Supporting Information

Synthesis of microgel sensors for spatial and temporal monitoring of protease activity

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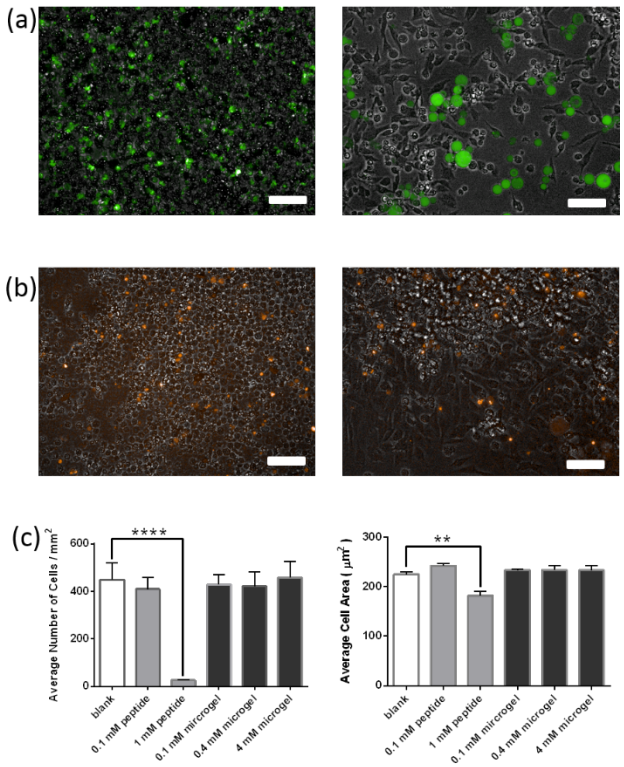


Figure S1. (a) Typical image of A375 cells after one day incubated with 1 mM sensor peptides (left) or sensor microgels containing 4mM peptide (excitation 460-490 nm, emission 500-550 nm). Scale bar: 50μm. (b) Representative image of cells labeled with ethidium homodimer to identify dead cells after one day. A375 cells were incubated with either sensor peptides (left) or sensor microgels (right). The cells incubated with the sensor peptide show a higher proportion of dead cells and smaller, less spread morphology. Scale bar: 50μm. (c) Average cell density (number/mm^2, left) and average cell area (right) of cells incubated for 3 days with sensor microgels (synthesized with 0.1 mM, 0.4 mM, 4 mM sensor peptides) or sensor peptides (0.1 mM, 1 mM). Cells incubated with 1 mM sensor peptides exhibit statistically significantly lower average cell area and the number compared to the control condition. The cells incubated with sensor microgels show no significant change in cell area or numbers after incubation regardless of the incorporated concentration of sensor peptide.

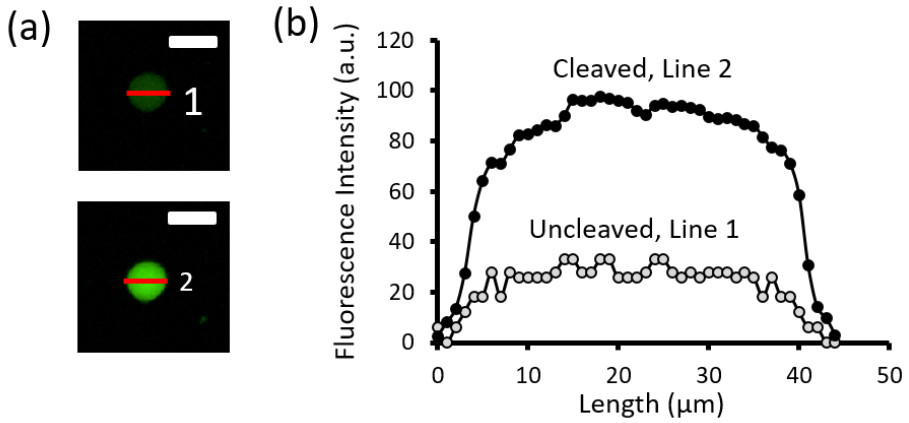


Figure S2. (a) Typical fluorescence image of a microgel sensor before cleavage by collagenase (above), and after reaching maximum fluorescence (below) by exposure to 10 μg/ml collagenase solution for 1 hour. Scale bars, 50 μm. **(b)** Fluorescence intensity profile of microgels along the line depicted in (a) were analyzed with image j software. The fluorescence intensity along the diameter before cleavage of the quencher group (Line 1) is evenly distributed in the center and decreases at the edges as it approaches z-axis resolution. After exposure to 10 μg/ml collagenase solution, an evenly distributed 5-fold fluorescence increase is observed along the diameter of microgel sensors (Line 2). This image analysis shows that the sensor peptides were evenly cleaved throughout the microgel sensors, and MMPs were not sterically excluded from any regions within the particles after reaching steady state.

