Synthesis of Conductive Polymer Thin Film Having Choline Phosphate Side Group and Their Bioadhesive Properties

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Materials

Lithium perchlorate, THF and acetonitrile were purchased from FUJIFILM Wako Pure Chemical Corporation. 3,4-ethylenedioxythiophene (EDOT), hydroxymethyl EDOT (EDOT-OH), sodium dodecyl sulfate (SDS), dioctyl sulfosuccinate sodium salt (AOT), and bovine serum albumin (BSA) were purchased from Sigma Aldrich. NIH3T3 cells were obtained from the Academia Sinica. *N*,*N*-dimethylmethanamine-3,4-ethylenedioxythiophene (EDOT-N(CH_3)$_2$), 2-methyl-1,3,2-dioxaphospholane 2-oxide (MeEP), EDOT-PC were prepared according to literature.[1-2]

Characterizations

Proton nuclear magnetic resonance (^{1}H-NMR), phosphorus-31 nuclear magnetic resonance (^{31}P-NMR) spectra of the synthesized compounds were measured with AVANCE III HD 400 MHz (Bruker Co. Ltd., Massachusetts, U.S.A.). X-ray photoelectron spectroscopy (XPS) was carried out with APEX (ULVAC-PHI Inc., Kanagawa, Japan) using a monochromatic Al-Kα X-ray source (1486.6 eV). Contact angel measurement carried out with Theta T-200 Auto 3 (Biolin scientific, Västra Frölunda, Sweden). Quartz crystal microbalance (QCM) measurements were performed at 25 ^{o}C on a Q-Sense AB system (Biolin Scientific) to monitor *in situ* the interactions of biomolecules with the functionalized conducting polymer substrates. Electrochemical experiments were performed using an Autolab PGSTAT128N potentiostat (Metrohm Autolab) and a three-electrode electrochemical cell, with a Pt electrode as the counter electrode and an Ag/$AgNO_3$ electrode (0.01M $AgNO_3$ and 0.1M Bu_4NPF_6 in acetonitrile) as the reference electrode. Each measurement was calibrated using a standard ferrocene/ferrocenium redox system. The conducting polymers were electrochemically deposited on the surface of a QSX 301 sensor crystal (Biolin Scientific) and then placed in the measurement chamber. Solutions of the biomolecules were delivered continuously

to the measurement chamber at a flow rate of 20 ml min^{-1}, pumping with an Ismatec ISM597D pump.

Assignment of 3,4-ethylenedioxythiophene having CP side group (EDOT-CP)

