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Sperm DNA-mediated reduction of nonspecific fluorescence during cellular imaging with quantum dots

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Experimental procedures

Cell culture plate coating with ECM components and polypeptides

Twenty-four-well cell culture plates were coated with gelatin (1 mg/mL), poly-D-lysine (0.2 mg/mL), poly-L-ornithine (20 μg/mL), fibronectin (5 μg/mL), or laminin (20 μg/mL) at 37ºC. Unbound coating material was completely removed, and the plates were washed with PBS.

Fluorescence intensity

Twenty-four-well plates were treated with various SSDNA concentrations (0, 0.01, 0.1, 1, 10, and 100 μg) for 2 hr at room temperature and washed three times with PBS. QD ITK carboxyl 525 or 705 (Invitrogen, Carlsbad, CA, USA) was added to each well (1 pmol of QD 525 and 5 pmol of QD 705 per well) and incubated for 1 hr at 4ºC. Remaining QDs were washed with PBS. To elute the attached QDs from the surface of the cell culture plates, the plates were treated with RIPA buffer (Thermo Fisher Scientific Inc., Waltham, MA, USA) for 30 min at room temperature with gentle shaking. The fluorescence intensity was measured with an Infinite M200 (Tecan, GmbH, SZ, Austria, scanning wavelength: 558/581nm).

For cell culture experiments with aptamer-conjugated QD 705, U343 cells were seeded on gelatin-coated 24-well plates at a density of 3 x 10^4 cells per well and incubated in a 5% CO_2-humidified chamber. U343 cells were cultured in Dulbecco's modified Eagle's medium, supplemented with 10% FBS and 1% antibiotics, at 37°C in humidified incubator with 5% CO_2. After 24 hr, SSDNA at different concentrations (0, 10, and 100 μg) was added to the cell culture plate and incubated for 2 hr at 37ºC. Aptamer-conjugated or non-conjugated QD 705 (5 pmol per well) were added and incubated for 1 hr at 4ºC. To measure the fluorescence intensity, cells were lysed with RIPA buffer and transferred to a 96-well plate (Chemicell GmbH, Germany). All data are represented as mean ± standard error (SE) of triplicated samples (* $P < 0.05$, ** $P < 0.01$, ** $P<0.001$).

Cytotoxicity assay

Cell viability was evaluated with a colorimetric WST-8 assay. U343 cells (3 x 10^4 cells/well) were cultured for 24 and 48 hr under basal condition or with various SSDNA concentrations (0.01, 0.1, 1, 10, and 100 μg/well). WST-8 was added to the cell culture plates and incubated for an additional 4 hr. Absorbance was measured at 450 nm and presented as mean ± SE of triplicate samples.

Confocal microscopy

U343 cells were plated at a density of 3 x 10^4 cells per well on gelatin coated glass coverslips and allowed to attach for 24 hr. After treating with SSDNA, non-conjugated QD 705 or aptamer-conjugated QD 705 was added for 1 hr at 4°C. Cells were fixed with 4% paraformaldehyde and washed 3 times with PBS. Cells were cover-slipped with mounting medium containing 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI) solution (Vector Laboratories, Inc, CA, USA). The cells were then imaged by confocal laser scanning microscopy (Carl Zeiss LSM 510, Weimer, Germany) with an excitation wavelength of 488 nm and emission wavelength of 705 nm for QD 705 signals and an excitation wavelength of 405 nm and emission wavelength of 460 nm for DAPI. To graph background signal on the bottom of the coverslip, a Z-stack image was taken with 1-μm steps.

