Electronic Supplementary Information for:

A biological breadboard platform for cell adhesion and detachment studies

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Biomimetic cell adhesion of biological breadboard

An adherent cell binds to substrates, extracellular matrix (ECM) proteins, or other cells using its cell adhesion molecules. An integrin, one of the most common transmembrane proteins, is known as a receptor that mediates cell adhesion to ECM proteins or other cells which have a cell adhesion site, for example an arginine-glycine-aspartic acid (RGD) peptide. An RGD peptide that serves as a cell adhesion motif constitutes a major recognition system for *in vivo* cell adhesion together with an integrin (Fig. S1A).[35,36] This integrin-mediated cell adhesion is known to regulate critical cellular behavior and function such as migration, growth, differentiation, and apoptosis.[37] Thus, this mechanism of *in vivo* cell adhesion is kept intact in the design of the biological breadboard (BBB). In detail, the RGD peptide is used to provide cells of interest with microenvironment for cell adhesion which is biologically identical to the *in vivo* one. The RGD peptide is tethered to thiol, and then is introduced to the gold electrodes of the BBB. The RGD peptide is therefore is chemically bound to the gold electrodes through the spontaneous chemisorption of gold and thiol, achieving a biomimetic cell adhesion site in the BBB (Fig. S1B).

This surface functionalization using RGD-terminated thiol (RTT) has key roles in the spatiotemporal manipulation of cell adhesion and detachment: attaching an adherent cell(s) to the gold electrodes of the BBB via RGD peptide; detaching the cell(s) (or part of the cell(s)) from the gold electrodes through the reductive desorption of a gold-thiol self-assembled monolayer (SAM) at a negative potential of -0.90 to -1.65 V (less than -2.00 V).

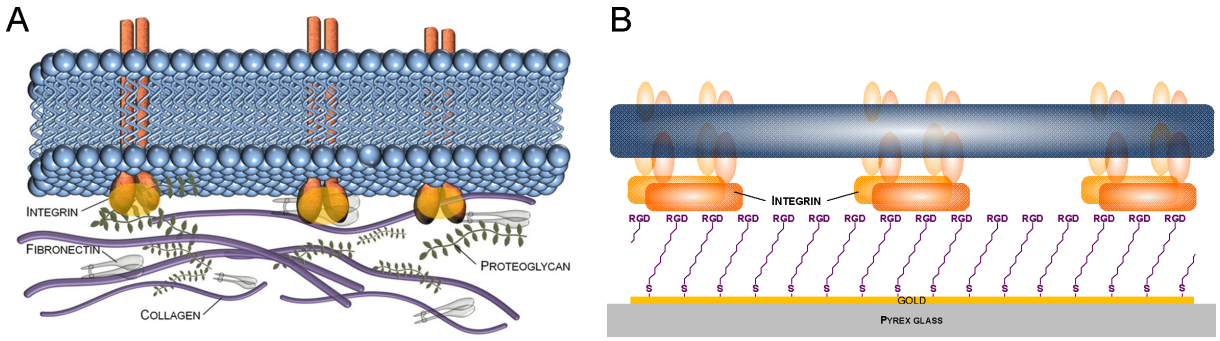

Fig. S1 Biomimetic cell adhesion of the BBB. (A) Adherent cells, in nature, adhere to substrates, ECM proteins, or other cells through the cellular interaction of an integrin to cell adhesion sites (e.g., RGD peptide). (B) Adherent cells, in the BBB, are tethered to the gold electrodes via RTT.

