Supporting Information

Nanoscopic Analyses of Cell-Adhesive Proteins Adsorption on Poly(2-methoxyethyl acrylate) Surfaces

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S1 Determination of amount of adsorbed proteins

PMEA substrate was incubated with PBS for 1 h at 37 °C. After removing the supernatant, PMEA substrate was immersed in solutions of fibrinogen (10 μgmL^{-1}) and fibronectin (10 μgmL^{-1}) dissolved in PBS at 37 °C for 10 min. After washing by PBS twice, proteins-adsorbed substrates were immersed in 5% sodium dodecyl sulfate (Bio-Rad) solution dissolved in PBS for 2 h. The extracted proteins were collected and reacted with micro BCA protein assay kit (Thermo Fisher Scientific). The absorbance was measured at a wavelength of 562 nm using Infinite 200PRO M Plex microplate reader (Tecan, Zürich, Switzerland).

S2 Elastic modulus measurement

Elastic modulus was acquired from force-distance curve of the protein-conjugated cantilevers contacted with PET substrates. The measurements were done at 20 - 50 points with the forwarding and retracting velocities of 1.0 μm s^{-1} and the setpoint of 1.0 nN. The elastic moduli of proteins were calculated by the JKR two-point method depends on the balance point and the maximum adhesion point of retraction curves.[1]

S3. Force measurement for urea-treated protein

The protein-conjugated cantilever was soaked in 4 M urea solution for 1 h at room temperature. The cantilevers washed by PBS three times. The force measurements with the protein-conjugated cantilevers were performed on the polymer-rich or polymer-poor regions of PMEA in PBS. In the case of PMEA, the measured points were classified into the polymer-rich or polymer-poor regions from

their height variation. The measurements were done at 50 points for PMEA polymer-rich and PMEA polymer-poor with the forwarding and retracting velocities of 1.0 μm s^{-1} and the setpoint of 1.0 nN. The adsorption force was obtained from the retraction curves.

S4. Reference

1. H. K. Nguyen, S. Fujinami, K. Nakajima, *Polymer,* 2016, **87**, 114–122.

Table S1. Characterization of polymers and substrates using present study.

Code	M_n	M_w/M_n	$T_{g,dry}$ [a)] [°C]	$T_{g,wet}$ [a)] [°C]	Contact angle Sessile drop [°] [b)]	Contact angle Captive air [b)] [°]
PET	-	-	-	-	74.8 ± 1.2	131 ± 2.9
PMPC[b)]	250,000	2.4	64	-	110 ± 2.4	149 ± 2.2
PMEA	18,000	3.1	-41	-49	36.0 ± 0.7	132 ± 2.3

a) Determined by differential scanning calorimetry performed at a rate of 5 °C/min a) Water in air (sessile water drop) and an air bubble in water (captive air bubble). Data were expressed as mean ± SD (n = 5). b) It was provided by NOF COMPANY (Tokyo, Japan): poly(2-methacryloyoxyethyl phosphorylcholine-*co*-*n*-butyl methacrylate) (30 : 70 mol%) (PMPC; Lipidure-CM5206).