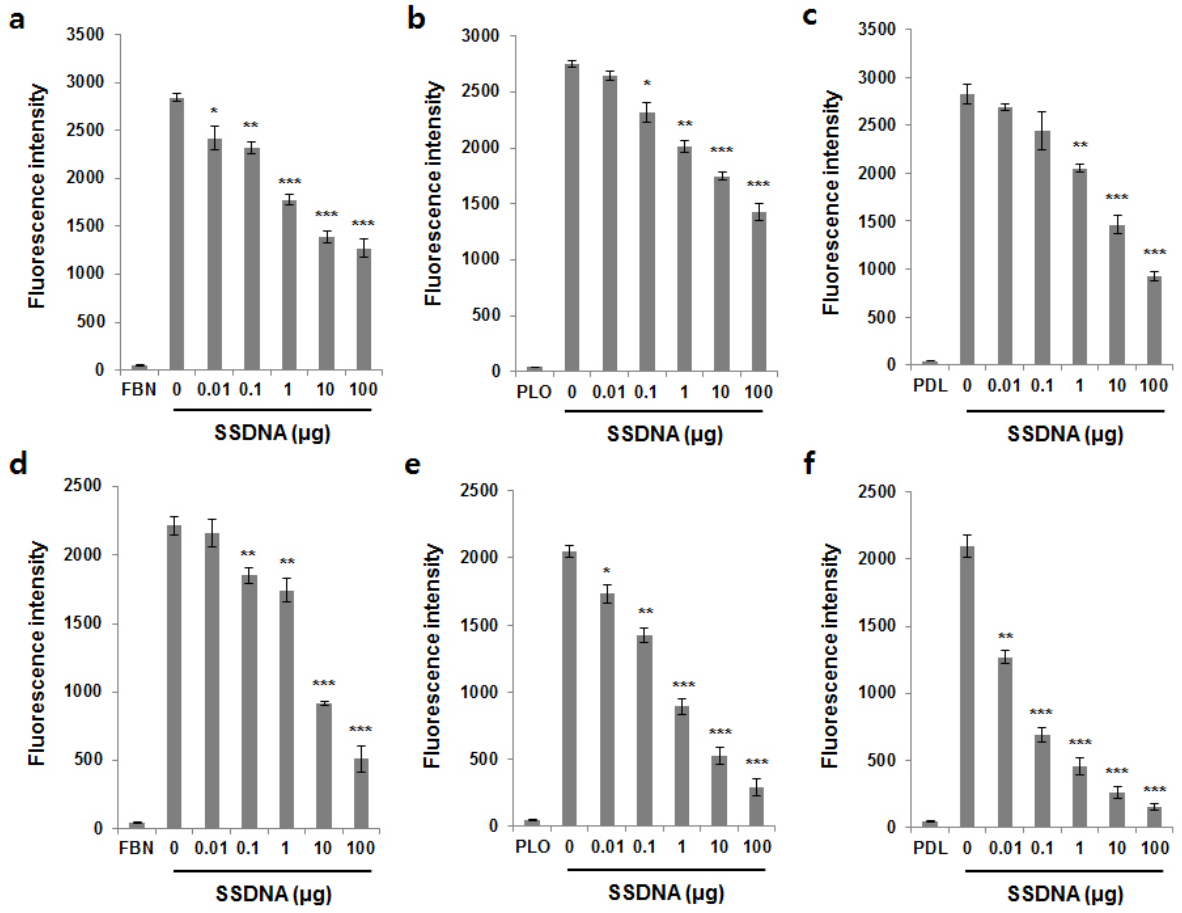

Figure S1. Decreased background fluorescence caused by SSDNA treatment. Decreased QD 525 signal on (a) fibronectin (FBN)-, (b) PLP-, and (c) PDL-coated surfaces. Decreased QD 705 signal on (d) FBN-, (e) PLO-, and (f) PDL-coated surfaces. Data are represented as mean ± standard error of triplicated samples (* $P < 0.05$, ** $P < 0.01$, ** $P<0.001$).