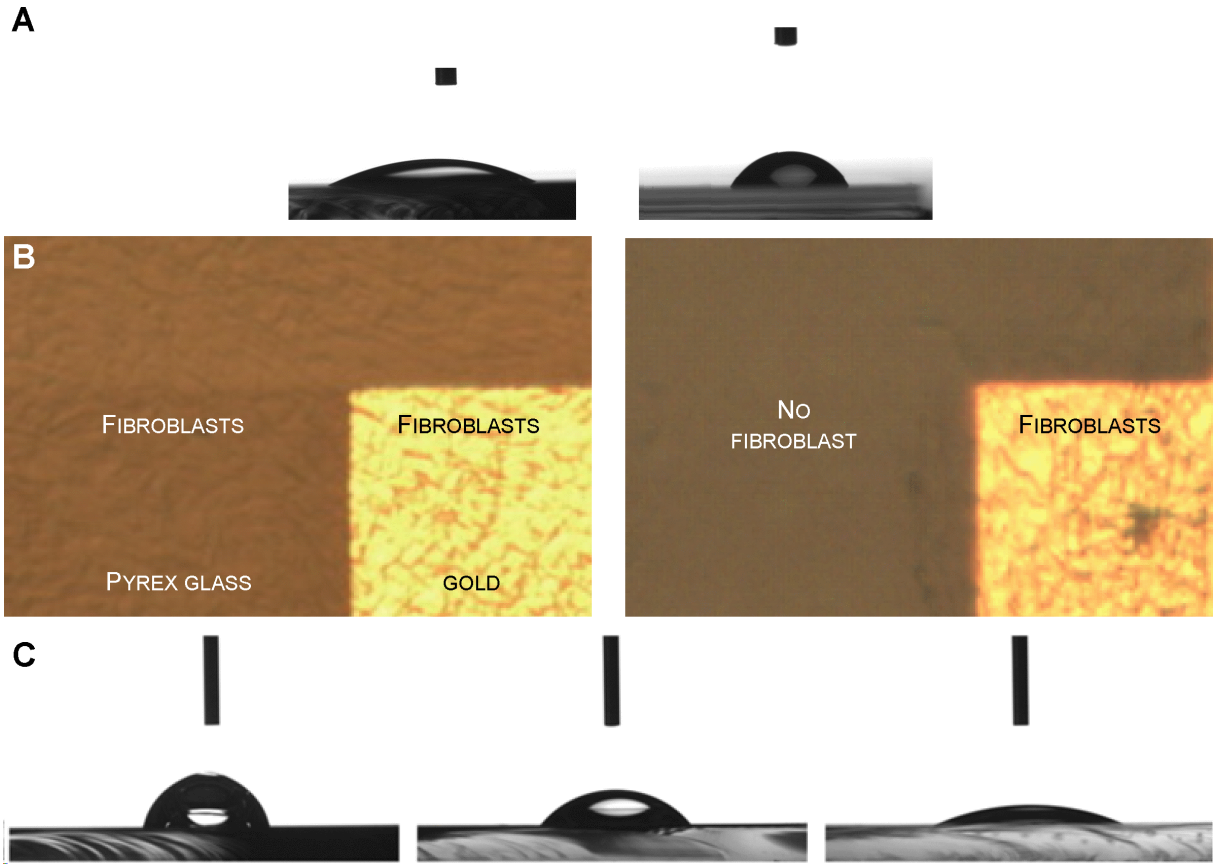

Fig. S2 Characterization of two surface modifications (i.e., PEG treatment on Pyrex substrate and RTT functionalization on gold surface) by contact angle measurements. (A) Measured contact angles of the Pyrex substrate before (left) and after (right) PEG treatment. The contact angle is changed from 25.7±1.5 to 61.5±3.8° through PEG treatment. (B) Cell loading on the Pyrex substrate before (left) and after (right) PEG treatment. The NIH 3T3 fibroblasts adhere to the untreated Pyrex substrate, while no cell adheres to the PEG-treated Pyrex substrate, indicating the Pyrex substrate is modified as a cell-resistive surface through PEG treatment. (C) Measured contact angles of untreated gold surface (67.3±2.5°, left), thiol-treated one (53.3±1.3°, middle), and RTT-functionalized one (24.6±2.8°, right), verifying the surface modification of the gold surface using RTT.

