Supplementary Material

Real-Time Monitoring Biomarker Expression of Carcinoma Cells by Surface Plasmon Resonance Biosensors

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Control experiments:

Calcium ionophore effects: We have tested calcium ionophore in the absence of cells as one of major control experiments. The result shows no significant interaction between calcium ionophore and surface immobilized anti-VEGF since there is no change on SPR signal observed by calcium ionophore injection. We have performed control experiments without antibody immobilization. Results show no significant SPR angle shift upon VEGF or calcium ionophore injections.

Calibration study: We have performed antibody saturation experiments and dynamic range experiments. The sensor was saturated at 4μg/mL VEGF. Linear response to VEGF can be obtained from 0.1μg/mL to 2.5μg/mL. Results have been added to Fig 1A and B. However, for better presentation and clarity of data plots, we did not show SPR curves for 3, 4μg/mL VEGF samples.

200 μM MitoTracker Red CMXRos dye stock solution and the 1.0 mM Hoechst 33342 dye were diluted into HBSS or appropriate cell medium with serum. The concentration for MitoTracker Red CMXRos dye is 10–50 nM; The concentration for Hoechst 33342 dye is 1.0 μg/mL. Both dyes may be combined in a single staining solution. Then apply a sufficient amount of labeling solution to cover cells adhering to substrates and the cell number is counted. We calculated the single cell VEGF release amount by the following equation: VEGF concentration (obtained from calibration curve) × flow chamber volume (2μL) / cell number. Incubate for 15 minutes at 37°C. When labeling is complete, remove the labeling solution and wash cells twice in cell medium. Labeled cells will then be fixed with 4% formaldehyde for 15 minutes at 37°C, followed by washes in buffer and staining with any additional counterstains.

The substrates were then removed and mounted on glass microslides with antifade reagent/mounting medium mixture. Then, the specimens were observed by fluorescence microscopy (Olympus IX81, Japan) with a 20x objective. The fluorescence was imaged at Hoechst channel (nucleus stain dye, ex/em: 358/461 nm), and λex (570), λem (590 nm) for MitoTracker Red. A CCD camera was used to capture the signals and the images were software-merged with pseudo color. The fluorescence microscope settings were kept the same throughout the experiment with the exception of the exposure time. The images were recorded at the same exposure time for Hoechst and MitoTracker.

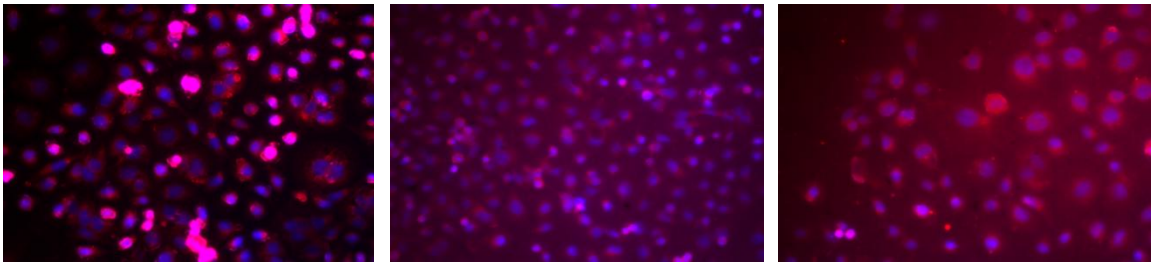

Fig. S1. Fluorescence images of SKOV-3 cell culture on: (left) petri dish, (middle) gelatin coated PDMS and (right) uncoated PDMS.