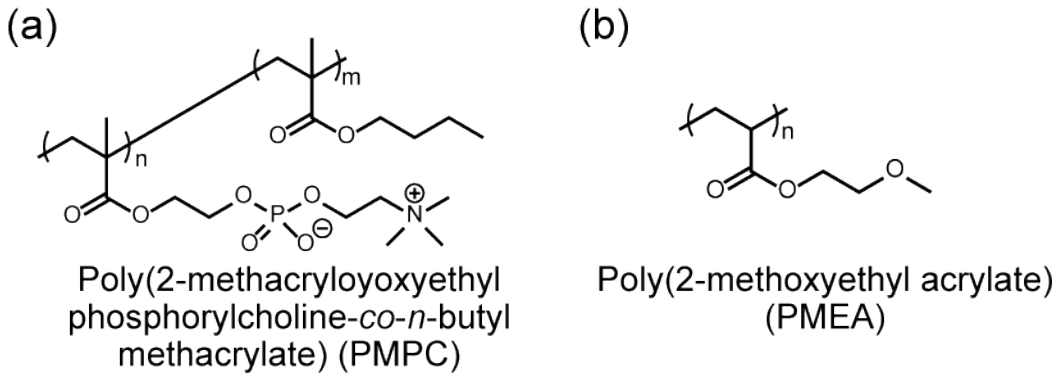

Figure S1 (**a,b**) Chemical structures of poly(2-methacryloyoxyethyl phosphorylcholine-co-*n*-butyl methacrylate) (30 : 70mol%) (PMPC) (b) and poly(2-methoxyethyl acrylate) (PMEA) (b).

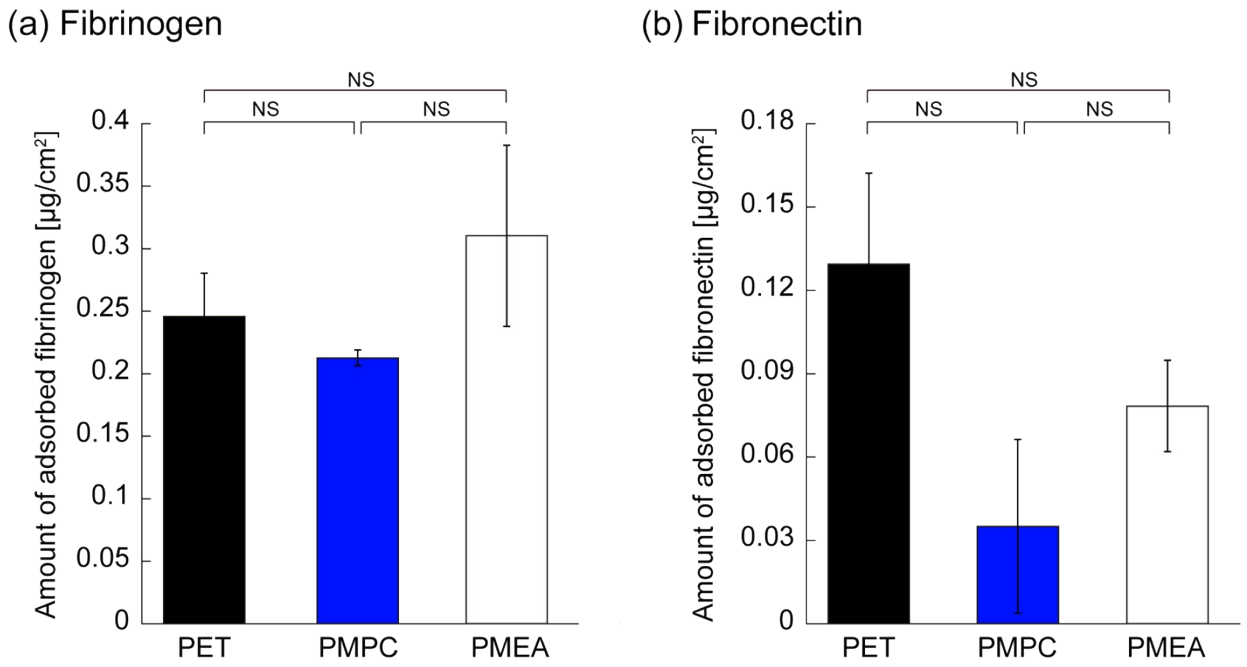

Figure S2. (a,b) Amount of fibrinogen (a) and fibronectin (b) adsorbed on PET, PMPC, and PMEA. Data are means ± SD (n = 3; NS, not significant).

