

[Vol. 4, No. 6, 2011-12-22/Focus 26-29](#)**Focus 26: Topics Close-Up <No. 29>**

---Kyushu Nanotechnology Network---

**Nanostructured enzyme-modified carbon electrodes and biofuel cells****Masato Tominaga, Graduate School of Science and Technology, Kumamoto University****Naotoshi Nakashima, Graduate School of Engineering, Kyushu University <sup>†</sup>**[↑](#)**1. Introduction <sup>†</sup>**

With energy and environmental concerns in mind, the interest in batteries such as solar batteries, fuel cells, and secondary batteries is increasing. Enzymatic biofuel cells are ideal batteries, characterized by safe power generation achieved under mild conditions like those in the living body, the availability of a wide array of fuels combining various kinds of enzymes, and high energy-conversion efficiencies. However, enzymes are less stable than inorganic catalysts, and are generally more expensive; therefore, the use of large amounts of enzymes for fuel cells is not feasible, and there are many problems to be solved before the stable operation of enzyme-based fuel cells for long periods can be realized. The practical use of enzyme-based biofuel cells will be favorable only if we can solve the problems described above, in other words, if we can reduce the cost by minimizing the amount of immobilized enzyme needed through the optimization of the performance of the enzyme immobilized on the electrode for electricity generation. We can consider an electrode design that enables direct electron transfer between the electrode and the enzyme immobilized on the electrode surface [1][2][3][4][5][6][7][8][9]. Here, the amount of enzyme required is only a monolayer on the electrode (of the order of pmol/cm<sup>2</sup>). We can obtain an extremely large electrode area by using a nanostructured carbon material. The applications of enzyme-immobilized electrodes will be extended if we can significantly reduce the cost of the enzyme and the electrode. The practical use of enzyme-based biofuel cells will also become a reality if we can realize the periodical exchange and the short-term use of disposable enzyme-immobilized electrodes. However, in most cases, under the present conditions, the amount of enzyme engaged in direct electron transfer is infinitesimal compared with the total amount of enzyme immobilized on the electrode. In other words, most of the enzyme on the electrode is inactive in the direct electron-transfer reaction. This is mainly because of the denaturation of the enzyme through its adsorption on the electrode and the inappropriate adsorption orientation of enzyme molecules for the electron-transfer reaction (there are many reports indicating that the adsorption orientation of enzyme molecules greatly influences their reactivity).

The outside user is studying the development and applications of nanostructured enzyme-modified carbon electrodes using nanotubes as the platform electrode, because this nanocarbon structure has excellent conductivity characteristics and a large specific surface area [4][5][6][7][8]. In this study, from the viewpoint of developing enzyme-based fuel cells and high-sensitivity biosensor electrodes, the outside user suggested that there is a demand for the fabrication and evaluation of nanostructured enzyme-modified carbon electrodes. In response to this demand, the "support of Kyushu Nanotechnology Network on Molecular Synthesis and Analysis" offered its support for the measurements required. This report discusses some of the results of this research.



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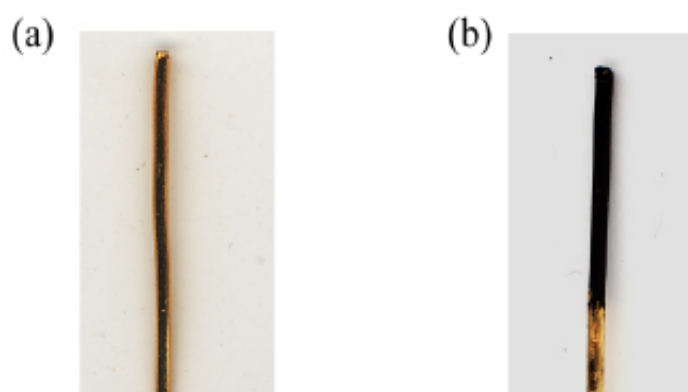
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## 2. Interface of carbon nanotubes and electrochemical reaction †

