Amperometric immunosensing using an indium tin oxide electrode modified with multi-walled carbon nanotube and poly(ethylene glycol)-silane copolymer

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Chemicals and reagents. The PEG-silane random copolymer (poly(TMSMA-r-PEGMA)) was synthesized as described previously.8 The initial feed ratio of two co-monomers, TMSMA as a surface anchoring group and PEGMA as a protein repelling group is 1:1. ITO-coated glass was obtained from Geomate (www.geomatec.co.jp). MWCNT (produced by CVD method, catalog number = 636487) and AP were purchased from Aldrich. Avidin, biotinylated goat antimouse IgG, mouse IgG from serum, ALP-conjugated goat antimouse IgG, FITC-labled goat antimouse IgG, and FITC-labled avidin were received from Sigma. H2O2, NH4OH, HNO3, and H2SO4 were obtained from Sigma-Aldrich. APP was purchased from Universal Sensors (Cork, Ireland). All reagents for buffer solutions were supplied by Sigma, Aldrich, or Fluka. Doubly distilled water was used for preparing aqueous solutions, and all chemicals were used without further purification.

The PBS buffer consisted of 0.01 M phosphate, 0.138 M NaCl, and 0.0027 M KCl (pH 7.4). The PBSB buffer contained all of the ingredients of PBS and additionally 1% (w/v) albumin-bovine serum (pH 7.4). The rinsing buffer (RB) consisted of 50 mM tromethamine, 40 mM HCl, 0.5 M NaCl, and 0.05% (w/v) albumin-bovine serum (pH 7.6). The Tris buffer for the electrochemical experiment contained 50 mM tromethamine, 10 mM KCl, 1 g/L MgCl2, and 7 mM HCl (pH 9.0).

Carboxylation of MWCNT. 0.1 mg MWCNT was added in a 100 mL of mixed solution of concentrated H2SO4 and HNO3 (3:1 by volume), and then the solution was sonicated for 8 h to generate carboxylic groups. Afterward, the mixture was diluted with water, hold still for 8 h, and then upper part of the diluted solution was decanted. The dilution and decantation procedure was repeated six times. The resulting mixture was filtered and washed with water. Finally Carboxylated MWCNT was dried at 60 °C for 24 h.

Construction of the ITO electrode modified with MWCNT and PEG-silane copolymer. ITO electrodes were cleaned with trichloroethylene, ethanol, and water successively, and dried at 60 °C. The cleaned substrate was pretreated in a 5:1:1 mixed solution of H2O/H2O2 (30%)/NH4OH (30%) at 70 °C for 1.5 h to ensure the presence of hydroxyl groups on the surface. Afterward, the substrate was washed with copious amount of water and dried at 60 °C for 20 min. The pretreated ITO electrode was immersed in an aqueous solution containing 1 mg/mL carboxylated MWCNT for 4 h,
and it was dried at 60 °C after washed with water. The MWCNT modified-ITO electrode was dipped in a methanol solution containing 10 mg/mL PEG-silane copolymer for 2 h, and then washed with methanol, followed by curing at 120 °C for 15 min.

**Construction of immunosensing layer.** The ITO electrode modified with MWCNT and PEG-silane copolymer was immersed for 6 h in PBS containing 100 μg/mL avidin, and then it was washed twice with PBS. The avidin-coated electrode was dipped in a PBSB solution containing 0.05% (v/v) Tween-20 (pH 7.4) for 30 min to prevent nonspecific adsorption of proteins. After washing with RB, the electrode was immersed for 30 min in PBSB containing 10 μg/mL biotinylated goat antimouse IgG, and washed with RB. Afterward, the substrate was incubated for 30 min in a PBSB solution containing variable concentrations of mouse IgG. After rinsing with RB, the resulting assembly was dipped in PBSB containing 10 μg/mL ALP-conjugated goat antimouse IgG for 30 min, followed by washing in RB.

**Electrochemistry.** The electrochemical experiment was performed using a CHI 617 (CH instruments). The electrochemical cell consisted of the modified ITO working electrode, a Pt counter electrode, and an Ag/AgCl reference electrode. The Tris buffer solution containing 1 mM AP was used to check electrocatalytic properties of the bare ITO and modified ITO electrodes. The Tris buffer solution containing 1 mM APP was used for immunosensing experiments.

**Fluorescence experiment.** The bare ITO or PEG-silane copolymer-modified ITO electrode was immersed in a PBS solution containing 100 μg/mL of FITC-labled goat antimouse IgG for 30 min or FITC-labeled avidin for 6 hr and then washed with water. Fluorescence images were taken using OLYMPUS, BX51 to measure the extent of nonspecific adsorption.

**SEM images of MWCNT-modified electrodes.** Figure S1 shows SEM images of MWCNT-modified electrodes obtained after immersing in an aqueous solution containing 1 mg/mL MWCNT for various times. There is no significant change on the surface coverage of MWCNT with time. The MWCNT submonolayer is stable to resist detachment even after sonication for 10 min in a water solution (g and h in Figure S1).

**Electrooxidation of APP.** Figure S2 shows a cyclic voltammogram obtained in a Tris buffer solution containing 1 mM APP. The electrooxidation of APP is considerable at potentials more positive than 0.4 V.
Figure S1. SEM images of MWCNT-modified electrodes after immersing in an aqueous solution containing 1 mg/mL MWCNT for (a) 1 min, (b) 10 min, (c) 1 hr, (d) 4 hr, (e) 1 day, and (f) 7 days and after sonication of the MWCNT-modified ITO electrode in a water solution for (g) 1 and (h) 10 min.
**Figure S2.** Cyclic voltammogram obtained in a Tris buffer solution containing 1 mM APP.