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Supporting Information

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Electrically Addressable Cell Immobilization Using Phenyl Boronic Acid Diazonium Salts

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Supporting Information

Experimental

Reagents

Aqueous solutions were prepared with 18 MΩ water using a Barnstead Nanopure water purifier (Boston, MA). Sodium periodate ($NaIO_4$), sodium chloride, anhydrous acetonitrile (ACN), tetrahydrofuran (THF), and toluene were purchased from Acros Organics (Beel, Belgium). Sodium phosphate monobasic, sodium phosphate dibasic, fructose, and 30% H_2O_2, and Trypan Blue solution (0.4%) were purchased from Sigma (St. Louis, MO). Nitrosonium tetrafluoroborate, diethyl ether, and tetrabutylammonium tetrafluoroborate (Bu_4NBF_4) were obtained from Aldrich. Sulfuric acid, ethyl alcohol (95% denatured) was purchased from Fischer Scientific (Pittsburgh, PA). Tris-HCl was purchased from Fluka (Buchs, Switzerland). All reagents were used as received unless otherwise noted.

Synthesis of Phenyl Boronic Acid Pinacol Ester Diazonium:

Nitrosonium Tetrafluoroborate (Aldrich, used as received, 0.315g 2.69 mmoles) was dissolved in anhydrous acetonitrile (5mL) under nitrogen and then cooled to -40 °C. The 4-aminophenylboronic acid pinacol ester (Aldrich, used as received, 0.53g 2.42 mmole) was dissolved in 12 mL of anhydrous acetonitrile under nitrogen. The amine solution was added slowly by cannula to the stirred -40 °C solution of the $NOBF_4$. The resulting solution was stirred 1 hour at -40 C and then allowed to warm to 0 °C and then stirred an additional 10 minutes. This solution was cannulated into 800 mL of rapidly stirred cold diethyl ether in air. The resulting precipitate was collected on a buchner funnel and dried under vacuum. 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzenediazonium 0.64 grams 2.0 mmoles 83%.

Synthesis of 1-(4-methoxyphenyl)-2-methylpropane-1,2-diol (MPMP-diol):

MPMP-diol was synthesized by a variation of the method described in reference 22. 2-(4-methoxyphenyl) ethyl acetate was allowed to react with excess methylmagnesium bromide in THF to provide after aqueous workup 1-(4-methoxyphenyl)-2-methylpropan-2-ol. This was brominated with NBS in carbon tetrachloride at reflux, 12 hours. Hydrolysis of the resulting 1-bromo-1-(4-methoxyphenyl)-2-methylpropan-2-ol was performed with freshly prepared silver carbonate in aqueous acetone. The resulting MPMP-diol was identical spectroscopically with that reported in reference 22.

Electrochemical Instrumentation

All electrochemical measurements were performed on a PGZ100 Voltalab potentiostat (Radiometer Analytical, Lyon, France) or an Autolab PGSTAT12 potentiostat (Eco Chemie, The Netherlands) and were measured versus a Ag/AgCl reference (3M, aqueous solutions) or a $Ag/AgNO_3$ reference (10 mM, non-aqueous solutions, -102 mV vs. ferrocene couple) and a Pt counter electrode from Bioanalytical Systems (West Lafayette, IN). All potentials reported herein are with respect to the relevant reference electrode. 250 μm diameter gold disk arrayed electrodes, spaced 1.5 mm apart, and 5 mm diameter gold disk electrodes were prepared via thermal evaporation of a 200Å Cr adhesion layer followed by 2000Å of Au onto a Pyrex wafer. Au electrodes were cleaned immediately before use with freshly prepared piranha (5:3 conc. sulfuric acid: 30% H_2O_2) for 5 min, washed with nanopure water, and dried under a stream of nitrogen.

