Cytochrome c assembly on fullerene nanohybrid metal oxide ultrathin films†

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The immobilization of Cyt. c (cytochrome c) on C60 (fullerene) nanohybrid TiO2 (titanium dioxide) gel films assembled with C60, Ti(O–t-Bu)4 and Cyt. c was realized by a surface sol–gel process. Interestingly, C60 was used without any surface modification of the film formation and exhibited good electrochemical performance. Additionally, the surface morphologies of the TiO2 and TiO2/C60 nanocomposite thin films showed remarkable uniformity over a wide area, however, the surface morphology depends on the deposition of Cyt. c, which densely covers the surface with adsorbed Cyt. c molecules. The cyclic voltammetry of the outer-most fullerene films revealed high stability, as well as, a pair of current peaks characteristic of a weak redox system with anodic and cathodic peak potentials at 0.262 V and 0.137 V. The electrochemical properties of thin films can be attributed to the redox system of the C60 layer deposited on the TiO2 gel layer. A pair of well-defined quasi-reversible redox peaks generated by Cyt. c are easily achieved because of the efficient assembly on the C60-modified electrode. In this work, we suggest the efficient introduction of C60 into a TiO2 gel matrix, evaluating the electrochemical characteristics of Cyt. c assembly.

1. Introduction

Nanocarbon materials, such as fullerenes, carbon nanotubes, and graphene have attracted a substantial amount of attention in a wide variety of fields because of their extraordinary thermal conductivity and electrical, optical, and mechanical properties.1–7 For the past decade, abundant research on nanocarbon materials has been conducted, including with regards to their use in nanoelectronic devices,8,9 electrical circuits,10–12 biomedical applications,13–15 energy storage,16,17 nanosensors,18,19 reinforcement materials,20,21 and solar cells.22–25 Since their discovery in 1985,26 fullerenes have been a significant subject of intensive research regarding both their unique chemical properties, which are not exhibited by any other existing compounds, and the practical applications of their highly symmetric structures with diameters of approximately 1.0 nm and abundant π-electron systems.27 Therefore, previous studies on fullerenes and their derivatives have focused on functions that are affiliated with superconductors28,29 and magnetic materials.30 Recent studies have demonstrated their performance as functional materials, including for biological applications, such as DNA-carrying devices31,32 and pharmaceuticals,33 and as fuel cells34,35 and solar cells.36–38

Moreover, fullerenes have the ability to significantly improve electron-transfer reactions and could be used as an electrode material in electrochemical devices.39,40 In particular, incorporating noble fullerenes into electrodes is a promising pathway for efficient electrical communications in bioelectronics systems. Thus, a number of studies have focused developing efficient methods for fullerene modification and their effective incorporation into electrodes.41–44 Chai et al. reported a novel approach for highly sensitive glucose detection using fullerene functionalized spherical Pd@Cys-C60 nanoparticles consisting of L-cysteine and Pd by an in situ spontaneous reduction process. The electrocatalytic activity of a Pd@Cys-C60 nanoparticle-modified electrode facilitated detection with excellent sensitivity and stability and a fast response to glucose.44 Zhang et al. demonstrated that fullerenes can promote electron transfer between hemeproteins and electrodes. They developed C60–MWCNT films as nanocomposite materials to facilitate the direct electron transfer of hemoglobin (Hb) more effectively than bare MWCNT films. They reported...
a faster electron transfer on the C$_{60}$-MWCNT film and
confirmed that the electron mediator and protein docking site
roles are played by C$_{60}$. Hb can transfer electrons to and from
electrodes relatively easily through C$_{60}$-MWCNT nano-
composite films.$^{43}$ Recently, Lanzellotto et al. reported a novel
electrochemical biosensing platform based on nanostructured
materials using gold nanoparticles and fullerenols. Modifying
the electrode enhanced of the electrochemical properties.$^{44}$ The
functionalization of fullerenes can be achieved through various
free-radical or cycloaddition reactions. Thus, chemical reac-
tions involving fullerenes follow essentially original structure
changes, and the $\pi$-electron systems deviate substantially from
the original ones.$^{45}$

