Supporting Information (SI)

Micropatterned Surfaces Functionalized with Electroactive Peptides for Detecting Protease Release from Cells

Dong-Sik Shin, Ying Liu, Yandong Gao, Timothy Kwa, Zimple Matharu, Alexander Revzin*

Department of Biomedical Engineering, University of California, Davis, CA 95616

arevzin@ucdavis.edu

Optimization of MB-peptide immobilization on Au electrode

Au electrodes were treated with various concentrations (0.05-5 mM) of MB-peptide solutions to measure a peptide concentration for a saturated point of immobilized peptide density. SWV signals were then measured on each electrode. The results presented in Figure S1 demonstrate that redox signal from the peptide becomes independent of peptide solution concentration above 0.5 mM, suggesting surface saturation.

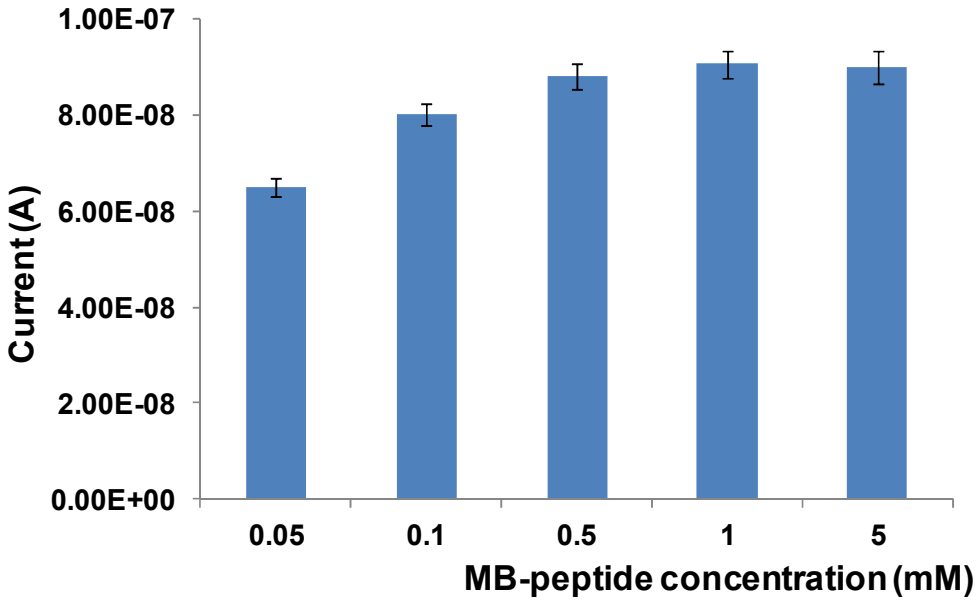

Figure S1. SWV signals on surfaces treated with various concentrations of MB-peptides.

MMP9 assay from cells using a Fluorimetric MMP9 assay kit

The degree of release of MMP9 was determined by using a commercial kit for MMP9 assay according to the manufacturer's instructions (SensoLyte 490 MMP9 Assay Kit, Fluorimetric, Anaspec, Fremont, CA). The fluorescence was detected emission at 490 nm with excitation at 340 nm using a fluorometer (Safire II Fluorometer, Tecan, Durham, NC) [1, 2]. All solutions

were prepared in a 96-well plate (Nunc black 96 well optical bottom plates, Thermo Scientific, Rochester, NY) in the dark.

Calibration curve was obtained using recombinant MMP9 with a range at 0.3-5.5 nM. MMP9 solution from U-937 cells was prepared as described in literature [3, 4]. Monocytes were cultured in 10% (v/v) fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin in RPMI-1640 media. The media was removed by centrifugation followed by washing the monocytes with PBS. PBS in the cell suspension was exchanged by serum-free and phenol red-free RPMI media. The density of monocytes was 360,000 cells/mL. PMA was added into the media adjusted a final concentration to 100 ng/mL and a control sample was prepared without PMA solution. Both cultures were incubated for 24 hr and solutions of cell cultured media were taken and centrifugedfor 10 min at 3000 rpm. Supernatants were collected and used for MMP9 assay.

