Supporting Information (SI)

Micropatterned sensing hydrogels integrated with reconfigurable microfluidics for detecting protease release from cells

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Specificity of peptide beacons for detection of MMP9

In our previous work, we showed the substrate specificity of MMP9 by challenging the MMP9 specific peptide with nonspecific proteases such as tumor necrosis factor-α-converting enzyme (TACE) and urokinases-type plasminogen activator (uPA).[1] Furthermore, we examined the dependence of sensing capabilities on configuration of methionine in MMP9-specific peptide. MMP9-specific peptide (Gly-Pro-Leu-Gly-Met-Trp-Ser-Arg-Lys-Cys; GPLGMWSRKC) and MMP9-nonspecific peptide (Gly-Pro-Leu-Gly-D-Met-Trp-Ser-Arg-Lys-Cys; GPLGmWSRKC) were used as sensing elements for protease detection. The sensing capability of MMP9-specific peptides depends on configuration of Met, which was demonstrated in Figure S-1. Two different types of peptides, which have D-Met and L-Met, were conjugated to maleimide-functionalized hydrogel. Challenged with 10nM recombinant MMP9 solution, the changes in fluorescence for both peptide hydrogels were monitored. In Figure S-1, peptides with L-Met exhibit significant fluorescence increase, while peptides with D-Met (inactive form) show only a slight increase. This result verifies that our peptide containing sensing hydrogels are cleaved specifically by MMP9 molecules, providing highly sensitive detection of proteases.

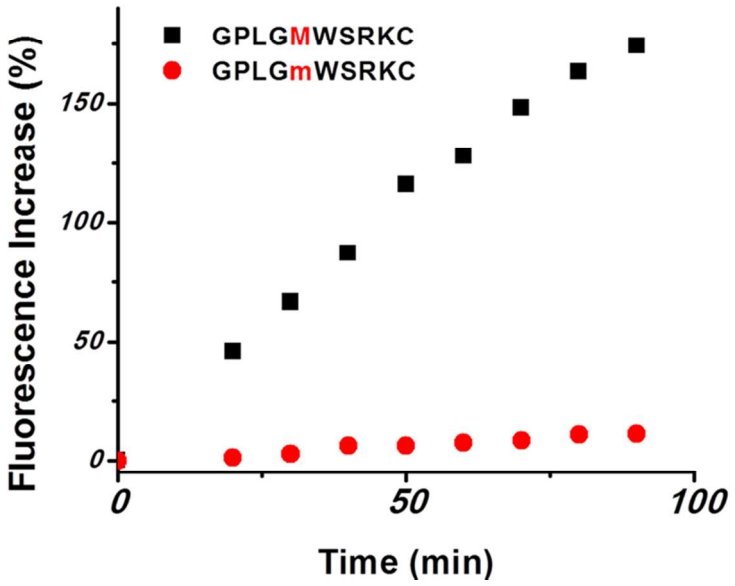

Figure S-1. Peptide-dependence of our sensing hydrogel biosensor: Responses of (black square) active peptide with L-Met and (red dot) inactive peptide with D-Met containing hydrogels to 10nM protease.

To verify the sensitivity of FRET-peptide, MMP9 release from different cell types was determined using fluorescence spectroscopy (Infinite M1000, Tecan, Durham, NC). The fluorescence of (FITC/Dabcyl) FRET-peptide was measured at the emission wavelength (525nm) with the excitation wavelength (490 nm). MMP9 solutions from U-937 and primary hepatocyte were prepared. Primary hepatocytes were isolated from adult female Lewis rats (Charles River Laboratories, Boston, MA, USA) and purified as described in previous literature[2], and cultured in 10% (v/v) fetal bovine serum (FBS), 200 U/ml penicillin , 200 µg/ml streptomycin, 7.5 µg/ml hydrocortisone, 20 ng/ml EGF, 14 ng/ml glucagon, and 0.5 U/ml insulin in DMEM media. U-937 lymphoma cells were cultured in RPMI-1640 media supplemented with 10% FBS, 100 U/mL penicillin and 100 µg/ml streptomycin. For the experiment, media for both cells were exchanged by serum-free and phenol red-free RPMI media and the density of cells was set at