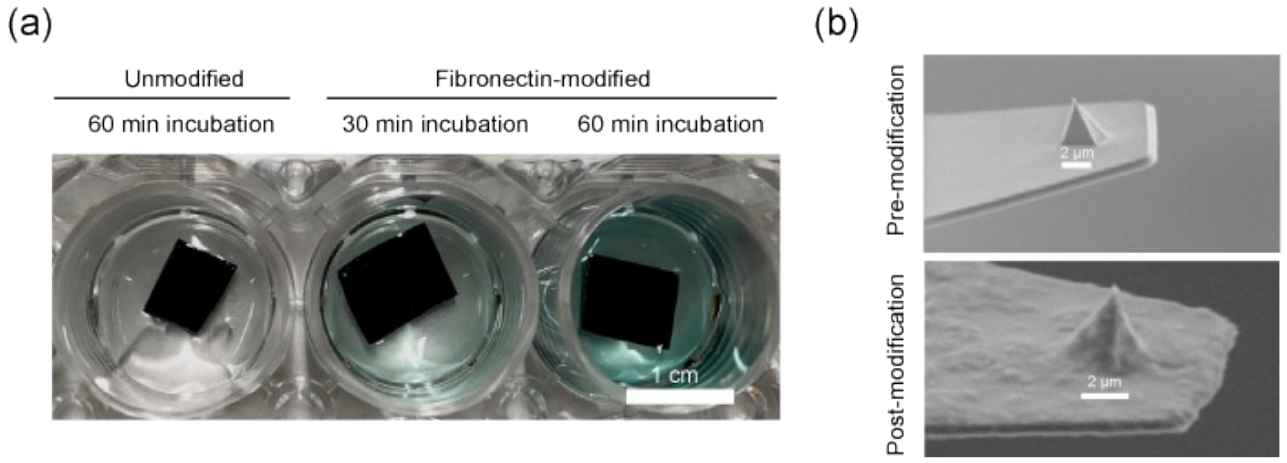

Figure S3. (a) Photograph of fibronectin-modified silicon wafer-immersed solution after ELISA. After 60 min of incubation with ABTS, the solution of fibronectin-modified silicon wafer showed color change from colorless to purple. **(b)** Scanning electron microscopic images of cantilever at pre- and post-modification by fibronectin.