Recently, we examined a novel TiO$_2$/fullerene nano-
composite thin film assembled with C$_{60}$ and Ti(O-$^+$Bu)$_4$, which
was fabricated by a surface sol–gel process to prepare ultrathin
metal oxide films. The elemental ratio of C/Ti within the TiO$_2$/
C$_{60}$ film was estimated to be 4.7 by X-ray photoelectron spec-
troscopy (XPS) measurements, indicating that one C$_{60}$ molecule
is surrounded by a matrix of 13 Ti atoms. An important feature
of this approach is that fullerene nanohybrid ultrathin films
could be controlled at nanometer thicknesses and used in
pristine C$_{60}$.46,47

In the present study, we explored a new approach for
immobilizing Cyt. c on the fullerene nanohybrid TiO$_2$ gel film,
which was developed using a nanoassembly approach, to obtain
bioelectrochemical systems of Cyt. c. This approach should
provide a potential pathway for not only creating novel bio-
electrochemical devices but also fabricating the elements of
nanostructure materials.

2. Experimental
2.1 Materials
Titanium(n)-n-butoxide (Ti(O-$^+$Bu)$_4$) was purchased from Acros
Organics (New Jersey, USA). Fullerene (C$_{60}$) was obtained from
Aldrich. Horse heart cytochrome c (Cyt. c) was purchased from
Wako Co. (Tokyo, Japan). Phosphate buffer solution (PBS) was
obtained from Samchum Pure Chemical Co. (Pyeongtaek,
Korea). 10 mM PBS, pH 7.0, was used for the preparation of Cyt.
c electrode, and served for recording of cyclic voltammograms.
All of these chemicals were guaranteed reagents, and used
without further purification. All of the other chemicals that
were used as solvents were of analytical-grade purity and ob-
tained from commercial sources. Pure deionized water
(18.2 M$\Omega$ cm) was obtained by reverse osmosis followed by ion
exchange and filtration (AquaMax$^\text{TM}$-Ultra, Younglin Instru-
ment, Korea).

2.2 Preparation of nanohybrid ultrathin films
Gold-coated quartz crystal microbalance (QCM, 9 MHz manu-
factured by USI System, Japan) electrodes, quartz plates, and
silicon wafers were used as substrates for the preparation of
thin films. Prior to film assembly, a gold-coated QCM elec-
trode was treated with piranha solution (96% sulfuric acid/
30.0–35.5% hydrogen peroxide, 3/1, v/v) and then rinsed with
pure water, washed with ethanol and dried under flowing
nitrogen gas. The electrode was then treated in an ethanol
solution of 2-mercaptoethanol (10 mM) for 12 h, washed with
ethanol and water, and dried under flowing with nitrogen gas.
Thus, the activated electrode contains free hydroxyl groups on
the surface. The QCM frequency was measured with a Hewlett-
Packard 53131 A counter (255 Hz), where a 1 Hz frequency
decrease corresponds to a mass increase of ca. 0.9 ng, according
to the Sauerbrey equation.$^{50}$

In the present work, nanohybrid ultrathin films were
prepared by the surfaces sol–gel process. First, a hydroxylated
QCM electrode was immersed in 100 mM Ti(O-$^+$Bu)$_4$ in toluene/
ethanol (1/1, v/v) for 5 min at 25 °C and washed twice with
ethanol for 1 min to remove the physically adsorbed Ti(O-$^+$Bu)$_4$.
After hydrolysis in deionized water for 1 min and drying under
flowing with nitrogen gas, the TiO$_2$ gel-deposited electrode was
immersed into 1 mM C$_{60}$ solution in toluene for 20 min at 25 °C;
next, we thoroughly removed the physically adsorbed C$_{60}$ with
toluene. By repeating the TiO$_2$ gel deposition procedures, the
outermost surface of the TiO$_2$/C$_{60}$ bilayer QCM electrode was
eventually coated with a TiO$_2$ gel layer. Subsequently, Cyt. c
(10 mM PBS, 1 mg mL$^{-1}$, pH 7.0) was adsorbed on the TiO$_2$/C$_{60}$
nanohybrid layered electrode by immersing for 20 min at 25 °C.
In the QCM measurements, a decrease in frequency, which
indicates the formation of one of the ultrathin layers of TiO$_2$,
C$_{60}$ and Cyt. c, was observed at each of the deposition steps. In
the case of the only-TiO$_2$ gel layer without the adsorption of
fullerene, the deposition procedure was repeated three times,
and then, the film was immersed in Cyt. c solution, rinsed
thoroughly with deionized water and dried with nitrogen gas,
as shown in Fig. 1.