300,000 cells/mL. Cells were stimulated by adding PMA into the cell media (final concentration = 100 ng/mL), and incubated for 2 hr. Cell culture media were centrifuged for 5 min at 4500 rpm and the supernatants were collected for MMP9 assay. All solutions were prepared in a 96 well plate (Nunc black 96 well optical bottom plate, Thermo Scientific, Rochester, NY) in the dark. Calibration curve was obtained using recombinant MMP9 with a range at 0.078-5 nM. As shown in Figure S-2, the calibration curve for recombinant MMP9 was obtained with a linear range at 0.078-5 nM of MMP9 concentration at 1 hr proteolytic reaction ($R^2 = 0.9672$). The fluorescence intensities were measured with supernatant solutions from PMA activated or non-activated cell cultured media. The MMP9 release from U-937 and primary hepatocytes was calculated using the calibration curve. Figure S-2B shows that stimulated U-937 cells secreted MMP9 10-fold more than any other cells.

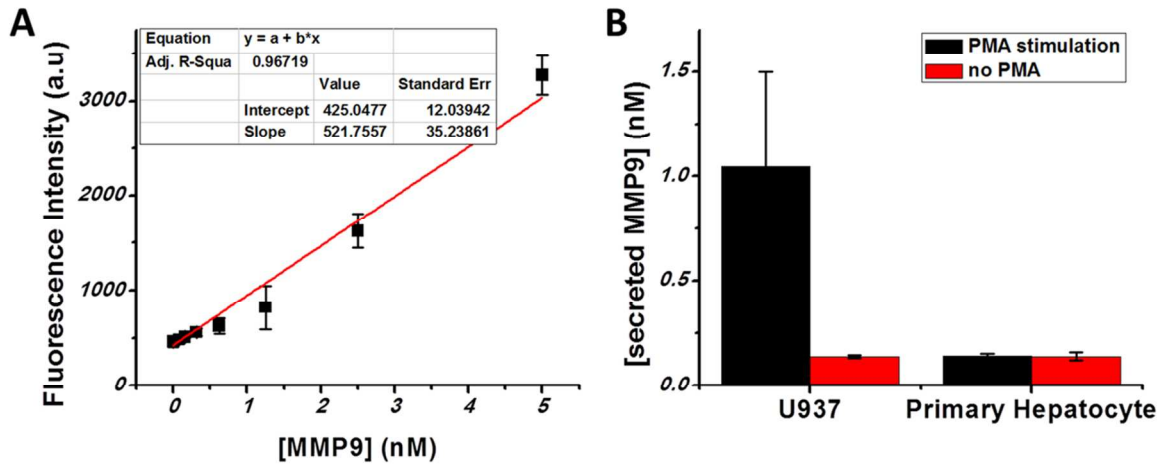

Figure S-2. Dependence of MMP9-sensing peptides on cell-type. (A) Calibration curve from fluorescence intensity at 525 nm of FITC/Dabcyl peptides using recombinant MMP9 (0 – 5 nM). (B) The concentration of released MMP9 from U-937 and primary hepatocytes (black, stimulated cells; red, quiescent cells).

Effect of Captured Lymphoma Cells Number on Detection of MMP9 Secretion

Different number of cells (28 and 11 lymphoma cells) was captured on the Ab-modified region inside the sensing hydrogel microwells (diameter = 100 μm) as shown in Figure S-3 (B). Figure S-2 (A) indicates time-lapse fluorescence images to monitor MMP9 secretion from mitogenically activated U-937 cells for 2 hr. Consequently, it was analyzed quantitatively by fluorescence increase in Figure S-3 (C), showing that a group of cells (28 cells) released more MMP9s and produced about 2-fold higher signal than other group of cells (11 cells). It is also of note that our sensing hydrogels are able to detect MMP9s secreted from a small group of cells (11 cells) with significant fluorescence signal (~ 30 %). Based on this result, our future works will be focused on creating high density gel sensor arrays for high-throughput single-cell analysis. Upon optimization, these sensing hydrogels could be used to monitor cancer cell-cell interaction and elucidate some signaling molecules involved in tumor metastasis.