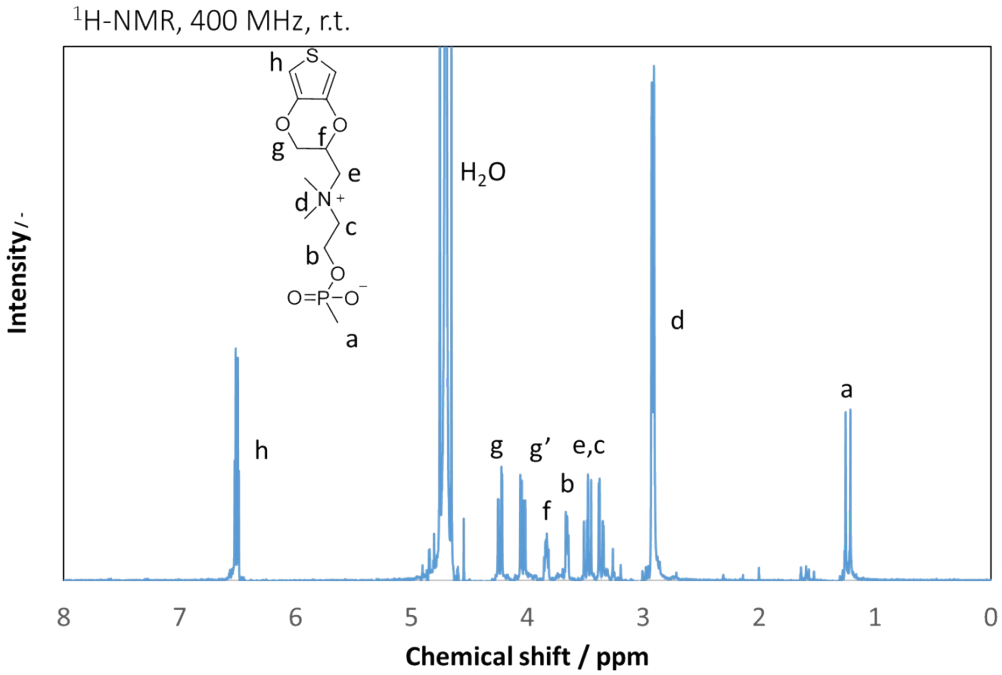

Fig. S1 ^{1}H-NMR spectra of EDOT-CP

^{1}H-NMR (400 MHz, D_2O): δ(ppm): 6.53-6.47(m, 2H, *thiophene*), 4.24(dd, J_1=3.8 Hz J_2 =12.2 Hz, 1H, -OCH_2CH(O-)CH_2), 4.04 (dd, J_1=3.8 Hz J_2 =12.2 Hz,1H, -OCH_2CH(O-)CH_2), 3.83(quin, J=4.4 Hz, 1H, -OCH$_2$CH(O-)CH_2), 3.65(t, J=4.3 Hz, 2H, CH_3P(=O)(-O$^-$)O-C$H_2$$CH_2$), 3.52-3.28(m, 4H, CH_3P(=O)(-O$^-$)O-CH_2CH_2N(CH_3)$_2$CH_2), 2.91 (s, 6H,CH_2CH_2N(CH_3)$_2$$CH_2$), 1.23 (d, J=16.6 Hz, 3H, CH_3P(=O)(-O$^-$)O-CH_2),

^{31}P-NMR (162 MHz, D_2O): δ(ppm):28.4 (CH_3*P*(=O)(-O$^-$)O-CH_2).

MS(m/z):[M+H$^+$], Calculated for $C_{12}H_{21}NO_6PS$, 322.09 Obs. 322.08

Assignment of poly(EDOT-CP) deposited on ITO.