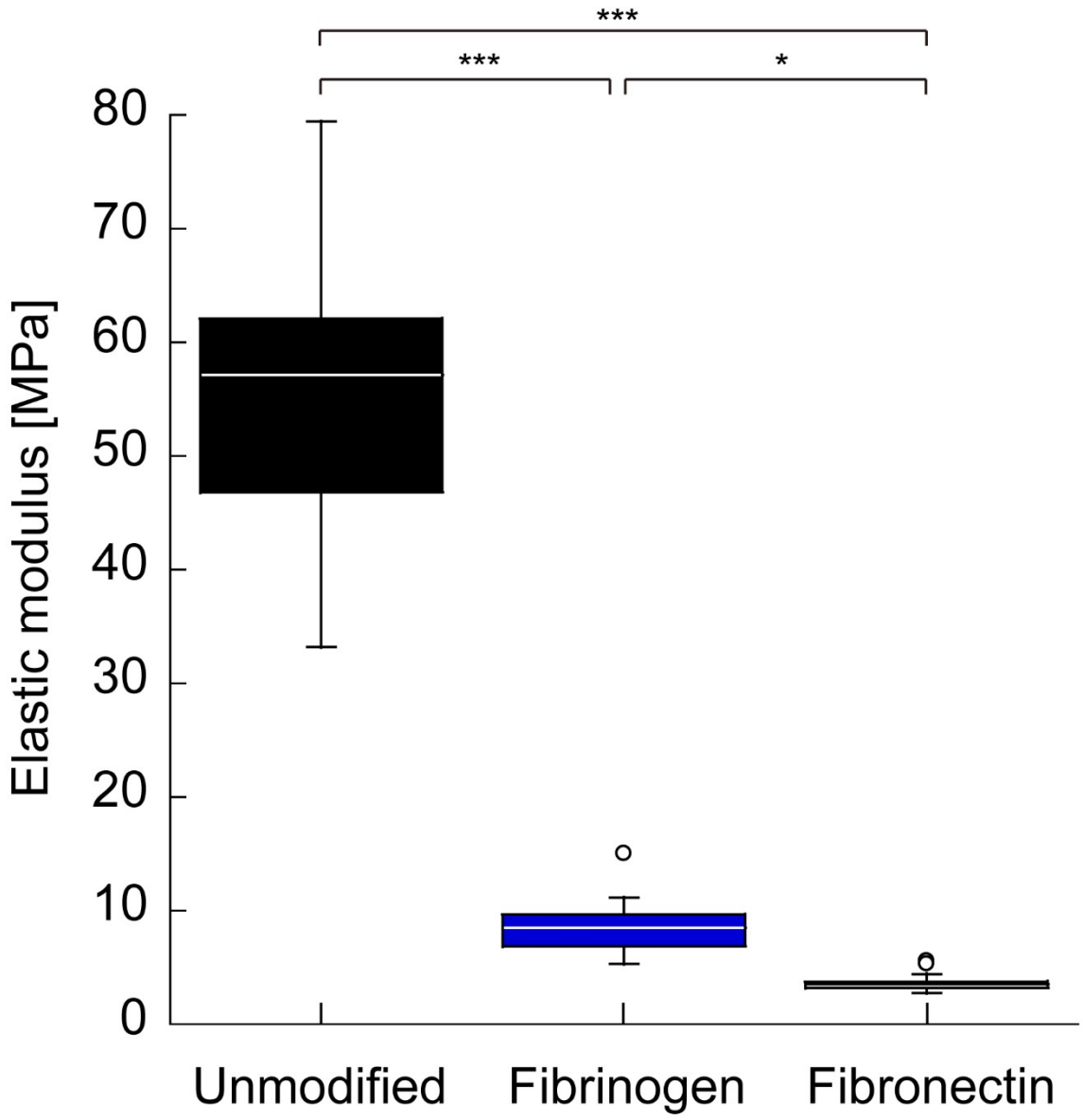

Figure S4. Elastic moduli on PET measured by the cantilevers unmodified and conjugated with fibrinogen and fibronectin. Elastic modulus was calculated by the JKR two point method from the retraction curve. Data are means ± SD (n = 20; $p^* < 0.05$, $p^{***} < 0.001$).

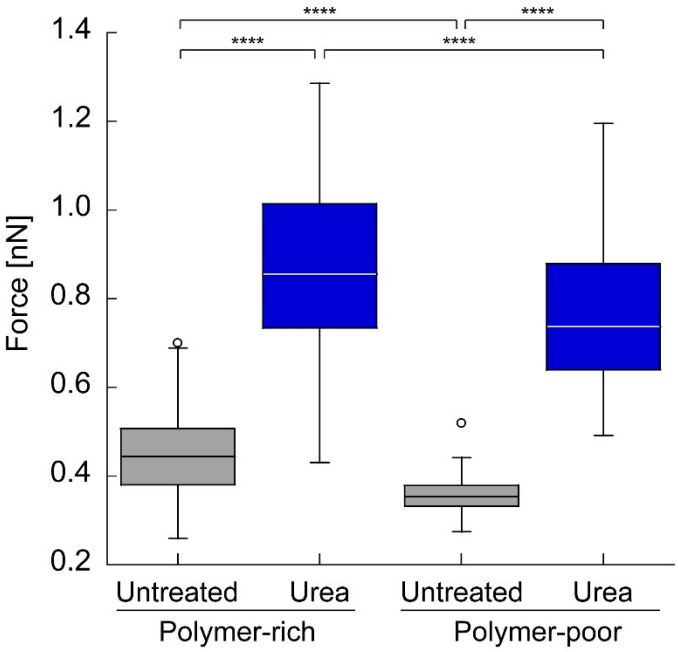

Figure S5 Adsorption force of the fibronectin conjugated cantilevers in contact with the PET substrate, the polymer-rich and polymer-poor regions of PMEA. The fibronectin was treated with 4 M urea solution before measurement. Data were expressed as mean ± SD (n = 20 - 50, ****p < 0.0001).