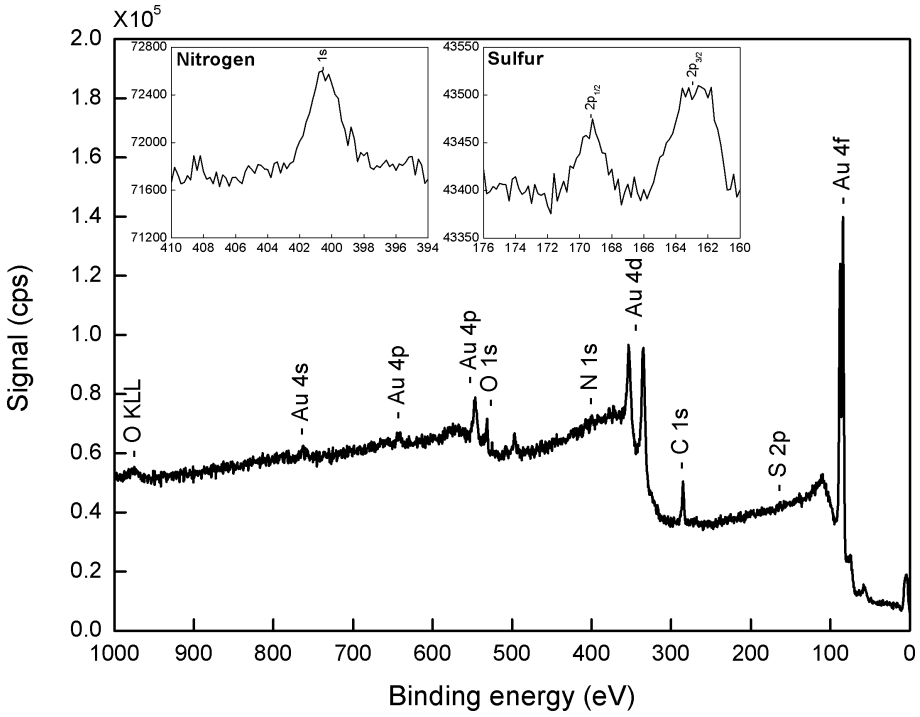

Fig. S3 XPS survey spectrum of the RTT-functionalized gold surface. Detected are gold peaks from the gold electrodes of the BBB, sulfur peaks from the thiol, and the peaks of carbon, oxygen, and nitrogen from the amine and carboxylic groups of the RGD peptide.