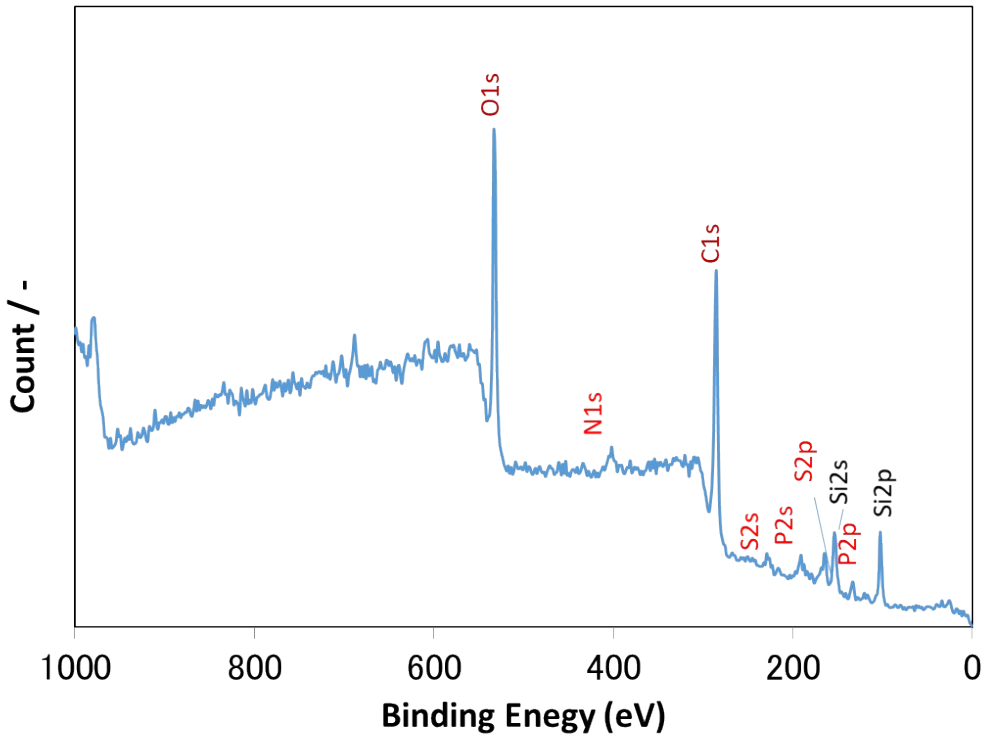

Fig. S2 XPS spectra of poly(EDOT-CP) deposited on ITO.

Stability of poly(EDOT-CP-co-EDOT) in water

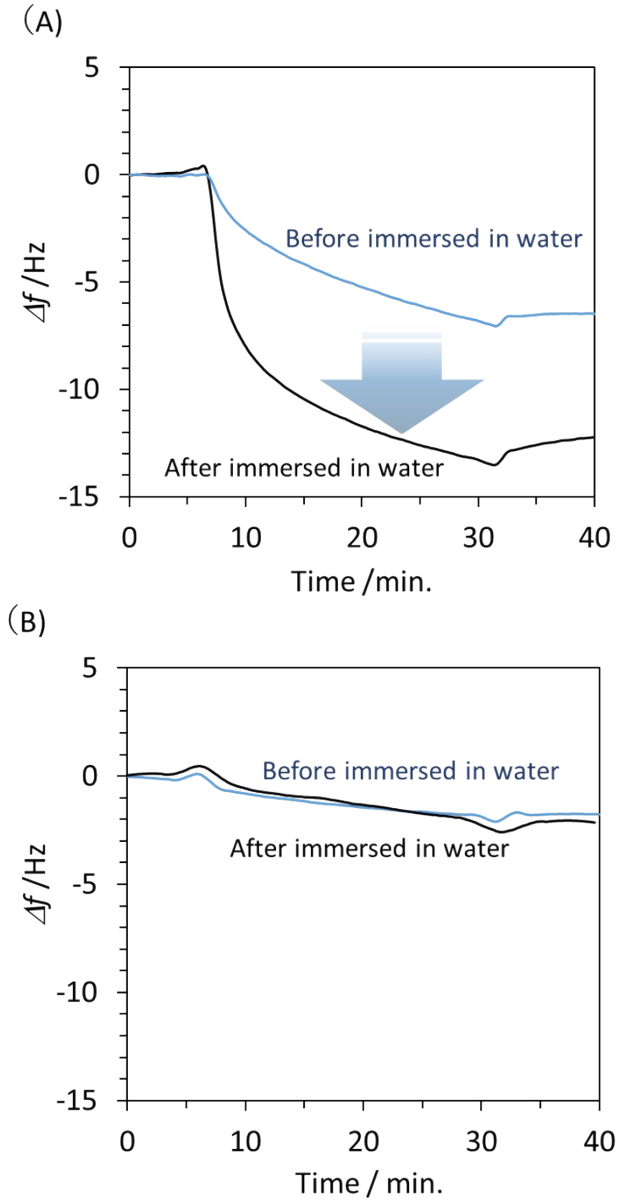

Fig. S3 Oscillation frequency change (*Δf*) of QCM due to before (blue line) and after (black line) immersed in water. The polymers on ITO electrodes were immersed in water for overnight before QCM measurements. QCM measurements carried out in bovine serum albumin (BSA) 1 wt% in phosphate buffered salts (PBS) buffer, 25 ^{o}C. (A) 9:1 poly(EDOT-CP-*co*-EDOT); (B) 7:3 poly(EDOT-CP-*co*-EDOT);