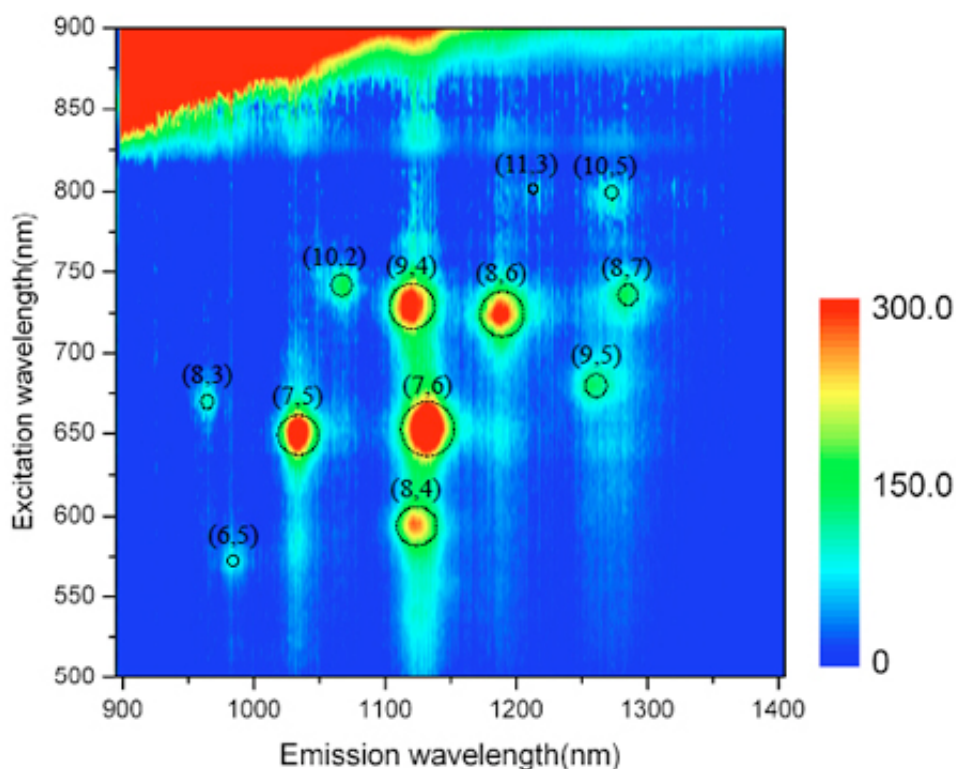
In an electrochemical reaction, electron transfer and mass transfer occur at the solid/liquid interface where a liquid is in contact with a solid (electrode). Therefore, the electrode interface has a very large influence on the electrode reaction. Attention should be paid to the electrochemical reaction at the interface of carbon nanotubes (CNTs), but there are some research reports in which the cleanliness of the CNT interface is not considered.

In this study, we evaluated the cleanliness of the CNT interface and studied the electrode reaction. We used CNTs immediately after their synthesis to achieve a clean CNT interface for the measurements. Figure 1 shows a CNT-modified gold electrode fabricated by the alcohol chemical vapor deposition (CVD) method. Electrochemical measurements could be made soon after the synthesis of CNTs on the gold electrode substrate, so that there was little CNT detachment from the gold substrate in the

solution. From Raman spectrometry and transmission electron microscopy (TEM) measurements, the formed CNTs were shown to be single-walled carbon nanotubes (SWCNTs) of approximately 1 nm in diameter. After the isolation of CNTs dispersed using a surface-active agent, the chirality of the semiconducting CNTs was evaluated using the photoluminescence (PL) caused by photoexcitation. Figure 2 shows the PL map of the SWCNTs synthesized in this study. The diameters of the SWCNTs as determined from their chirality were 0.7-1.1 nm, in good agreement with the results of the TEM measurements. Assuming a 20- $\mu\text{m}$  SWCNT layer on the gold electrode, the CNT surface area, as evaluated from the capacity of the electric double layer, was approximately 1,000 times the apparent electrode area.