UV-vis measurements were performed using a Lambda
35 spectrophotometer to monitor the stepwise film assembly.
Before the deposition process, a quartz plate was cleaned with
concentrated sulfuric acid (96%), thoroughly washed with pure
deionized water, and subjected to sonication treatment with
1 wt% ethanolic KOH (ethanol/water = 3 : 2, v/v) for 30 min. It
was then washed with ethanol and pure deionized water and
dried under flowing nitrogen gas.

2.3 Surface morphology and contact angle
The surface morphology of the prepared films was investigated
using an atomic force microscope (AFM). AFM measurements
were conducted in non-contact mode using a MicroMash
NSC12/Ti/PT-15 silicon cantilever (curvature tip radius < 40 nm,
tip length 15–20 μm, Spain) on a Scanning Probe Microscope
JSPM-5200 at room temperature. Contact angle measurements
were conducted using a CAM 200 optical contact angle meter
(KSV Instrument, USA). The contact angles described in this
study are static contact angles, which were measured with
the standard sessile drop technique. A water drop was made on
the tip of a syringe and placed on the film surface by moving the
substrate vertically until contact between the water drop and
sample was achieved. The volumes of the water droplets
were maintained at 1 μL, and the image was obtained using a
CCD camera.
2.4 Cyclic voltammetry measurement

A CompactStat electrochemical analyzer (Ivium Technologies, Netherlands) was used for cyclic voltammetry (CV) measurements with a typical three-electrode cell. The reference and counter electrodes consisted of a 3 M NaCl Ag/AgCl electrode and a 0.5 mm diameter platinum (Pt) wire, respectively. The QCM electrode modified with the thin film was used as the working electrode. CV measurements were collected in phosphate buffer solution (10 mM, pH 7), which was thoroughly purged with nitrogen gas before performing measurements.

3. Results and discussion

3.1 Immobilization of Cyt. c on the C₆₀ hybrid TiO₂ gel layer

As shown in Fig. 2, the sequential adsorption of Ti(O-⁻nBu)₄, C₆₀, and Cyt. c result in QCM frequency changes. The change in frequency shown in Fig. 2a represents the change that occurs because of the adsorption of three cycles of TiO₂ and Cyt. c. The QCM frequency decreases linearly as the adsorption cycle increases, indicating consistent growth of the TiO₂ gel multilayer film and efficient immobilization of Cyt. c on the TiO₂ gel layer. The average frequency change by Ti(O-⁻nBu)₄ adsorption was 14 ± 2 Hz, and that by Cyt. c adsorption was 144 ± 18 Hz. In the current setup, a QCM frequency decrease of 1 Hz is estimated to correspond to an increase in the thickness (the unit is angstroms, Å) of 0.273/ρ, where ρ is the density (g cm⁻³) of the adsorbed film. The thickness of the TiO₂ gel layer was measured to be 0.67 ± 0.1 nm based on the frequency 42 ± 6 Hz calculated from the bulk density of the TiO₂ gel (1.7 g cm⁻³). Similarly, the thickness of the Cyt. c layer adsorbed on the TiO₂ gel layer is estimated to be approximately 3 nm when the density of the protein layer was assumed to be approximately 1.3 g cm⁻³. Furthermore, the adsorption density of the Cyt. c can be calculated from the QCM frequency change (Δf = 144 Hz) by the mass of the adsorbed Cyt. c, corrected for the molecular weight (MW: 12 384) and size (2.5 × 2.5 × 3.7 nm³) of the Cyt. c as a globular shape with a diameter of ~3 nm and a surface area of 0.32 cm² for both sides of the QCM electrode. Therefore, we postulated that the Cyt. c layer was densely absorbed on the TiO₂ gel, with 0.196 Cyt. c molecules per 1 nm². This value was estimated to correspond to 153% of the theoretical adsorption density (0.128 Cyt. c molecules per nm²) of the Cyt. c adsorbed on the TiO₂ gel and can thus be considered to exhibit a very densely covered protein layer.

