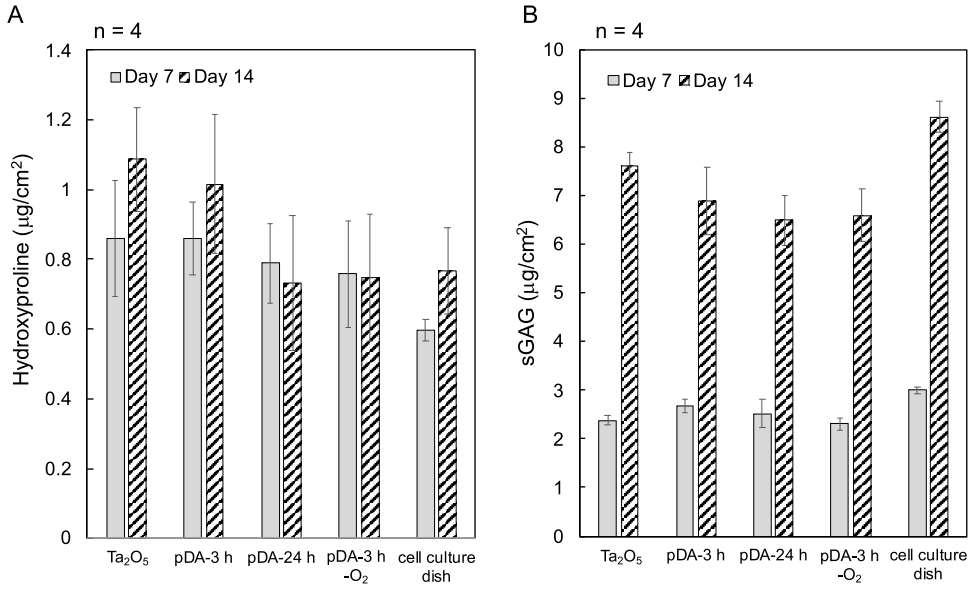

Figure 6. Colorimetric analysis of (A) hydroxyproline or (B) sGAG production on different substrates on days 7 and 14: native Ta_2O_5 membrane, pDA-3 h membrane, pDA-24 h membrane, pDA-3 h-O_2 membrane, and cell culture dish.

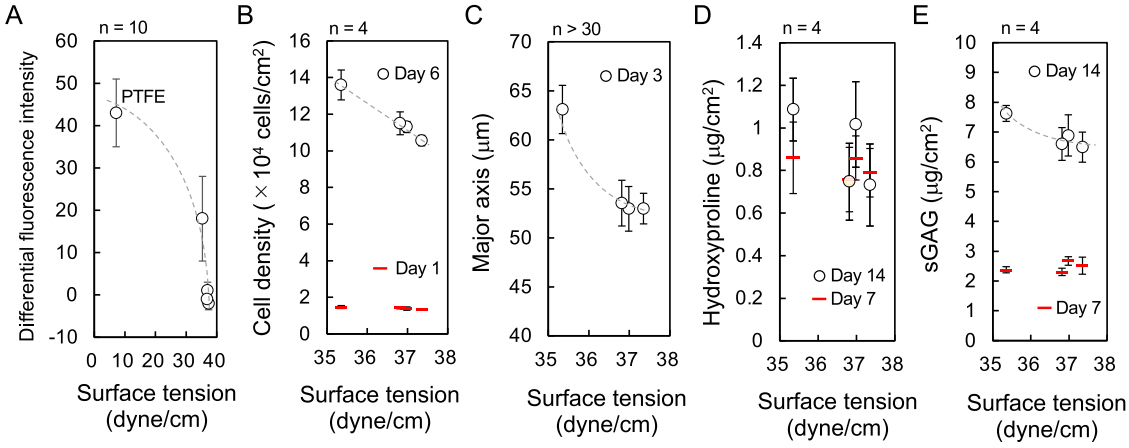

Figure 7. Cell adhesion parameters with change in surface tension: (A) differential fluorescence intensity for nonspecific adsorption of serum proteins, (B) cell density, (C) major axis of extended cells, (D) hydroxyproline production, and (E) sGAG production. These data were based on those shown in Figures 1−6.