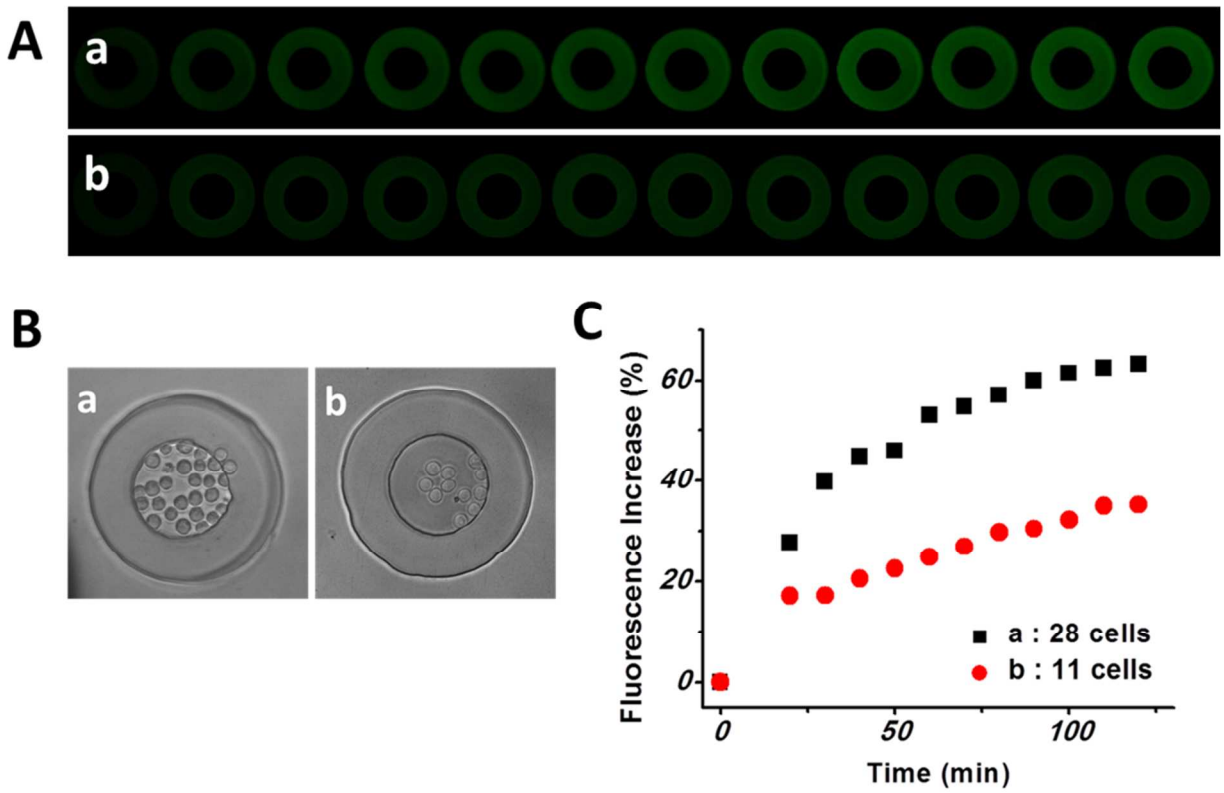

Figure S-3. Comparison of differences in fluorescence increase depending on the number of captured lymphoma cells. (A) Time-lapse fluorescence images of sensing hydrogel with a. 28 cells captured and b. 11 cells captured (0 – 120 min). (B) The bright-field images: a. 28 cells captured and b. 11 cells captured. (C) Quantitative analysis: fluorescence increase profile of each well with different number of captured U-937 cells.

Fluorescence-based Detection of Peptide Cleavage.

Proteolytic cleavage of MMP9-specific peptide was monitored using fluorescence microscope as shown in Figure S-4. The fluorescence signal of peptide containing sensing hydrogels increases as concentration of MMP9 solution increases, demonstrating that cleavage of peptide by MMP9

generates fluorescence increase. Fluorescence images illustrated in Figure S-4 were quantitatively analyzed in Figure 5A.