Fig. 2b shows the change in the QCM frequency for the sequential adsorption of Ti(O-⁻nBu)₄, C₆₀, and Cyt. c. As expected, the QCM frequency linearly decreases during the adsorption process of the above mentioned molecules, indicating the extremely regular growth of the TiO₂/C₆₀/TiO₂ gel film and the successful immobilization of Cyt. c on the fullerene hybrid TiO₂ gel layer. The frequency change from the TiO₂ layer formed by the first cycle of Ti(O-⁻nBu)₄ was measured to be 11 ± 3 Hz. Then, the frequency changes for C₆₀ and the third cycle of Ti(O-⁻nBu)₄ were 25 ± 7 Hz and 15 ± 4 Hz, respectively, and, eventually, 146 ± 25 Hz for Cyt. c. Corresponding to the frequency change by the adsorption of each molecule, the change in the thickness during the adsorption process was also investigated. The thicknesses of the TiO₂ gel layers were calculated to be 0.18 ± 0.05 and 0.24 ± 0.06 nm for the first and third cycles, respectively. The thickness of the TiO₂ gel layer generated during the third cycle was 1.36 times thicker than that of the first cycle. The discrepancy in the thickness might be induced by either desorption of the TiO₂ gel during the washing and hydrolysis step or by the enhanced surface area according to the formation of the C₆₀ adsorption layer.
The thickness of the C\textsubscript{60} layer was calculated to be 0.43 ± 0.12 nm based on the frequency, which was 25 ± 7 Hz, as calculated from the C\textsubscript{60} layer density (1.6 g cm\textsuperscript{-3}). The occupied C\textsubscript{60} molecules on the TiO\textsubscript{2} gel layer show an adsorption density of 0.47 C\textsubscript{60} molecules per nm\textsuperscript{2}.

This value was estimated to correspond to 59% of the theoretical adsorption density (0.79 C\textsubscript{60} molecules per nm\textsuperscript{2}) of the C\textsubscript{60} molecules adsorbed on the TiO\textsubscript{2} gel. This finding can be attributed to the separation between the C\textsubscript{60} molecules caused by the repulsive force during the adsorption on the TiO\textsubscript{2} gel layer while maintaining a sufficient distance between the C\textsubscript{60} molecules. Therefore, we can conclude that the C\textsubscript{60} molecules constitute a closely packed monolayer of spherical particles with diameters of ca. 1 nm, similar to the adsorption of Cyt. c on the TiO\textsubscript{2} gel. The thickness and adsorption density of the Cyt. c layer adsorbed on the TiO\textsubscript{2}/C\textsubscript{60}/TiO\textsubscript{2} layers were estimated to be reach to 3.1 nm and 0.199 Cyt. c molecules per nm\textsuperscript{2}, corresponding to 155% of the theoretical adsorption density, which is 0.128 Cyt. c molecules per nm\textsuperscript{2}. Fig. 2c and d show that the QCM frequency shifts caused by the adsorption of C\textsubscript{60} and Cyt. c on the TiO\textsubscript{2} gel films with different thicknesses depend on the cycles. The total QCM frequency shifts that occurred during the TiO\textsubscript{2} gel film preparation were 11 ± 3 Hz, 40 ± 7 Hz, and 68 ± 6 Hz for 1-cycle, 3-cycle, and 5-cycle TiO\textsubscript{2} gel films, respectively. As the adsorption cycle increases, the thickness of the prepared films also obviously increases.