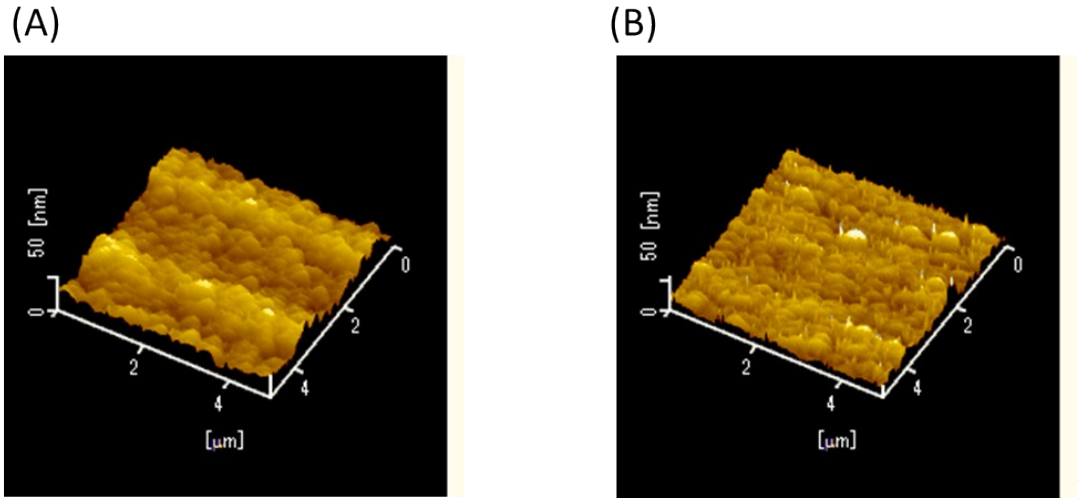

Fig. S4 Scanning probe microscopy (SPM) images of image of (A) 9:1 poly(EDOT-PC-*co*-EDOT) and (B) 7:3 poly(EDOT-CP-*co*-EDOT).