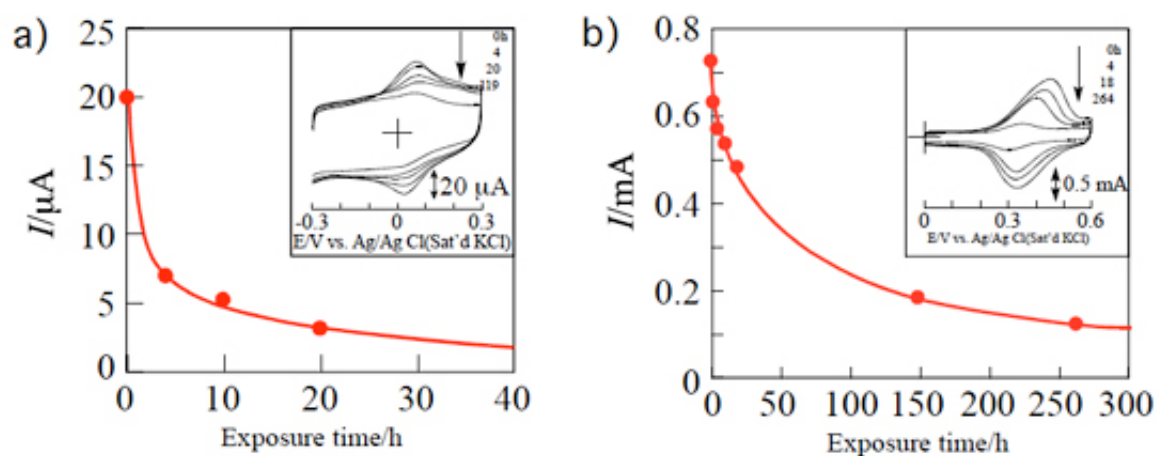


**Fig. 1** Gold wire (a) before and (b) after the synthesis of SWCNTs.



**Fig. 2** PL map of SWCNTs synthesized on the gold electrode. The dispersant was sodium cholate.

Cytochrome c and ferrocenecarboxylic acid were used as the electrochemical measurement probes. Both materials are representative biological molecules and complexes for electrochemical measurements. As shown in Fig. 3, reversible oxidation/reduction waves were observed for cytochrome c and ferrocenecarboxylic acid when using the electrode immediately after the SWCNT synthesis. The oxidation/reduction potential was about the same as that measured using a general electrode. When the electrode was left in the atmosphere of the laboratory, the oxidation/reduction peak current decreased with increasing exposure time, indicating that the electrode reaction was suppressed. The influence was particularly significant in the case of cytochrome c. It is known that the electrode interface has a great influence in the electrode reaction of cytochrome c [8][9]. It is thought that the above result applies to CNTs in general. Thus, in electrochemical measurements using commercially available CNTs, it seems reasonable to assume, especially when considering the measurement results, that the interface is in some way contaminated.



**Fig. 3** Influence of SWCNT surface contamination on the electrode reaction of (a) cytochrome c and (b) ferrocenecarboxylic acid, evaluated using an SWCNT-modified gold electrode. In the case of the cytochrome c measurements, an oxidation-treated electrode was used for the SWCNTs. The concentrations of cytochrome c and ferrocenecarboxylic acid were 0.1 mM and 1 mM, respectively, in phosphate-buffered solution (pH 7). The apparent electrode area was 0.25 cm<sup>2</sup>.