Regardless of the changes in the film thickness, the QCM frequency shifts resulting from the adsorption of C\textsubscript{60} on the TiO\textsubscript{2} gel films was saturated within approximately 20 min, and almost the same frequency change was observed: QCM frequency shifts of 24, 26, and 25 Hz were observed for 1-cycle, 3-cycle, and 5-cycle TiO\textsubscript{2} gel films, respectively, as shown in Fig. 2c. These results revealed that the C\textsubscript{60} molecules only adsorbed on the surface of the TiO\textsubscript{2} gel films. As observed for the C\textsubscript{60} adsorption on the TiO\textsubscript{2} gel films, the adsorption of Cyt. c on the TiO\textsubscript{2} gel films was also saturated within approximately 20 min and showed very similar frequency changes for all of the TiO\textsubscript{2} gel films with different thicknesses (Fig. 2d). As mentioned above, the QCM frequency shift is caused by the adsorption cycles, and thus, the frequency shifts for the TiO\textsubscript{2} gel films subjected to 1-cycle, 3-cycles, and 5-cycles were 147 Hz, 149 Hz, and 143 Hz, respectively. These results indicate that Cyt. c...
adsorbed on the obtained on the surface of the TiO2 gel films only. To verify the efficient immobilization of Cyt. c on the TiO2 gel layer, we also measured the UV-vis absorption spectra of the Cyt. c-adsorbed TiO2/C60/TiO2 nanohybrid film. The absorption peak was observed at 409 nm and was clearly demonstrated to be the ‘Soret’ band, which originates from heme protein, which exhibits a strong absorption band.23 In Fig. 3a, the UV-vis absorption spectra display the absorption changes resulting from the adsorption of Ti(O–Bu)4 and Cyt. c. A broad absorbance band at approximately 230 nm is attributed to the formation of the TiO2 gel layer and linearly increased with the deposition cycles, indicating the preparation of regular TiO2 gel films during the consecutive deposition steps. An increase in the UV-vis absorbance intensity upon Cyt. c adsorption was clearly observed at 409 nm and is attributed to the ‘Soret’ band and thus originates from a heme unit. The absorption peak of the ‘Soret’ band has the same wavelength range as that of native Cyt. c in phosphate buffer solution (10 mM, pH 7.0). This result clearly shows that the native characteristics of Cyt. c is preserved on the TiO2 gel layer without any conformational change or degradation. The increase in the absorbance intensity \( \Delta \text{Abs} = 0.0068 \) and the molar extinction coefficient at 409 nm \( (\varepsilon = 106 \text{ M}^{-1} \text{ cm}^{-1}) \) correspond to the number of immobilized Cyt. c molecules on the TiO2 gel layer.23 The density of the Cyt. c adsorbed on one side of the quartz plate was found to be 0.192 Cyt. c molecules per nm2, corresponding to 150% of the theoretical adsorption density of the Cyt. c (0.128 molecules per nm2); this result is almost consistent with that determined based on the QCM frequency data (0.196 Cyt. c molecules per nm2). The UV-visible spectral changes resulting from the adsorption of Ti(O–Bu)4, C60, and Cyt. c also showed increased absorbance intensity during the consecutive deposition process, confirming the completely regular nanohybrid film assembled using TiO2 gel, C60, and Cyt. c (Fig. 3b). The broad absorbance bands observed at 200–300 nm are ascribed to the TiO2 gel layer. Additionally, after the deposition of C60 on the TiO2 gel layer, the UV-visible spectrum displayed an increase in absorption in the near UV and visible regions, although the absorption of the TiO2 gel increased only in the near UV region. Interestingly, the UV-visible spectrum collected after the additional deposition of Ti(O–Bu)4 on the C60 layer displayed an increased absorption peak from the only-TiO2 gel layer in the near UV region, without any change in the absorbance characteristics of C60, as shown in the inset in Fig. 3b. Assuming that the C60 molecules strongly absorb on the TiO2 gel layer through chemical interactions, this finding can be considered to step from the interaction between the double bond of C60 and the ionic species −Ti–O− in the TiO2 gel layer (Fig. S3†). If C60 molecules are adsorbed on the TiO2 gel layer by relatively weak interactions, such as van der Waal’s forces and/or physical adsorption, C60 molecules could be readily desorbed during the deposition process of Ti(O–Bu)4 in toluene/ethanol (1/1, v/v) solution. Although the complex is formed by strong chemical interactions with the TiO2 gel layer (the carboxylic groups can be covalently bound to the titanium atoms, −Ti–O–OC–R), we occasionally observe a decrease in the absorbance after the adsorption of Ti(O–Bu)4.23 An increase in absorbance \( \Delta \text{Abs} = 0.0069 \) resulting from Cyt. c adsorption was observed at 409 nm. The Cyt. c molecules adsorb at a density of 0.195 molecules per nm2 on one side of the quartz plate, corresponding to 152% of the theoretical adsorption density of the Cyt. c; this finding indicates the adsorption of an excess amount of Cyt. c on the TiO2 gel layer. This excess adsorption of Cyt. c is considered to be the result of a significant strong electrostatic interaction between the positively

![Fig. 3](image_url)
charged Cyt. c and the negatively charged TiO$_2$ gel layer. To further establish the effect of C$_{60}$ adsorption, we next examined the UV-vis spectrum of 10 $\mu$M C$_{60}$ in toluene and C$_{60}$ immobilized onto the TiO$_2$ gel layer. As shown in Fig. 3d, two small bands at 261 and approximately 330 nm were observed after the deposition of C$_{60}$. Unfortunately, the band at approximately 330 nm is difficult to identify because it overlaps with the noise peak of the spectrophotometer. Nonetheless, these bands are very similar to those detected at 284 and 338 nm in a 10 $\mu$M toluene solution of C$_{60}$ (Fig. 3c). The blue shift suggests that the C$_{60}$ molecules are strongly adsorbed on the TiO$_2$ gel layer by chemical interactions.