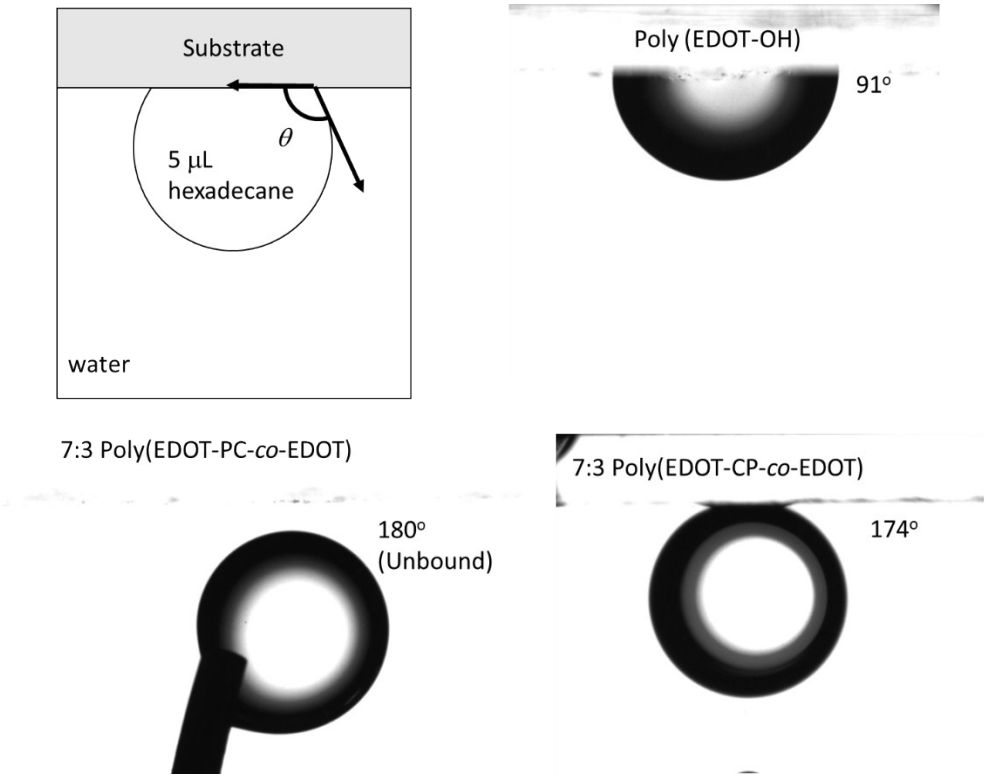

Fig. S5 Hexadecane contact angel images of poly(EDOT-OH), poly(EDOT-PC-*co*-EDOT) and poly(EDOT-CP-*co*-EDOT) surface in water.

Scheme for protein binding by QCM

The protein binding was monitored by a QCM-D E1 (Biolin Scientific, Västra Frölunda, Sweden) system. The polymer thin films were coated on the surface of a QSX 301 QCM chip by electropolymerization before being placed into the Q-sense flow module. All measurements were performed at 25 °C. The flow rate was maintained at 75 μL/min using an Ismatec ISM829B pump. In this study, all QCM tests were repeated for at least three times to ensure the accuracy of our data and confirm the results were highly reproducible. The binding test was performed in PBS buffer. Protein solutions at concentrations 1 wt% were used to assess protein binding. When protein adsorbs on the surface of QCM chip, the shifts in frequency and dissipation could be recorded directly.

Cell experiments

NIH3T3 fibroblasts were used as the model to evaluate cell attachment. Polymers electrodeposited on ITO-coated glass were placed in tissue culture plate and NIH3T3 fibroblasts were seeded on each sample. The base medium for this cell line is ATCC formulated Dulbecco's Modified Eagle's Medium with 10% bovine calf serum. The plate was then incubated at 37 °C with 5% CO_2. Attached cells were observed with a phase contrast microscope after 2 days cell culture.

Electrochemistry Impedance Spectroscopy (EIS) Measurements

EIS was performed on an Autolab PGSTAT128N potentiostat with a FRA32 M module. The three-electrode setup was made of a Au electrode as a working electrode, Ag/AgCl as a reference electrode, and a platinum wire as a counter electrode in $[Fe(CN)_6]^{3-/4-}$ solution. The $[Fe(CN)_6]^{3-/4-}$ solution contained 5 mM $K_3Fe(CN)_6$ and 5 mM $K_4Fe(CN)_6$ in 1X PBS. The EIS spectra were recorded at frequencies ranging from 10^{-1} to 10^5 Hz at 0.21 V vs Ag/AgCl.

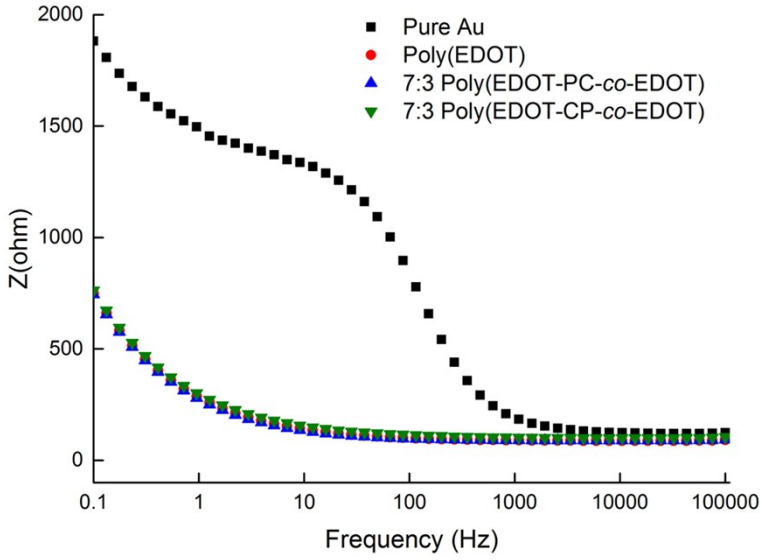

Fig. S6 Electrochemical impedance spectroscopy (EIS) Bode plot of pure gold surface, poly(EDOT), 7:3 poly(EDOT-CP-*co*-EDOT) and 7:3 poly(EDOT-CP-*co*-EDOT).