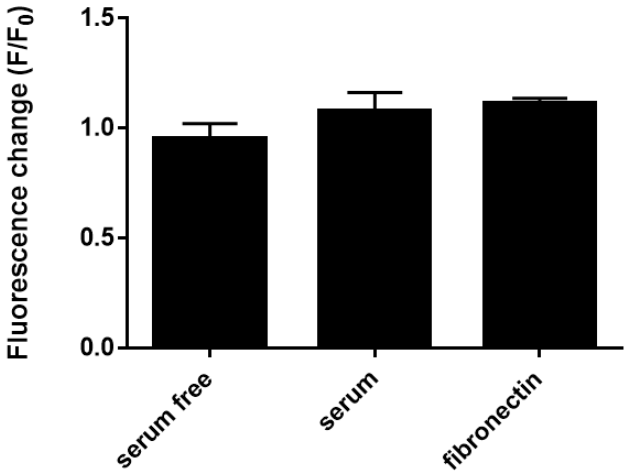
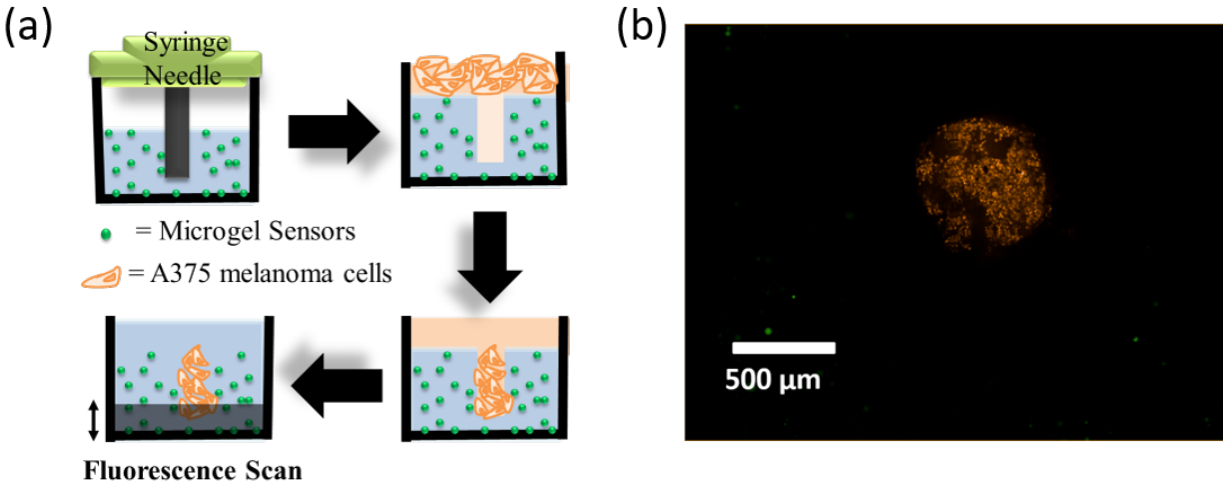


Figure S3. Fluorescence change (F/F_0) in microgel sensors incubated without A375 melanoma cells in serum free, serum (10% FBS) supplemented, and 33 μg/ml of human fibronectin supplemented RPMI media for 4 days.



5. (a) Preparation of cell aggregates inside 3D hydrogels. 0.5 wt% microgels were suspended in a 15 wt% gelatin solution and placed in a 97 °C dry block heater to dissolve. 20 µl of this suspension was added to a well plate and solidified in a 4 °C refrigerator. A hollow cylinder was bored out of the center of the gelatin gel using a syringe needle. An A375 cell suspension (10^6 cells/ml) was added on top of each hydrogel and the plate was centrifuged. The remaining supernatant was removed and 10 µl of the 15 wt% gelatin solution was overlayed on top of the cell-laden hydrogel and solidified. The fluorescence intensity of each microgel located in the bottom 75 µm of the hydrogel was imaged and then compiled in a maximum intensity projection of z-stack images. The distance from the center of the cell aggregate to each microgel was then calculated and compiled. (b) Representative image of a prepared cell aggregate. A375 cells are labeled with CellTracker™ red