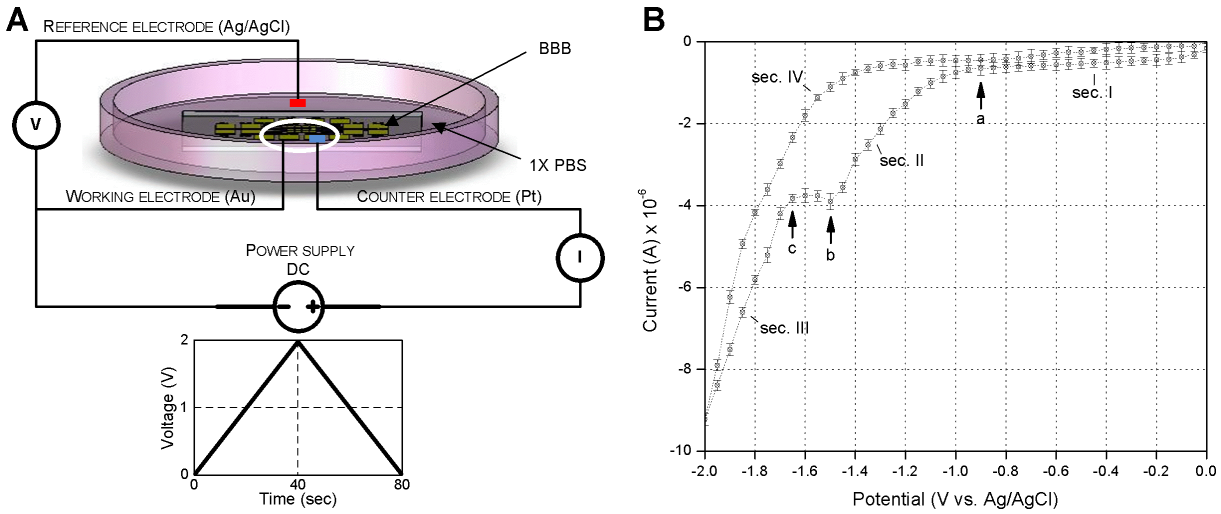

Fig. S4 Potentiodynamic electrochemical characterization of the reductive desorption of a gold-thiol SAM in DPBS solution (pH 7.4). (A) Experimental setup for the measurement of cyclic voltammetry where the gold electrodes of the BBB, a platinum electrode, and an Ag/AgCl electrode are worked as working, counter, and reference electrodes, respectively. (B) Measured cyclic voltammetry, showing the reductive desorption of a gold-thiol SAM begins and ends at -0.90 V and -1.65 V respectively, and get maximized at -1.50 V. All points indicate mean ± standard error (of the mean) values, obtained from five independent measurements.

Reusability characterization of BBB

The reductive desorption of a gold-thiol SAM yields bare gold again, following the electrochemical reaction of $R{-}S{-}Au + H^+ + e^- \rightarrow R{-}S{-}H + Au$. This electrochemical reaction lays the foundation of the reusability of the BBB. Thus, the BBB can be said as a cost-effective and environment-friendly technology for the spatiotemporal manipulation of cell adhesion and detachment.

The programmable cell patterning of NIH 3T3 fibroblasts was conducted using a single four-by-four BBB to demonstrate the reusability of the BBB (see Fig. 4F). In the programmable cell patterning, the BBB was cleaned after each ease in the following method (Fig. S5): (i) all cells were detached by activating all gold electrodes of the BBB after each experiment; Trypsin-EDTA solution 0.5% (1X) (Sigma-Aldrich) was added to the BBB to completely remove cellular material known as integrin remnants;[38] the integrin remnants were also removed by sonificating the BBB in acetone (Fisher Scientific) at room temperature for 8 minutes; the BBB was rinsed 3 times in ethanol (Fisher Scientific), and then was rinsed 7 times in deionized (DI) water. The cleaned BBB was inspected with an optical microscope to examine the surface (i.e., gold electrodes) of the BBB. One or two remnants were occasionally observed on the gold electrodes of the cleaned BBB (Fig. S5C, circle) which did not cause any problem in the reuse of the BBB. This shows that the proposed cleaning method is enough to secure the reusability of the BBB. The BBB is thought to contribute in minimizing the waste of disposable experimental stuffs in biological experiments, thus solving a major concern (i.e., protection of the environment) among all of us.