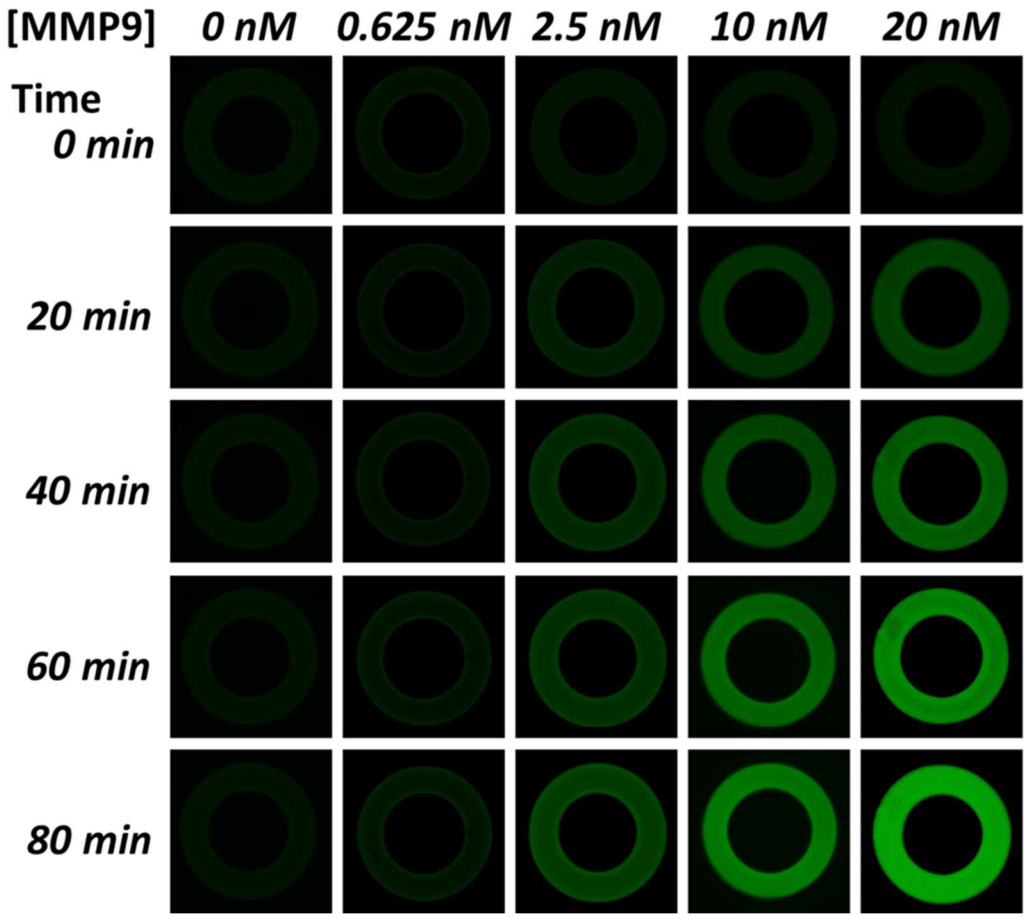

Figure S-4. The fluorescence images of FRET-peptide containing sensing hydrogels in various concentration of MMP9 solutions (0 – 20 nM) over time.

Analysis of Cross-Talk Between Adjacent Sensing Wells

Microstructured reconfigurable microfluidic device was exploited to enhance sensitivity as well as to restrict cross-talk between wells. When the device was actuated, the membrane with microchambers (radius = 500 μm) descended on the sensing hydrogel microwells, isolating microwells as shown in Figure 3B. To prevent the diffusion of MMPs to adjacent sensing wells,

the distance (d_*) between wells was set to 2 mm. Figure S5 (A) illustrates the simulation verifying diffusion of MMPs secreted from one sensing well to the next. For simplicity, only cells in chamber (I) were assumed to release MMPs. The concentration profile of MMPs in Figure S5 (B-C) was developed by solving Equation (3) – (6). These results show that no cross-talk exists between the two sensing gel wells separated by 2 mm – the distance that was used for all of the experiments reported here. The analysis of distance dependence on cross-reactivity of adjacent wells shows that at closer distance one would expect to observe cross-diffusion (Figure S5D). We note that bringing the sensing wells closer together will lead to cross-diffusion between the wells as shown in Figure S5D. However, we envision this being less of a problem for experiments utilizing small groups of cells. In addition, gel composition may be tailored in the future to slow the diffusion through the gel and to further minimize cross-reactivity between the wells.