tension (Figure 7), considering the above results. The pDA modification on the native Ta_2O_5 membrane slightly increased the surface tension (roughly 35−37 dyn/cm). Although this change seemed to be minimal, the pDA coating prevented serum proteins from being nonspecifically adsorbed on the native Ta_2O_5 membrane (Figure 7A). In other words, the native Ta_2O_5 membrane moderately allowed serum proteins to function as the scaffold of cells to be adsorbed on its surface, different from the PTFE substrate with low surface tension (~7 dyn/cm). This was clarified from the cell adhesion; that is, the cell density and the major axis of extended cells on the substrates decreased with the increasing surface tension (Figure 7B,C). This means that the nonspecific adsorption of serum proteins on the native Ta_2O_5 membrane enhanced cell attachment and migration because these adsorbed proteins would not have also undergone conformational changes on the native Ta_2O_5 membrane, maintaining their molecular structures that interact with ligands at the cell membrane. Thus, the control of surface tension with even a slight difference contributes to the cell adhesion characteristics including cell migration. Moreover, the amounts of hydroxyproline and sGAGs produced on the substrates, which are related to the functions of chondrocytes, decreased with increasing surface tension (Figure 7D,E); that is, such ECM components were more effectively produced on the native Ta_2O_5 membrane than on the pDA membranes. This means that more binding sites such as those in the adsorbed serum proteins enhanced cell adhesion and migration to the substrates; therefore, cell proliferation was promoted to a greater extent and the subsequent cell functions were enhanced more effectively. On the other hand, the hydrophilic effect was clearly found at a surface tension of around 37 dyn/cm or higher because serum proteins were hardly adsorbed on the pDA membranes (Figure 7A). Even when no serum proteins were adsorbed on the substrates, some functional groups and nanostructures would contribute to the interaction of substrates with ligands at the cell membrane, considering the cell adhesion onto the pDA membranes.

The surface tensions measured in this study were different among Ta_2O_5, pDA, PTFE, and cell culture dish depending on

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the surface properties. On the other hand, they were not significantly different among the pDA membranes fabricated under the different conditions despite the fact that their thickness and surface roughness varied. A nanostructured surface of the substrate efficiently induced cell adhesion and proliferation, depending on some parameters such as substrate rigidity and nanostructure dimensions that would contribute to the balance between adhesion energy and deformation energy of the cell membrane.[30,31] However, the nanostructures of the pDA surfaces obtained in this study might not markedly affect the cell adhesion properties and functions (Figure 7), considering that the surfaces showed similar surface tensions. If some nanostructures such as nanopillars can be fabricated on the native Ta_2O_5 membrane, we will be able to examine in the future the effect of the nanostructures of the Ta_2O_5 surface as well as the surface tension in cell adhesion and functions. Moreover, the polymerization degree of pDA membranes fabricated under different conditions did not appear to contribute to the change in the surface tension (Figure 1). The surface tensions for the pDA-3 h and pDA-3 h-O_2 membranes were relatively closer than that for the pDA-24 h membrane. Although the roughness of the pDA membrane surfaces was reduced by polymerization under the O_2 bubbling condition,[23] the thickness of pDA membranes depended on the polymerization time (Table S1). That is, the surface tension of pDA membranes might slightly vary depending on their thicknesses, regardless of their roughness (Figure 1 and Table S1). The larger the thickness of the pDA membrane, the more hydrophilic its surface is owing to the more functional groups in the membrane (i.e., the pDA-24 h membrane). As a result, the cell density for the pDA-24 h membrane was slightly lower than those for the pDA-3 h and pDA-3 h-O_2 membranes (Figures 3 and 7B) owing to the relatively hydrophilic nature of the pDA-24 h membrane. However, the subsequent production of ECM proteins related to cell functions for the pDA membranes did not seem to be affected by their thicknesses, that is, the polymerization time. This may be because a certain number of chondrocytes adhered to the substrates and then the cell−cell interactions induce their functions.

3. CONCLUSIONS

In this study, we showed that the native Ta_2O_5 membrane was sufficiently adequate as the gate electrode of the cultured-chondrocyte-gate ISFET biosensor. In particular, among various living cells, we focused on chondrocytes in our study of the characteristics of cell adhesion to substrates. The pDA-modified Ta_2O_5 membranes as well as the native Ta_2O_5 membrane had sufficient (critical) surface tension of 35−40 dyn/cm. In particular, the surface tension of the native Ta_2O_5 membrane induced the nonspecific adsorption of ECM proteins while maintaining their conformations and contributing to chondrocyte adhesion to the substrates. Moreover, the native Ta_2O_5 membrane with a slightly smaller surface tension resulted in a higher chondrocyte density owing to the larger amount of ECM proteins on it as the chondrocyte adhesion sites than on the pDA-modified Ta_2O_5 membrane. As a result, the native Ta_2O_5 membrane promoted cell proliferation to a greater extent and then the production of ECM proteins related to chondrocyte functions was more effectively activated. However, the nonspecific adsorption of proteins to the electrodes should be prevented when using biosensors for general applications because it would generate electrical noises, which makes it difficult to discriminate the electrical signal of a target biomolecule. Nevertheless, this is not a problem for the cultured-cell-gate ISFET biosensor. This is because the change in pH, that is, the change in the concentration of the smallest ions (hydrogen ions), which pass through nonspecifically adsorbed bio-macromolecules such as proteins to the Ta_2O_5 gate electrode surface, is monitored using the cultured-cell-gate ISFET, as shown in a previous study.[12] On the other hand, the pDA membrane enables not only the prevention of the nonspecific adsorption of interfering proteins but also the easy immobilization of specific probe molecules such as aptamers and antibodies as target biomolecules and the chondrocyte attachment because of its various functional groups.