Grazing Angle FTIR, Contact Angle, and Ellipsometry Measurements

Lithographically defined gold disk electrodes, 5 mm diameter, were used for grazing angle FTIR, contact angle, and ellipsometry measurements. FTIR measurements were obtained with a Nicolet 6700 Fourier transformed infrared spectrometer with a liquid nitrogen cooled mercury-cadmium-telluride (MCT) detector. An external specular reflectance attachment (SMART SAGA) was used to obtain an incidence angle of 80° with unpolarized light. 1024 scans were collected for each spectrum with a spectral resolution of 4 cm^{-1} using Happ-Genzel apodization. Background reference spectra were obtained immediately before collecting sample spectrum. All spectra are reported as $\log(R/R_0)$ where R is the reflectivity of the modified gold and R_0 is the reflectivity of the unmodified gold. Contact angel measurements were obtained using a

Video Contact Angle System, VCA 2500, (Advanced Surface Technology, Inc., Billerica, MA) and analyzed using VCA 2500 software, ver. 2.05. Ellipsometry measurements were performed using a Gaertner Scientific Corporation L166 S Stokes Ellipsometer with a 2 mW HeNe (λ = 632.8 nm) laser, an incidence angle of 70º, and film refractive index, n_f = 1.5. Initial substrate measurements were performed on each electrode following piranha cleaning.

Electrode Functionalization

Phenyl boronic acid pinacol ester thin films were assembled onto clean gold electrodes using cyclic voltammetry in a solution of 1 mM phenyl boronic acid pinacol ester diazonium and 0.1 M Bu_4NBF_4 in ACN. After electrodeposition, the electrodes were briefly rinsed with ACN, followed by a rinse with ethanol and a 15 second sonication in ethanol to remove any absorbed phenyl boronic acid pinacol ester diazonium. After sonication the electrodes were again rinsed in ethanol and dried under a stream of nitrogen. The electrodes were then treated with 100 µl 50 mM $NaIO_4$ (4:1 Water:THF) for 30 min to remove the pinacol blocking ester, rinsed with water and dried under nitrogen. The unblocked electrode was then treated with 100 µl of 10 mM 1-(4-methoxy-phenyl)-2-methyl-propane-1,2-diol (MPMP-diol) reprotection unit in anhydrous toluene for 30 min. The second deprotection was performed electrochemically by applying a + 0.6 V potential to the electrode in 0.1 M phosphate buffer, pH 7.4, for 60 sec followed by rinsing with water and drying under nitrogen.

Cell Immobilization Procedure

Prepared electrodes were first conditioned for one hour in 100 mM Tris-HCl, pH 8.5, washed with water and dried with nitrogen. 100 µl of yeast cells (1×10^7 cells/ml) or macrophage cells ($1\text{-}3 \times 10^7$ cells/ml) in 0.1 M phosphate buffer, pH 7.4, were placed onto the electrodes for two min, and gently washed three times with buffer.

Cell preparation

A single colony of S. cerevisiae strain INVSc1 (Invitrogen, Carlsbad, CA) was inoculated into 5 ml of YPD (Becton, Dickinson, Sparks, MD). Solution was cultured overnight at 30°C in a shaking incubator at

250 rpm. OD_{600} was measured. Overnight culture was then diluted into fresh YPD to an OD_{600} equal to 0.5 in a total volume of 1ml of YPD. Murine macrophage strain RAW 264.7 (ATCC, Manassas, VA) was maintained according to the manufacture protocol.

Cell Release and surface regeneration and reconditioning

Electrodes were treated with 100 µl of 20 mM fructose in 100 mM Tris-HCl, pH = 8.5 for 30 min and washed three times with buffer. The regeneration of the boronic acid groups was accomplished by removing bound fructose with 100 µl of 0.1 M phosphate solution, pH = 3.3, for 30 min followed by rinsing with buffer and conditioning the electrodes in 0.1 M Tris-HCl, pH = 8.5, for 12 hours.

Microscope Imaging

Microscope images were obtained using a WESCO CXRII upright microscope and recorded using a Sony XCD-SX910 camera. Cell viability tests were performed using trypan blue dye staining according to the manufacture's protocol.