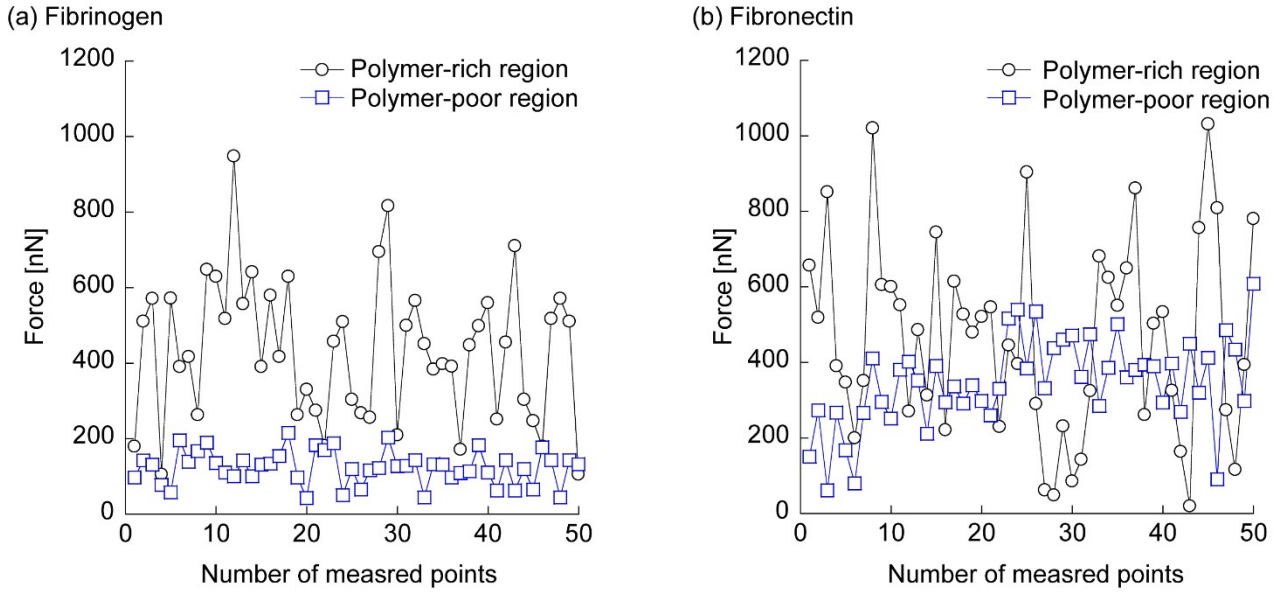

Figure S6 Relationship between number of measured points and adsorption force of the fibrinogen- or fibronectin-conjugated cantilevers in contact with the polymer-rich and polymer-poor regions of PMEA.

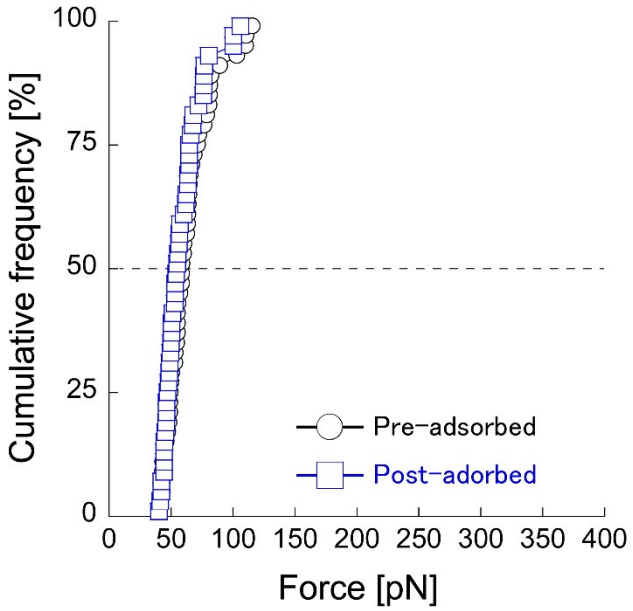

Figure S7. Cumulative frequency plots of adhesion force; between the anti-fibronectin monoclonal antibody-conjugated cantilever (FN-cantilever) and the pre- or post-fibronectin-adsorbed PMPC substrates. Data were expressed from 50 points of force curves.