Furthermore, the effect of surface tension on cell differentiation on various substrates is worth investigating. In particular, the quality of stem cells should be evaluated noninvasively using biosensors with a variety of electrodes in the fields of tissue engineering and so forth. In this study, the chondrocyte adhesion and functions on different substrates were mainly examined because the capability of the Ta_2O_5 gate insulator for ISFET sensors should be first elucidated. In the future, the effect of surface properties such as surface tension on cell differentiation should be examined with different substrates such as Ta_2O_5 and pDA for biosensing.

4. EXPERIMENTAL SECTION

4.1. Chemicals. The following chemicals and materials were used in this study. Sodium dihydrogen phosphate (NaH_2PO_4), sodium hydrogen phosphate (Na_2HPO_4), potassium chloride (KCl), phosphate-buffered saline (PBS), tris(hydroxymethyl)aminomethane (Tris), sodium lactate, sucrose, fluorescein isothiocyanate (FITC), glycerol, ethyleneglycol, dimethyl sulfoxide (DMSO), pyridine, and ethanol were purchased from Wako Pure Chemicals Industries, Ltd. 3-Hydroxytyramine hydrochloride (dopamine; DA) was purchased from Tokyo Chemical Industry Co., Ltd. Phalloidin, Triton X-100, and bovine serum albumin (BSA) were purchased from Sigma Aldrich. Dulbecco's phosphate-buffered saline (DPBS), Dulbecco's modified Eagle's medium (DMEM), and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Thermo Fisher Scientific. 1-Methoxy-5-methylphenazinium methylsulfate (1-methoxy-PMS) and water-soluble tetrazolium salt (WST-1) were purchased from Dojindo.

4.2. Preparation of Substrates. First, Cr was sputtered to 15 nm thickness as an adhesive layer on a glass substrate using a radio-frequency (RF) sputtering system (ULVAC, Inc.). Then, an Au thin film (100 nm thickness) was sputtered on it and then sandwiched with the adhesive layer of Cr, leaving the electrical contact with the gate of FET. To form a native Ta_2O_5 membrane (100 nm thickness), Ta_2O_5 was sputtered on the Cr/Au/Cr electrode. Next, pDA was polymerized on the Ta_2O_5 membrane by the self-oxidation of DA monomers at a concentration of 2 mg/mL in 10 mM Tris buffer (pH 8.5) for 3 and 24 h in air ambient at 25 °C, which were named pDA-3 h and pDA-24 h, respectively. In addition, pDA was polymerized for 3 h while bubbling O_2 under the same condition as described above to control the surface roughness, which was named pDA-3 h-O_2. The surface characteristics of the native and modified Ta_2O_5 membranes were analyzed by AFM (Keysight Technologies) and XPS (JPS-9010MC, JEOL). The pDA film polymerized on the Ta_2O_5 surface was obtained by covering a part of the Ta_2O_5 surface with a poly(dimethylsiloxane) (PDMS) sheet. The covered part was not modified in the subsequent modification processes, and the PDMS sheet was peeled off from the substrate after polymerization. The surface roughness based on arithmetical mean height (Sa) was analyzed by AFM.

4.3. Surface Tension Analysis. The static contact angle (θ) was measured on the native Ta_2O_5 membrane, pDA-3 h membrane, pDA-24 h membrane, pDA-3 h-O_2 membrane, cell culture dish, and

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poly(tetra-fluoroethylene) (PTFE) substrate using a contact angle measuring device (DSA25S, KRÜSS). The conventional cell culture dish used in this study was made by Falcon (353002). To analyze the critical surface tension ($\cos\theta_c = 1$) with the Zisman plot, six types of solvents were dropped for the measurement on each substrate, namely, water, glycerol, ethyleneglycol, dimethyl sulfoxide, pyridine, and ethanol.