3.2 Surface morphology and contact angle

To increase the accuracy of the observations of the surface morphology, AFM measurements were conducted for each of the films prepared on a silicon wafer. Fig. 4 and S2† show the 3D and 2D AFM images of the film after the deposition of Ti(2-nBu)$_4$, C$_{60}$, and Cyt. c. While the surface image of the only-TiO$_2$ gel layer reveals extremely smooth morphology with a root-mean-square (RMS) roughness of 0.163 nm (Fig. 4a), there is no significant change in the overall morphology of the films before or after C$_{60}$ adsorption (Fig. 4b). Although the presence of C$_{60}$ molecules was not identified, the RMS roughness slightly increased to 0.212 nm after the deposition of the C$_{60}$ molecules. We suggest that the C$_{60}$ molecules might be adsorbed into the TiO$_2$ gel layer at the single particle level without aggregation.

Additionally, the surface after another deposition of Ti(2-nBu)$_4$ on the TiO$_2$/C$_{60}$ layer was observed to be extremely smooth, and the RMS roughness was slightly decreased to 0.198 nm compared with the C$_{60}$ layer (Fig. 4c). Interestingly, in the final process, the RMS roughness increased to 0.438 nm after the deposition of Cyt. c, and the surface was densely covered with the adsorbed Cyt. c molecules (Fig. 4d).

The contact angle facilitates better understanding of the surface properties in terms of the interactions between the prepared surfaces and liquids. The water droplets on the film after the deposition of Ti(2-nBu)$_4$, C$_{60}$, and Cyt. c on a quartz palate were formed as in Fig. 5a. While the contact angle of 21 $\pm$ 5$^\circ$ for the TiO$_2$ gel layer is 5$^\circ$, which agrees with the high hydrophilicity of the abundant hydroxyl group (~Ti–OH moieties) in the TiO$_2$ gel (Fig. 5a), the deposition of the C$_{60}$ molecules on the hydrophilic TiO$_2$ gel layer sharply increased the surface contact angle to 93 $\pm$ 6$^\circ$ (Fig. 5a and S2†). The C$_{60}$ layer exhibits very different wetting behavior than the TiO$_2$ gel layer. This significant change in the surface chemical features is caused by perfect modification of the TiO$_2$ layer by C$_{60}$ molecules.

In contrast, the deposition of Ti(2-nBu)$_4$ on the C$_{60}$ layer dramatically decreases the contact angle of the C$_{60}$ layer to 24 $\pm$ 4$^\circ$ (Fig. 5c), almost the same as that of the first TiO$_2$ gel layer on a quartz palate. Based on the contact angle data, we confirmed that the change in the contact angle arises from uniform and complete coverage by TiO$_2$ gel and C$_{60}$ deposition. The Cyt. c immobilized on the TiO$_2$ gel layer also alters the surface properties. The increase in the contact angle of the immobilized Cyt. c film (55 $\pm$ 3$^\circ$) could be ascribed to an increase in the hydrophilic moieties on the protein surface and hydrophobic protein backbones.

3.3 Electrochemistry of Cyt. c

To investigate the electroactivity of the C$_{60}$ modified electrodes, the QCM electrodes were used as working electrodes to collect