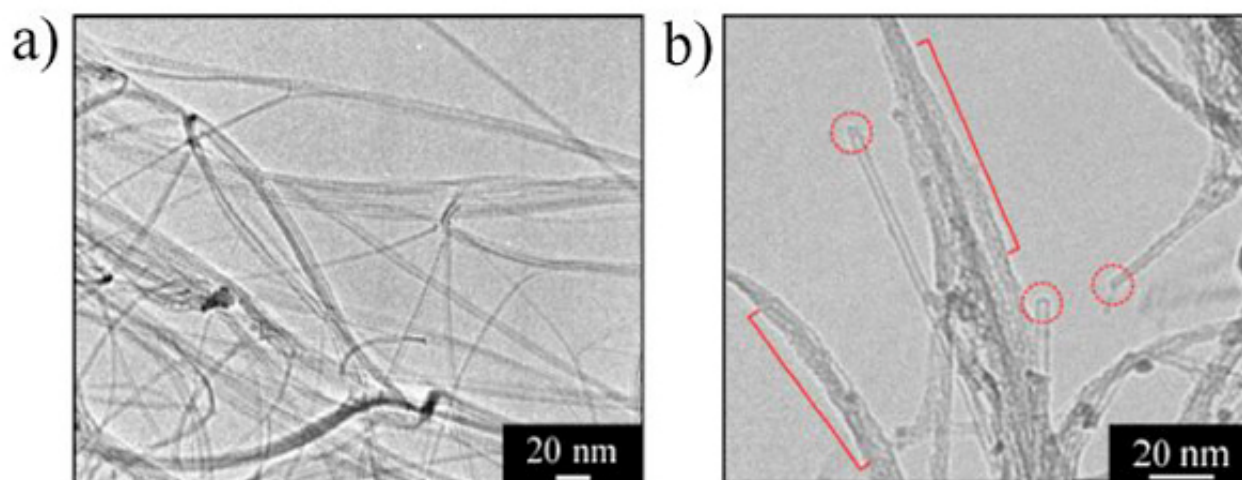
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### 3. Influence of SWCNT oxidation treatment on the electrode reaction behavior †

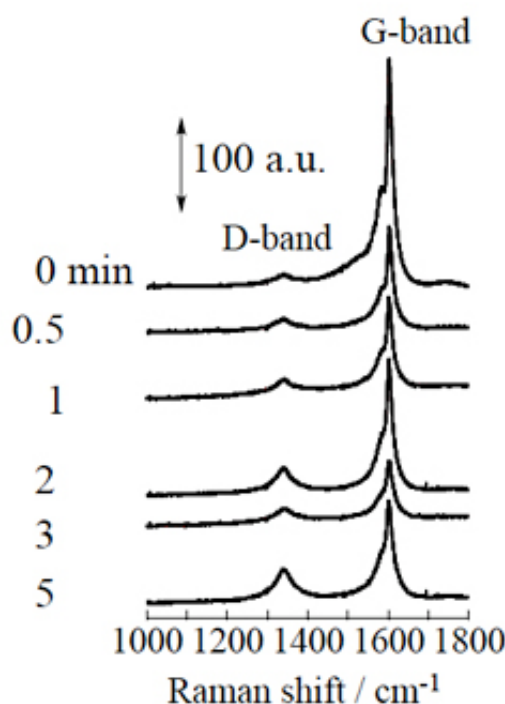
The number of structural defects in CNTs varies with the synthetic conditions. Such

structural defects include functional groups of carbon oxide such as C-O, C=O, and O-C=O. For the general carbon electrode, this carbon oxide has a significant influence on the electrochemical reaction. In this study, we examined the influence of the oxidation process on the electrochemical characteristics of high-quality SWCNTs, using the electrochemical method and the UV-ozone method.

Figure 4 shows TEM photographs of CNTs before and after UV-ozone irradiation (UV power:  $11 \text{ mW cm}^{-2}$ ; ozone density: 100 ppm). After the UV-ozone treatment, we observed the appearance of collapsed structures on the CNT surface and opened caps on the ends of the CNTs. The G-band peak obtained from Raman measurements of the SWCNTs decreased with the UV-ozone treatment time (Figure 5). Since the G-band is a peak derived from the graphite structure of CNTs, it is understood that this graphite structure of SWCNTs is damaged more as the treatment time increases. From the surface X-ray photoelectron spectroscopy (XPS) measurements, it was found that functional groups such as C-O, C=O, and O-C=O increased in number with increasing treatment time. During the electrochemical oxidation process (in neutral solution; cyclic potential width 0-1.5 V vs. silver/silver chloride electrode), the G-band decreased and the number of CO functional groups increased with the increasing number of potential cycles, just as for the UV-ozone process.



