Supporting Information

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Bio-orthogonal Polymer Coatings for co-Presentation of Biomolecules

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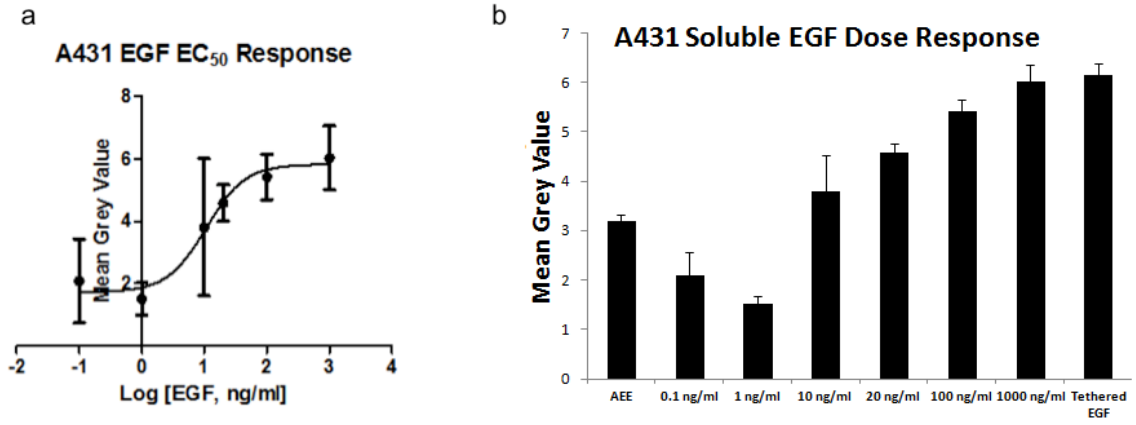

Figure S1. (a) EC_{50} plot for EGF (individual trial); (b) Quantification of pEGFR immunfluorescence of phosphorylated EGFR in A431 cells in bar graph form (individual trial). The cells were cultured on CVD-coated surfaces passivated with AEE and in media with soluble EGF from 0-1000 ng/ml, in comparison with surface with tethered EGF in soluble EGF-free media.

Adhesion and Spreading of Endothelial Cells

Copolymer **3** was deposited via CVD onto silicon and modified as outlined above to present tethered EGF, cRGD, EGF+cRGD, or passivated with 2-(2-aminoethoxy)ethanol (AEE). Once modified, these surfaces were washed thoroughly and placed in a 24-well plate. Human umbilical vein endothelial cells (HUVEC, Lonza) were cultured on the modified surfaces at a concentration of 5×10^4 cells/ml for 4 hours in serum-free EBM (Lonza). Passages 3-6 were used for all experiments. The cells were then fixed with 4% paraformaldehyde in DPBS and stained for actin with Alexa Fluor® 568-phallodin (Invitrogen). The surfaces were mounted with ProLong Gold + DAPI (Invitrogen) and imaged using an Olympus BX-51 fluorescence microscope. The average area of the cells was measured via ImageJ, and performed by dividing total cell area by total number of cells (equated to total number of individually counted DAPI stains). The results show three independent trials averaged; error bars are standard error (standard deviation divided by the square root of the total number of images taken over three trials), and p-values are calculated using all images from all trials.

Phosphorylated EGFR Immunofluorescence

The epidermal carcinoma line A431 (ATCC, Manassas, VA) was cultured in polystyrene flasks (Corning, Lowell, MA) at 37 ºC and in a humidified 5% CO_2 atmosphere until 100% cell confluence were observed. Passage 3 was used for all experiments. 18 hours prior to incubation with the modified surfaces, the A431 culture was serum-starved. A431 cells were trypsinized, spun down at 100xg, and resuspended with serum-free DMEM (ATCC) to a cell concentration of 5×10^4 cells/ml. A volume of 0.5 ml of A431 suspension was added to each well containing a surface. After 90 minutes of incubation at 37 ºC/5% CO_2, the cells were briefly washed with PBS, then fixed with 4% paraformaldehyde. After three five-minute PBS washes, the surfaces were incubated with blocking buffer (5% normal goat serum (Invitrogen, Carlsbad, CA), 0.3% Triton-X 100 (Sigma-Aldrich) in PBS) for one hour followed by

overnight incubation with primary antibody Phospho-EGF Receptor (Tyr1068) (D7A5) XP™ Rabbit mAb (Cell Signaling Technology, Danvers, MA). Incubation with secondary antibody Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 488 Conjugate) for two hours followed; finally the surfaces were cured on glass slides with ProLong Gold + DAPI (Invitrogen) and imaged using an Olympus BX-51 fluorescence microscope. The quantity of immunofluorescence as a ratio of total grey pixilation to cell area minus threshold was measured via ImageJ. The results show three independent trials averaged; error bars are standard error (standard deviation divided by the square root of the total number of images taken over three trials), and p-values are calculated using all images from all trials.

EGF EC_{50} measurement

A431 cells were cultured as previously described, and starved for 18 hours prior to incubation. Cells were trypsonized and cultured on AEE-modified surfaces, then incubated with a range of soluble EGF concentrations from 0.1 to 1000 ng/ml for 1 hour. pEGFR was then measured via the immunofluorescence protocol described above, including the same primary and secondary antibodies. EC_{50} data was fitted and analyzed using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego California USA) using the modified variable slope model.

Image and Statistical Analysis

The software package ImageJ (NIH) was used to analyze all images. Normalized cell spreading was quantified by measuring the total cell area and dividing by the total number of cells (counted by DAPI spot). Magnitude of fluorescence quantification was performed by converting the images to a 32-bit gray scale and then measuring the mean gray value. All error bars with the exception of the EC_{50} graph (which show standard deviation) represent standard error (one standard deviation from the mean divided by the square root of the total number of images averaged). Statistical significance was determined using Students two-tailed T-test assuming unequal variance, with $p<0.05$ considered significant.