The calibration curve for recombinant MMP9 concentration vs. fluorescence intensity at 490 nm showed a linear range at 0.3-5.5 nM of MMP9 concentration at 1 hr proteolytic reaction (Figure S2). The fluorescence intesities were measured with supernatant solutions from activated or non-activated cell cultured media. The fluorescence intensities were 3,598 A.U. from activated monocytes and 1,674 A.U. from non-activated monocytes. The MMP9 release from U-937 cells was calculated using the equation obtained from the curve. U-937 cells activated by PMA secreted MMP9 3.5-fold more than those without activation (0.00575 pg $cell^{-1}$ hr^{-1} with PMA activation vs. 0.00165 pg $cell^{-1}$ hr^{-1} without PMA activation).

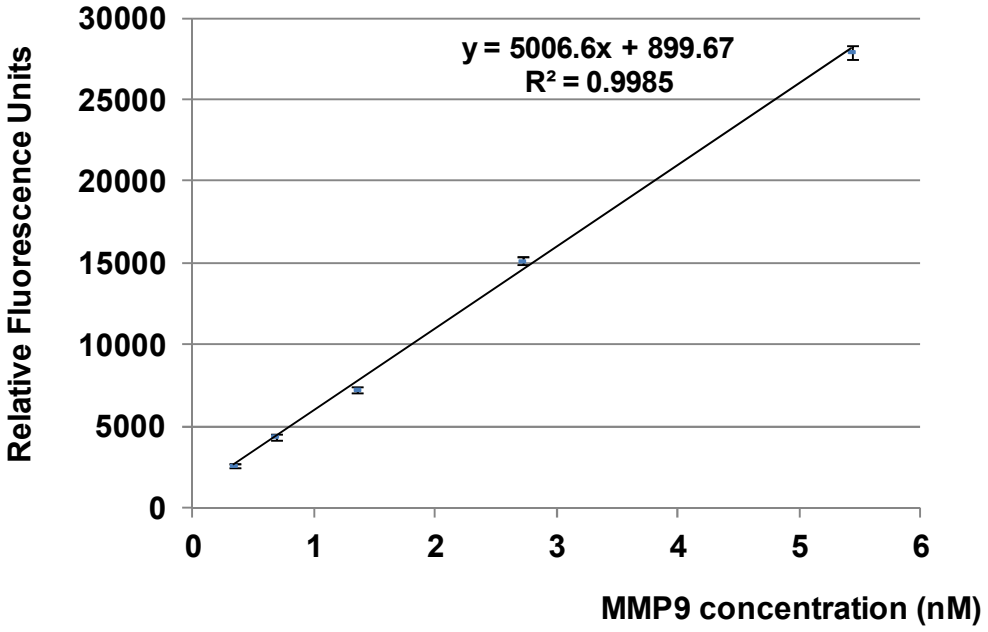

Figure S2. Calibration curve from fluorescence intensity at 490 nm of EDANS-Dabcyl peptide substrate using recombinant MMP9.

References

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[2] J.H. Oh, J.H. Kim, H.J. Ahn, J.H. Yoon, S.C. Yoo, D.S. Choi, I.S. Lee, H.S. Ryu, C.K. Min. Syndecan-1 enhances the endometrial cancer invasion by modulating matrix metalloproteinase-9 expression through nuclear factor κB. *Gynecol. Oncol.* **2009**, *114*, 509–515.

[3] M.W. Roomi, J.C. Monterrey, T. Kalinovsky, M. Rath, A. Niedzwiecki. Patterns of MMP-2 and MMP9 expression in human cancer cell lines. *Oncol. Rep.* **2009**, *21*, 1323-1333.

[4] M.W. Roomi, J.C. Monterrey, T. Kalinovsky, M. Rath, A. Niedzwiecki. Inhibition of invasion and MMPs by a nutrient mixture in human cancer cell lines: A correlation study. *Exp. Oncol.* **2010**, *32*, 243-248.