**Fig. 4** TEM images of CNTs (a) before and (b) after the UV-ozone process. The treatment time was 15 minutes.

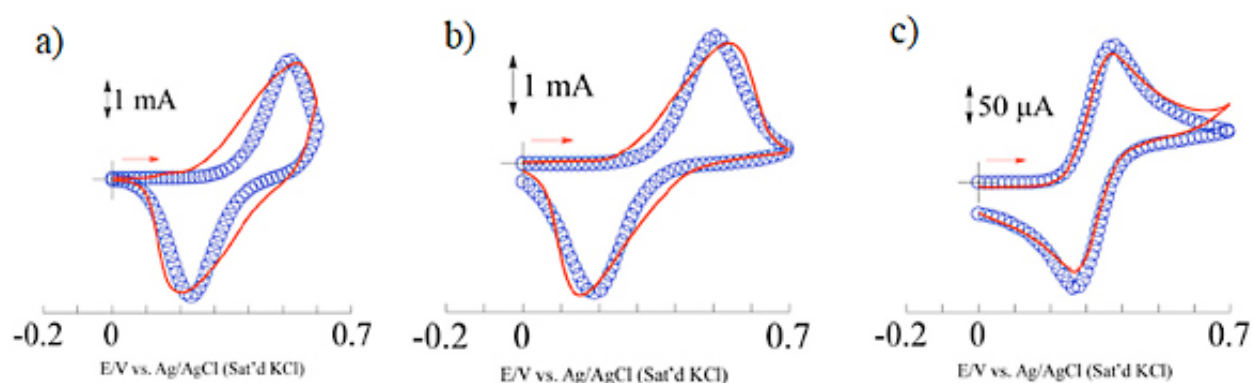


**Fig. 5** UV-ozone treatment time vs. Raman peak of SWCNTs.

The cyclic voltammogram of ferrocenecarboxylic acid was measured using a SWCNT-modified gold electrode subjected to UV-ozone oxidation and electrochemical oxidation; a simulation analysis was also carried out (Figure 6). The electron-transfer reaction rate of the SWCNTs processed by electrochemical oxidation ( $7.6 \times 10^{-3} \text{ cm s}^{-1}$ ) was about 1/20 of those for SWCNTs that had not undergone any processing ( $0.45\text{--}1.4 \times 10^{-3} \text{ cm s}^{-1}$ ) and UV-ozone-processed SWCNTs ( $0.45 \times 10^{-3} \text{ cm s}^{-1}$ ). Note here that a rate of  $16 \times 10^{-3} \text{ cm s}^{-1}$  was obtained for glassy carbon, which is generally used in carbon electrodes. More importantly, we found a difference between the voltammograms measured and the simulated results. The agreement was poor for the SWCNTs without any processing and the UV-ozone-processed SWCNTs, whereas the simulation and measurement agreed well for the SWCNTs processed by electrochemical oxidation. It is thought that this is due to the difference in homogeneity at the electrode interface, and that all the SWCNTs were oxidized uniformly in the electrochemical treatment. Since some defect structures exist in the unprocessed SWCNTs, it is assumed that this interface is not uniform.

In the UV-ozone-processed SWCNTs, considering the overall results from these and other measurements, it is thought that the surface SWCNT layer (approximately 20  $\mu\text{m}$ ) is processed, whereas deeper parts of the layer remain unprocessed. The above result shows that the defect structures of SWCNTs have a significant influence on the electrochemical reaction.





**Fig. 6** Cyclic voltammograms measured (solid line) and simulated (circles) for 1 mM ferrocenecarboxylic acid (phosphate buffer solution, pH 7) on SWCNT-modified gold electrodes. a) As-grown SWCNTs, b) UV-ozone-processed SWCNTs, c) electrochemically processed (20 cycles) SWCNTs. Scan rate:  $320 \text{ mV s}^{-1}$ . Apparent electrode area:  $0.25 \text{ cm}^2$ .