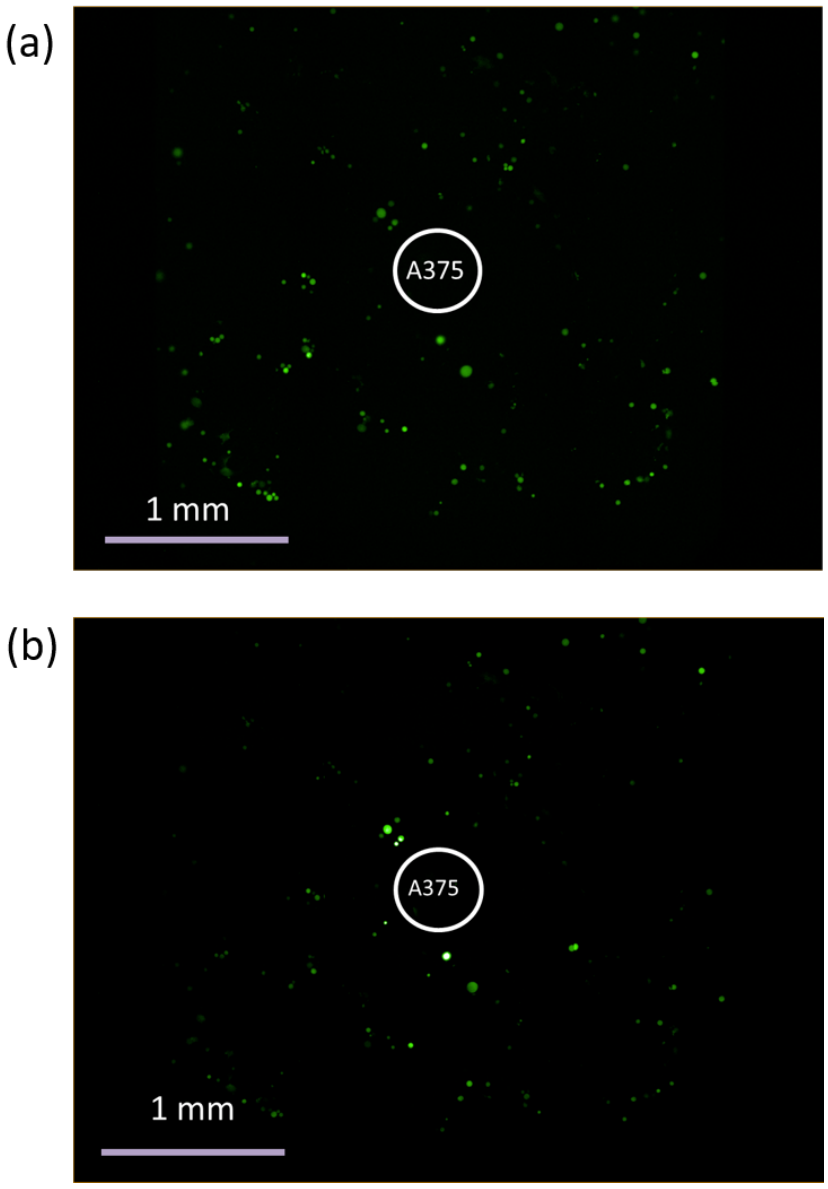


Figure S5. Typical image of the microgel sensors surrounding an A375 cell aggregate (a) at day 0 and (b) after 3 days. Microgel sensors shows green fluorescence and the location and size of the cell aggregate is indicated as white circle in this picture. This image shows that the microgel sensors were spatially localized in the gelatin gel for 3 days and shows selective increase in fluorescence intensiy of microgel sensors located nearer to the cell aggregate.

Table S1. LOD less than 10 ng/ml was derived from on IUPAC's suggestion for calculating LOD (i.e., Signal intensity at LOD = Mean signal Intensity at blank samples + 3 × standard deviation of blank signals[1]). Positive value of [Mean F/F0 of microgel sensors in 10 ng/ml - (Mean F/F0 of microgels in PBS) × 3] shows LOD is less than 10 ng/ml.

	Measurement time points (minutes)	5	10	15	20	25	30	35	40	45	50	55	60
a	Mean F/F0 of microgel sensors in 10 ng/ml collagenase solution	1.16	1.21	1.22	1.24	1.25	1.26	1.28	1.29	1.30	1.30	1.30	1.32
b	Mean F/F0 of microgel sensors in PBS	1.03	1.03	1.03	1.04	1.03	1.04	1.04	1.04	1.04	1.04	1.04	1.05
c	Standard deviation of F/F0 in PBS	0.02	0.03	0.04	0.04	0.04	0.04	0.05	0.04	0.05	0.05	0.05	0.05
	a - (b + 3×c)	0.06	0.09	0.08	0.09	0.11	0.09	0.09	0.12	0.11	0.11	0.12	0.12

REFERENCES

(1) Thomsen, V.; Schatzlein, D.; Mercuro, D. Limits of detection in spectroscopy. *Spectroscopy* **2003**, *18* (12), 112–114.