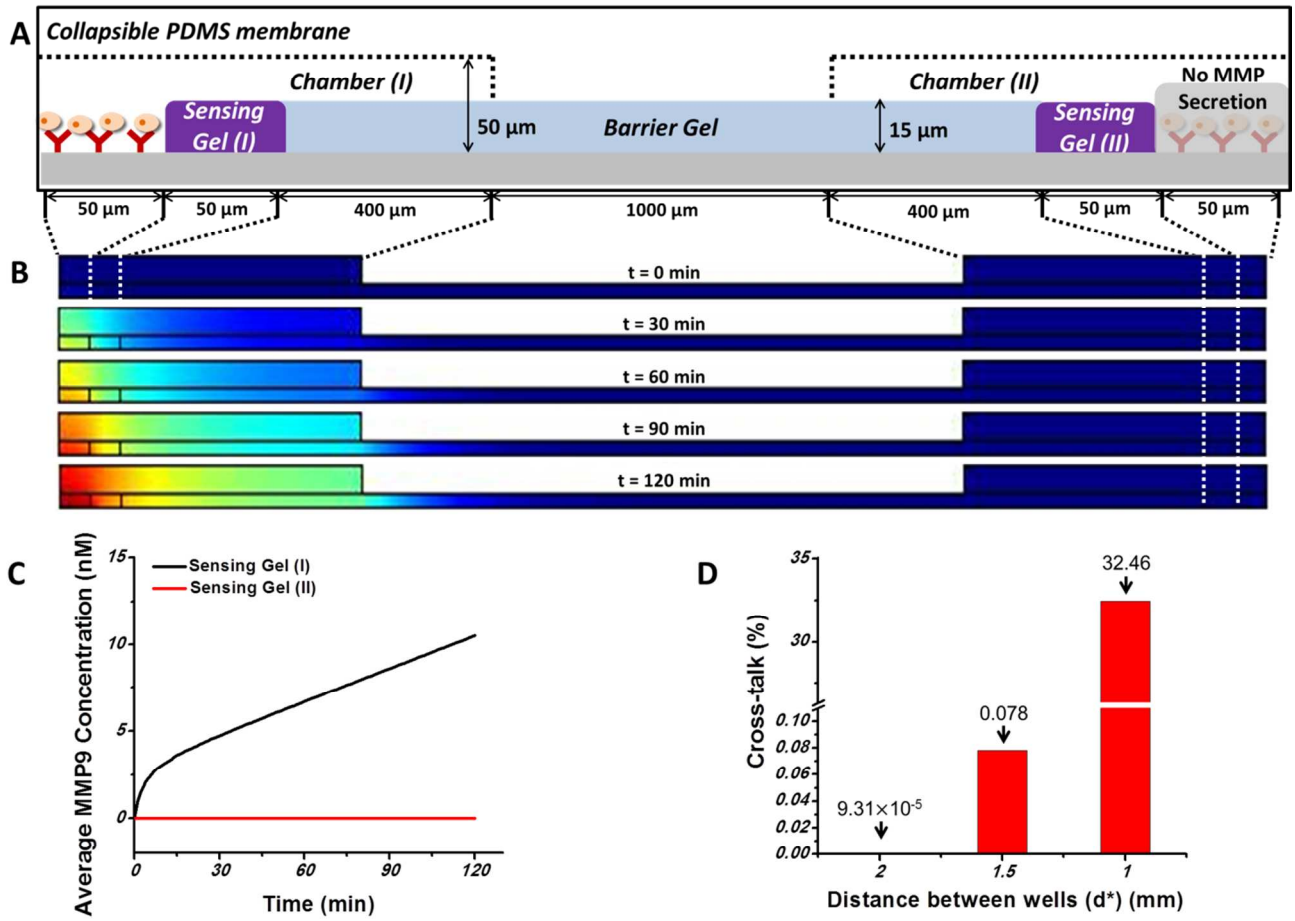

Figure S5. Numerical simulation of cross-reactivity between the adjacent wells. Secretion, diffusion and reaction are modeled in concert to determine concentration profiles. (A) Cross-sectional view of the two adjacent sensing wells being simulated. (B) Simulation results showing MMP9 concentration profile over time. MMP9s are secreted by a group of cells (n = 24) in Chamber I with a secretion rate of 0.575 pg/hr/cell, and diffused to sensing/barrier hydrogel. (C) Change in average MMP9 concentration inside the sensing hydrogel matrix: sensing gel (I) (black) vs. sensing gel (II) (red). (D) Percentage of cross-talk with respect to distance between wells. Cross-talk (%) = (Fluorescence increase in sensing gel (II)) / (Fluorescence increase in sensing gel (I))×100.

(1) Shin, D. S.; Liu, Y.; Gao, Y. D.; Kwa, T.; Matharu, Z.; Revzin, A. *Anal Chem* **2013**, *85*, 220-227

(2) Dunn J. C.; Yarmush, M. L.; Koebe, H. G.; Tompkins, R. G. *FASEB J* **1989**, 3174–3179