Simulation parameters: diffusivity  $5.8 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ ; inhomogeneous electron-transfer velocity constants: a)  $0.45\text{--}1.4 \times 10^{-3} \text{ cm s}^{-1}$ , b)  $0.45 \times 10^{-3} \text{ cm s}^{-1}$ , c)  $7.6 \times 10^{-3} \text{ cm s}^{-1}$ .

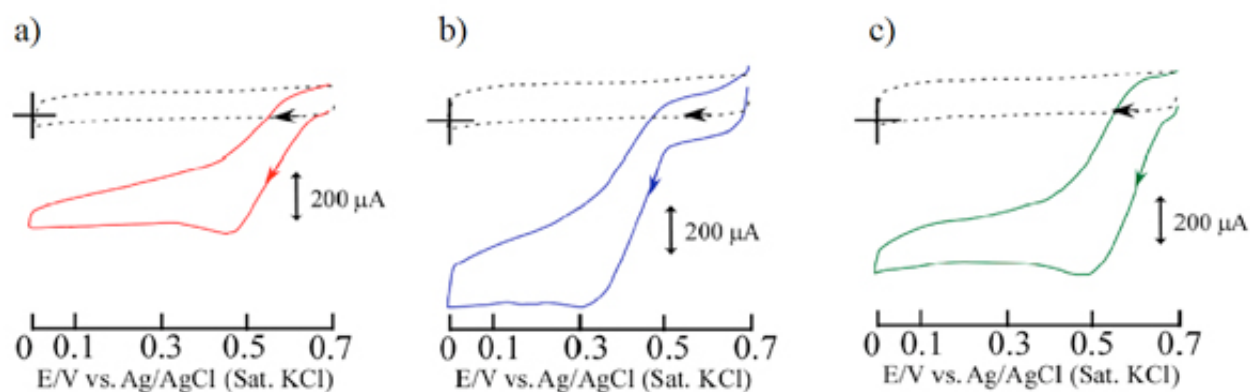
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#### 4. Application to enzyme-immobilized SWCNTs and fuel cells †

CNTs are nanocarbon structures with superior conductive characteristics and large specific surface areas, and they provide a superior platform electrode for the fabrication of enzyme-immobilized electrodes. This report introduces some of the results of an examination using laccase. Laccase is a multi-copper enzyme, and has active centers of Types I, II, and III, which are comprised of copper complexes. Laccase can reduce oxygen molecules to water through a four-electron reduction process, without the production of hydrogen peroxide (a usual intermediate in the oxygen reduction process). It has a reduction potential of  $+0.6\text{--}0.7 \text{ V}$  (vs. silver/silver chloride electrode) and a superior oxygen-reducing ability in neutral solution. Therefore, it is a promising enzyme for use as the oxygen reduction electrode in enzymatic biofuel cells [1][2].