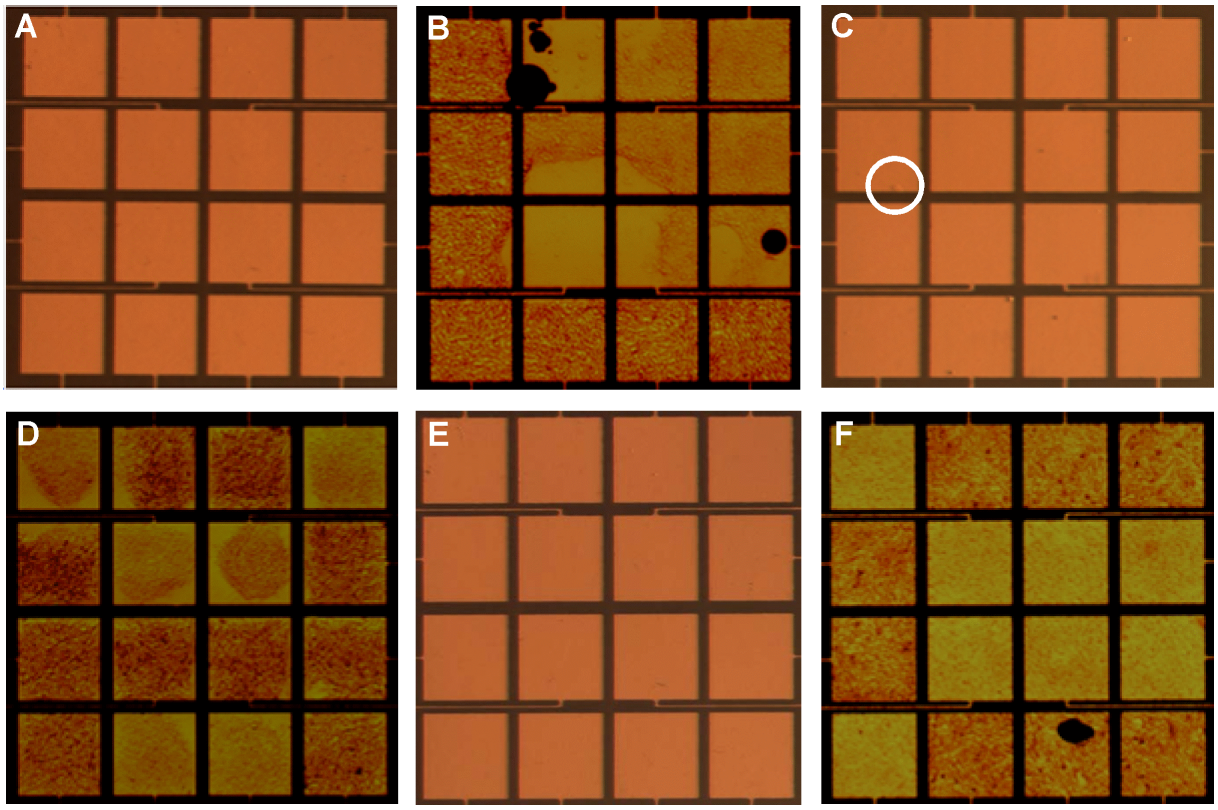

Fig. S5 Reusability characterization of the BBB. (A) Unused BBB. (B) First use of the BBB. (C) First cleaning of the BBB. (D) Second use of the BBB. (E) Second cleaning of the BBB. (F) Third use of the BBB.

Table S1 Biochemical reagents for surface modifications.

Solution	Reagent	Manufacturer	Amount	Comments
PEG solution	Polyethylene glycol (PEG), $C_3H_9O_3Si(C_2H_4O)_{9\text{-}12}CH_3$	Gelest, Inc.	2 ml	2% v/v
	Hydrochloric acid, HCl	Fisher Scientific	1 ml	1% v/v
	Anhydrous toluene, $C_6H_5CH_3$	Fisher Scientific	97 ml	
RTT solution	*cyclo* (Arg-Gly-Asp-D-Phe-Lys), $C_{27}H_{41}N_9O_7$	Peptides International, Inc.	3.02 mg	1mM aliquot
	Dimethyl sulfoxide (DMSO), $(CH_3)_2SO$	Sigma-Aldrich	5 ml*	
	Triethylamine, $N(CH_2CH_3)_3$	Fisher Scientific	50 µl	1% v/v
	Dithiobis(succinimidyl undecanoate), $C_{30}H_{48}N_2O_8S_2$	Dojindo, Inc.	3.14 mg	1mM aliquot
	Dimethyl sulfoxide (DMSO), $(CH_3)_2SO$	Sigma-Aldrich	5 ml**	

* DMSO used for 1mM RGD aliquot.
** DMSO used for 1mM thiol aliquot.

Videos of cell detachment

Video S1. Cell detachment of two cells (0% cell confluence) at an activation potential of -1.5 V.

Video S2. Cell detachment of subconfluent cells (25% cell confluence) at an activation potential of -1.5 V.

Video S3. Cell detachment of confluent cells (100% cell confluence) at an activation potential of -1.4 V.

Video S4. Cell detachment of confluent cells (100% cell confluence) at an activation potential of -1.6 V.

Video S5. Cell detachment of confluent cells (100% cell confluence) at an activation potential of -1.8 V.

References

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38 S. P. Palecek, C. E. Schmidt, D. A. Lauffenburger and A. F. Horwitz, *Journal of Cell Science*, 1996, **109**, 941-952.