Stability and anti-fouling properties of poly(EDOT-CP-*co*-EDOT)

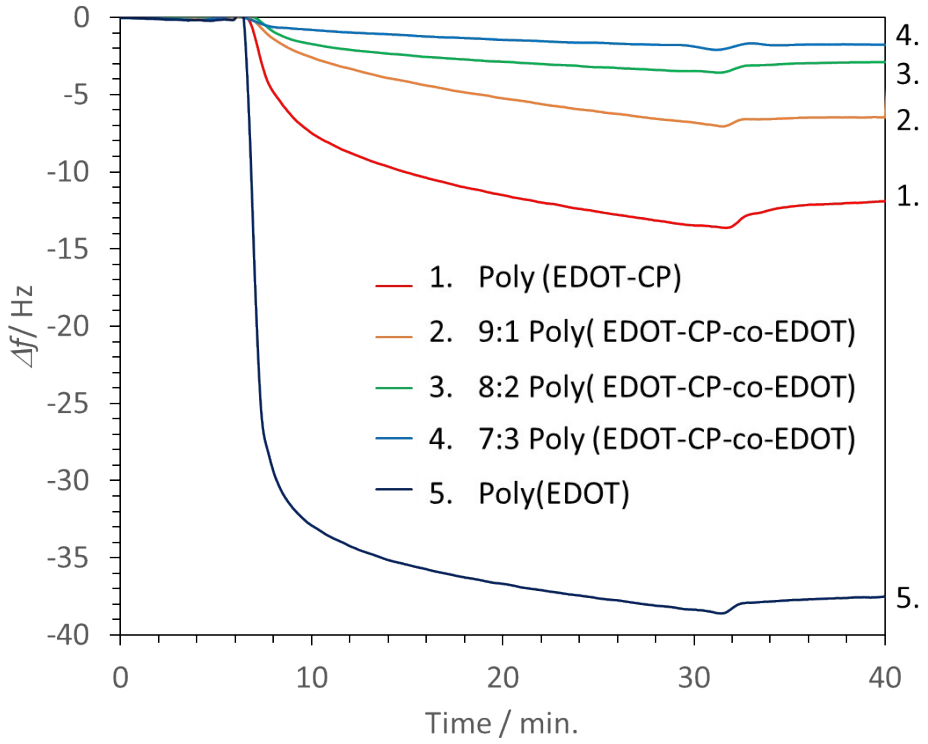

Fig. S7 Oscillation frequency change (*Δf*) of QCM due to difference feed ratio of EDOT-CP deposited on poly(EDOT-OH) layer. QCM measurements carried out in bovine serum albumin (BSA) 1 wt% in phosphate buffered salts (PBS) buffer, 25 °C. Poly(EDOT-CP), 9:1 poly(EDOT-CP-*co*-EDOT), 8:2 poly(EDOT-CP-*co*-EDOT), 7:3 poly(EDOT-CP-*co*-EDOT) and poly(EDOT) means poly(EDOT-CP-*co*-EDOT) with a 100%, 90%, 80%, 70% and 0% feed ratio of EDOT-CP deposited on poly(EDOT-OH) layer, respectively.

Reference

1. Cao, B.; Lee, C.-J.; Zeng, Z.; Cheng, F.; Xu, F.; Cong, H.; Cheng, G., Electroactive poly(sulfobetaine-3,4-ethylenedioxythiophene) (PSBEDOT) with controllable antifouling and antimicrobial properties. *Chemical Science* **2016,** *7* (3), 1976-1981.

2. Steinbach, T.; Wurm, F. R., Degradable Polyphosphoester-Protein Conjugates: "PPEylation" of Proteins. *Biomacromolecules* **2016,** *17* (10), 3338-3346.