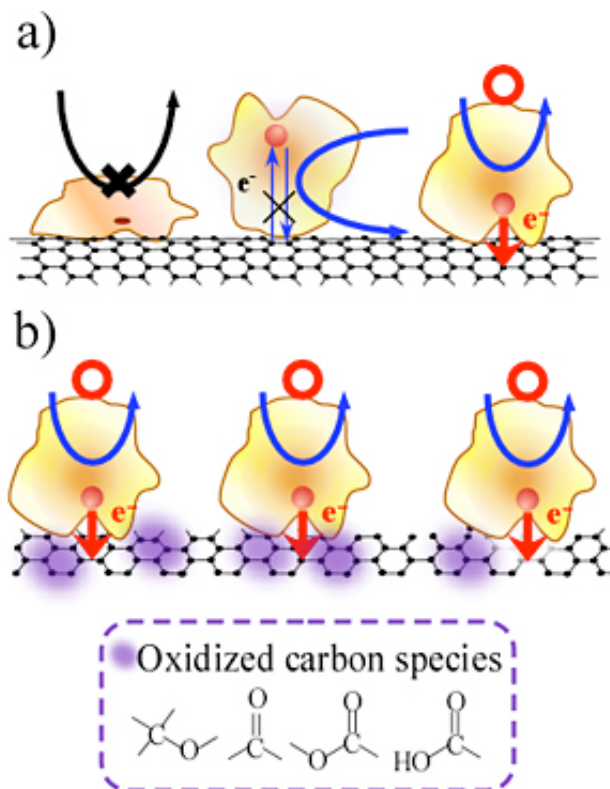
The purchased laccase (Lac), derived from *Trametes* sp., was refined by removing the stabilizer through chromatography. Immediately after synthesis, the SWCNT-modified gold electrode was immersed in a  $5 \text{ }\mu\text{M}$  Lac solution (phosphoric acid solution, pH 5) to immobilize Lac on the SWCNT surface. A catalytic reduction current of oxygen from the direct electron-transfer reaction between Lac and the SWCNTs was observed with the electrode manufactured as described above (Figure 7). The magnitude of the catalytic reduction current of Lac varied depending on the preprocessing of the SWCNTs, with the SWCNTs to which we had applied an electrochemical oxidation process (five cycles) showing the largest catalytic current. Since the amounts of enzyme adsorbed on the

SWCNTs and the enzyme activities were the same for all the electrodes, the difference in the catalytic current could not be related to these factors. The adsorption orientation of the enzyme on the interface is important in the direct electron-transfer reaction of the enzyme on the electrode. It is thought that Lac adsorbs with an orientation best suited for electron transfer on the SWCNTs treated by moderate oxidation (Figure 8).



**Fig. 7** Catalytic reduction waves of Lac based on the direct electron-transfer reaction between Lac and SWCNTs, as observed on the Lac-immobilized SWCNT-modified gold electrode. a) As-grown SWCNTs; b) electrochemically processed (five cycles) SWCNTs; c) electrochemically processed (ten cycles) SWCNTs. Oxygen-saturated phosphorus solution (pH 5): solid line. Argon-saturated phosphoric acid solution: dashed line. Scan rate:  $20 \text{ mV s}^{-1}$ . Apparent electrode area:  $0.25 \text{ cm}^2$ .