4.4. Confirmation of Nonspecific Adsorption of Serum Proteins. The prepared substrates were immersed in DMEM containing 10% FBS, 50 U/mL penicillin, and 50 μg/mL streptomycin in an incubator (37 °C, 5% CO_2) for 1 h. After washing the substrates three times with DPBS, they were immersed in the mixed solution [phosphate buffer (95 v/v %) and ethanol (5 v/v %)] including 100 μM FITC molecules for 1 h at 37 °C. As a control, the substrates interacted with the 100 μM FITC solution without the FBS treatment. After washing the substrates three times with DPBS and then drying them, fluorescence intensities were measured for the sample (with serum proteins) and control (without serum proteins) substrates at 488 nm excitation using a microplate reader (Corona Electric Co., Ltd., SH-9000). The difference in the fluorescence intensity between the sample and control substrates was evaluated for the nonspecific adsorption of serum proteins in the culture medium on the native and pDA-modified Ta_2O_5 membranes.

4.5. Evaluation of pH Sensitivity. The change in the interfacial potential (ΔV_{out}) at the native and pDA-modified Ta_2O_5 electrode/solution interface was measured at various pHs from 6 to 8 using an extended-gate FET. Also, a conventional ISFET with the Ta_2O_5 gate insulator (ISFETCOM Co., Ltd.) was utilized to investigate the pH responsivity. A transparent polycarbonate ring (inner diameter of 10 mm/outer diameter of 12 mm) was encapsulated on the native and pDA-modified Ta_2O_5 electrode (see Section 4.2 on Preparation of Substrates) using an epoxy resin (ZC-203T, Pelnox Ltd., Japan) except for the sensing surface. The native and pDA-modified Ta_2O_5 surfaces were in contact with the measurement solution. The Au (/Cr) surface outside the ring was connected to the gate of a junction FET (Toshiba) as the extended-gate electrode. ΔV_{out} was measured in real time using a source−follower circuit,[32] where the Ag/AgCl reference electrode with the saturated KCl solution (3.3 M), which was connected to a measurement solution through a salt bridge, was maintained at 0 V by connecting to the ground. Electrical measurements were performed at a constant I_D of 700 μA and V_D of 1 V (Figure S4). To investigate the pH sensitivity, phosphate buffer solutions were prepared with pHs from 5.9 to 8.2 by controlling the mixing ratio of Na_2HPO_4 to NaH_2PO_4.

4.6. Quantitative Evaluation of Cell Density and ECM Production by Colorimetry. Bovine chondrocytes were prepared and then preserved under optimized conditions, according to the method (see "Preparation of Bovine Chondrocytes and Culture" in Section 4) in a previous work.[13] After preculturing chondrocytes in a cell culture dish, they were transferred to the native and pDA-modified Ta_2O_5 substrates at a density of 1×10^4 cells/cm^2 and cultured for 1 and 7 days in an incubator (37 °C, 5% CO_2). To count the number of cells and estimate the cell density on the substrates, the amount of lactate dehydrogenase (LDH) in living cells was determined.[20] The substrates with adhering cells were immersed in DPBS containing 0.5 v/v % Triton X-100 to extract LDH by solubilizing the cells (LDH-DPBS). To measure the amount of LDH released, a mixture of 12 mg/mL sodium lactate, 1 mg/mL β-NAD + 100 μg/mL 1-methoxy-PMS, 1 mg/mL WST-1, 1 mg/mL BSA, and 4 mg/mL sucrose was prepared in DPBS, 50 μL of which was mixed with 50 μL of LDH-DPBS. Then, the amount of LDH released was quantified by absorbance measurement (435 nm) of the formazan dye in the microplate reader.

For the ECM analysis, bovine chondrocytes were cultured for 7 and 14 days on the native and pDA-modified Ta_2O_5 substrates. According to the method (see "Quantitative Evaluation of ECM Production by Colorimetry" in Section 4) in a previous work,[13] sGAGs were isolated, the amount of which was measured by the colorimetric method after the pretreatment. Similarly, hydroxyproline was isolated from collagen, the amount of which was measured by the colorimetric method, followed by the pretreatment, according to a previously described method.[13] To observe the cell distribution on different substrates on day 3, the nucleus and actin filament were stained with DAPI and phalloidin, respectively, and then observed by fluorescence microscopy (LSM510; Carl Zeiss Co., Ltd.).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.langmuir.1c01044.

Thickness and surface roughness of polydopamine (pDA) membranes analyzed by AFM (Figure S1 and Table S1); elemental analyses by X-ray photoelectron spectroscopy (Figure S2); pH sensitivities of native and pDA-modified Ta_2O_5 electrodes based on FET measurement (Figure S3 and Table S3); FET real-time measurement (Figure S4); and static contact angle (θ) on different substrates for surface tensions of various solvents (Figure S5) (PDF)

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Notes

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