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![Fig. 4 3D AFM images (1 $\mu$m × 1 $\mu$m) of the 1-cycle (a) TiO$_2$, (b) TiO$_2$/C$_{60}$, (c) TiO$_2$/C$_{60}$/TiO$_2$, and (d) (TiO$_2$/C$_{60}$/TiO$_2$)/Cyt. c layers deposited on a plasma-treated silicon substrate.](image-url)
CVs in PBS buffer (10 mM, pH 7) at a scan rate of 20 mV s\(^{-1}\). Fig. 6a shows the CV results for the TiO\(_2\), TiO\(_2\)/C\(_{60}\), and TiO\(_2\)/C\(_{60}\)/TiO\(_2\)-modified QCM electrodes. No obvious electrochemical response was observed for the TiO\(_2\) gel-layered electrode in the potential range from -0.3 to 0.6 V. In contrast, the electrochemical response after the deposition of C\(_{60}\) shows a pair of current peaks that are characteristic of a weak redox system with an anodic peak potential of 0.262 V and a cathodic peak potential of 0.137 V, which are attributed to the redox system of the deposited C\(_{60}\) on the TiO\(_2\) gel layer.

Interestingly, the electrochemical response after the deposition of Ti(O-n-Bu)\(_4\) on the C\(_{60}\) layer has a redox peak that disappeared and induced a broadening of the capacitive current, which reveals a decrease in the electrode’s effectiveness. This finding indicates that the surface is covered by TiO\(_2\) gel, which acts as a shielding material. Fig. 6b shows the CV results of (TiO\(_2\))\(_3\)/Cyt. c-, (TiO\(_2\))/Cyt. c-, and (TiO\(_2\)/C\(_{60}\)/TiO\(_2\))/Cyt. c-modified QCM electrodes in PBS buffer (pH 7) at a scan rate of 20 mV s\(^{-1}\). The (TiO\(_2\))\(_3\) gel electrode shows no obvious electrochemical response in the potential range of -0.3 to 0.6 V. This observation is very similar to that of the 1-cycle TiO\(_2\) gel-layered electrode. After immobilizing Cyt. c on the (TiO\(_2\))\(_3\) gel-layered electrode, the CVs shows a pair of redox peaks with anodic and cathodic peak potentials at 0.226 V and 0.162 V, respectively. Cyt. c immobilization results in irreversible behavior and weakly electrochemical response for the only-TiO\(_2\) gel-layered electrode. The formal potential (\(E^o\)) of Cyt. c obtained from the (TiO\(_2\))\(_3\) gel matrix was estimated to be 0.197 V (vs. SCE). In addition, a peak-to-peak potential separation (\(\Delta E_p\)) of the redox peaks was approximately 0.064 V at a scan rate of 20 mV s\(^{-1}\). However, a pair of well-defined quasi-reversible redox peaks of Cyt. c is clearly visible for the (TiO\(_2\)/C\(_{60}\)/TiO\(_2\))/Cyt. c-modified electrode. The anodic and cathodic peak potentials are located at 0.222 V and 0.176 V, respectively. The formal potential (\(E^o\)) of Cyt. c obtained from the C\(_{60}\) hybrid gel matrix was estimated to be 0.199 V (vs. SCE), and the peak-to-peak potential separation (\(\Delta E_p\)) of the redox peaks was approximately 0.046 V at a scan rate of 20 mV s\(^{-1}\). The small \(\Delta E_p\) indicates a fast electron transfer reaction between the Cyt. c and the electrode. The reversibility and capacitive currents of Cyt. c were greatly improved compared to those of the only-TiO\(_2\) gel-modified electrode. These results suggest that the introduction of C\(_{60}\) into the TiO\(_2\) gel matrix increased in the electrochemical characteristics of Cyt. c. Therefore, the C\(_{60}\) nanohybrid TiO\(_2\) gel matrix can provide a favorable environment prompting the electrochemical characteristics of Cyt. c.

4. Conclusions

A noble method for immobilizing Cyt. c on a fullerene nanohybrid TiO\(_2\) gel film was developed using a nanoassembly approach for improved bioelectrochemical systems of Cyt. c. The immobilization of Cyt. c and film growth were characterized by QCM, UV-vis spectroscopy, AFM, contact angle, and CV. A pair of well-defined and improved quasi-reversible redox
peaks of Cyt. c is clearly visible for the C_{60}-modified electrode. The fullerene nanohybrid TiO_{2} gel matrix can provide a favorable environment prompting the electrochemical characteristics of hemeprotein. Although improvement are still required, this new approach is a simple and useful method to prepare bioelectrochemical systems. The combination of a variety of biomaterials and fullerene nanohybrid ultrathin films will provide potential pathways for not only creating novel bioelectrochemical devices but also fabricating the elements of nanostructure materials.

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References