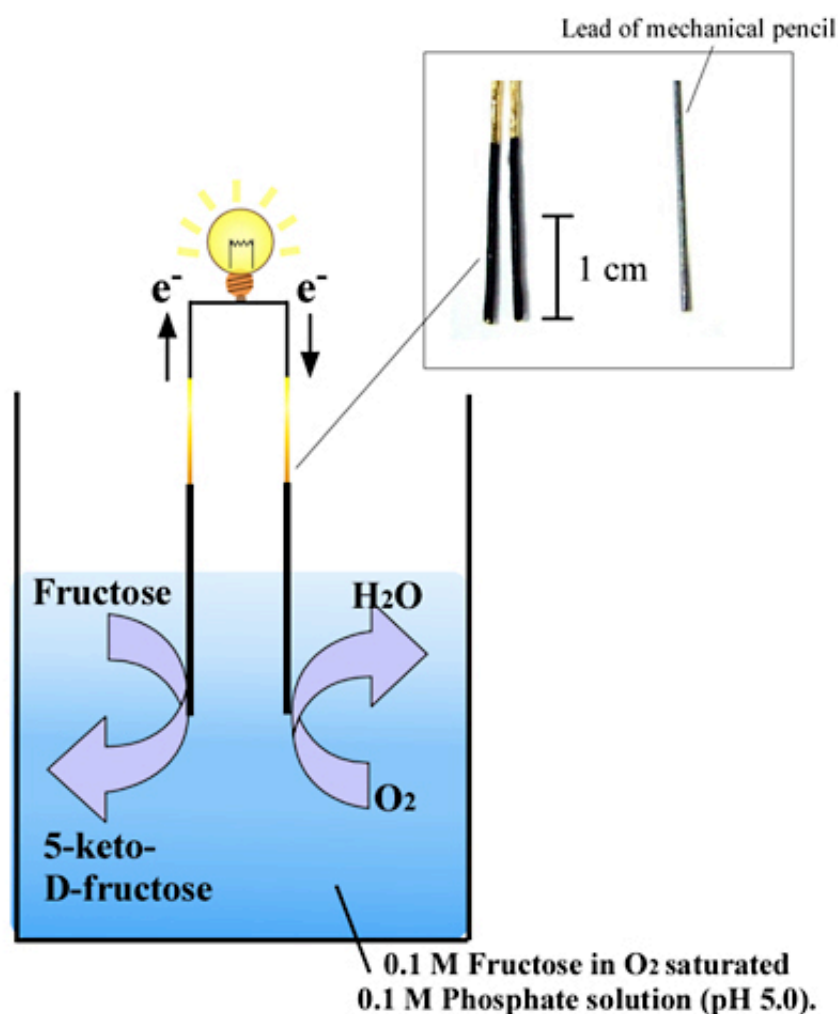


**Fig. 8** Schematic view of the adsorption orientation of Lac on (a) as-grown SWCNTs, and (b) oxidation-processed SWCNTs.

The catalytic reduction current as observed on the Lac-immobilized SWCNT-modified gold electrode gradually decreased with time. This was probably because the enzyme became detached from the SWCNTs or because the enzyme was denatured because of the strong adsorption of the enzyme on the SWCNTs. One week later, the catalytic reduction current had reduced to about half its initial value. Meanwhile, for an electrode that uses a lipid as a modulating agent, it is known that the immobilized enzyme is stabilized without disturbing the oxidation/reduction reaction of the immobilized enzyme [10]. When Lac was immobilized on SWCNTs modified in phosphatidylcholine from a biological lipid, the material was found to retain nearly 80% of its activity after a week, so such lipid-film-modified SWCNTs are useful for maintaining enzyme activity.

A fructose-oxygen biofuel cell was fabricated by using SWCNT-modified gold electrodes to which fructose dehydrogenase (FDH) and Lac were immobilized [6][7]. As shown in the schematic view in Figure 9, we were able to fabricate a simple fuel cell that gave an open-circuit voltage of 0.78 V, a maximum current density of  $0.6 \text{ mA cm}^{-2}$ , and a maximum power of  $0.2 \text{ mW cm}^{-2}$ , without using a separator to separate the reduction and oxidation poles. This was achieved simply by immersing the SWCNT-modified gold electrodes (with diameters equal to that of the core of a mechanical pencil), on which FDH and Lac had been immobilized by immersion, into a solution containing fructose

and oxygen.



**Fig. 9** Schematic view of a fuel cell using FDH- and Lac-immobilized SWCNT-modified gold electrodes as the fructose oxidation pole and the oxygen reduction pole, respectively.

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## 5. Final Remarks †

Nanocarbon materials are expected to find wide-ranging applications such as in electronic wiring, electrode materials, cell scaffolds for regenerative medicine, and drug-delivery systems, in diverse fields from electronic materials to medical care. In this report, considering the superior conductivity and specific surface area of carbon nanotubes, we have introduced our recent study on the application of nanocarbon materials as platform electrodes for enzyme immobilization. In addition, we have discussed the interface characteristics of carbon nanotubes when used as electrode materials for electrochemical measurements. We have addressed the topic of enzyme-based fuel cells as an application of nanostructured enzyme-modified carbon electrodes, in which the total amount of enzyme immobilized on the oxidation and reduction poles is just a few tens of nanomoles. As discussed in the Introduction,

reduction poles is just a few tens of nanometers. As discussed in the introduction section, it is expected that the output could be further improved if the enzyme immobilization on the electrode interface was optimized. We believe that enzyme-based fuel cells using disposable enzyme-immobilized electrodes will be put to practical use in the future.

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