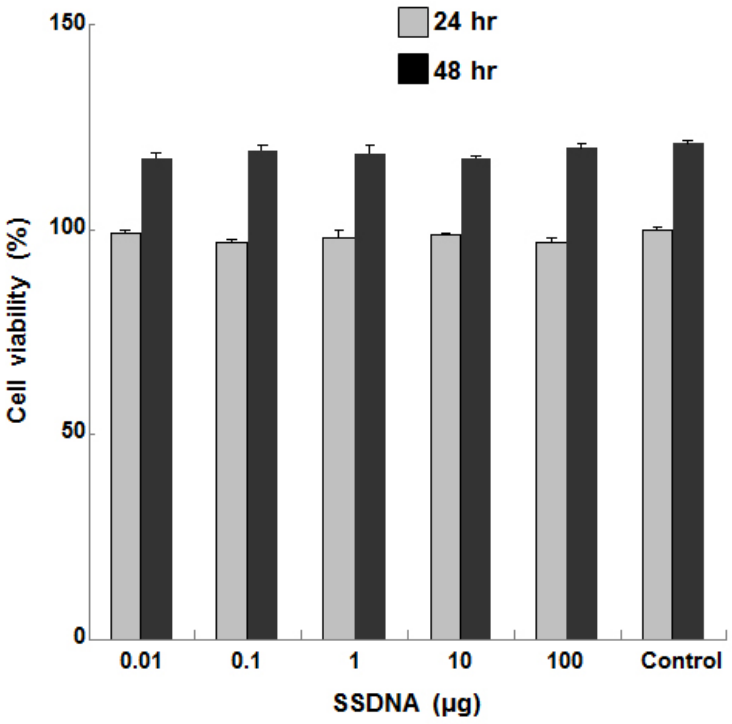

Figure S2. Cytotoxicity assay of SSDNA.

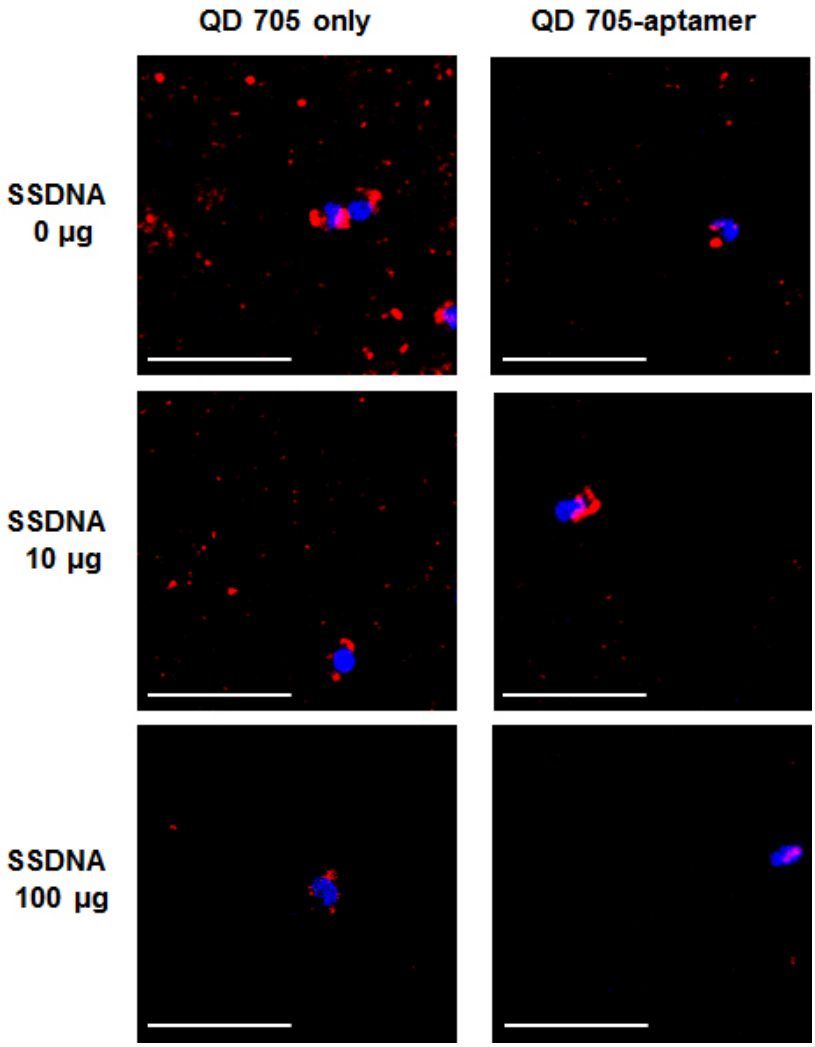

Figure S3. High magnification confocal microscopy images of Fig. 2b. Scale bars indicate 100 µm.

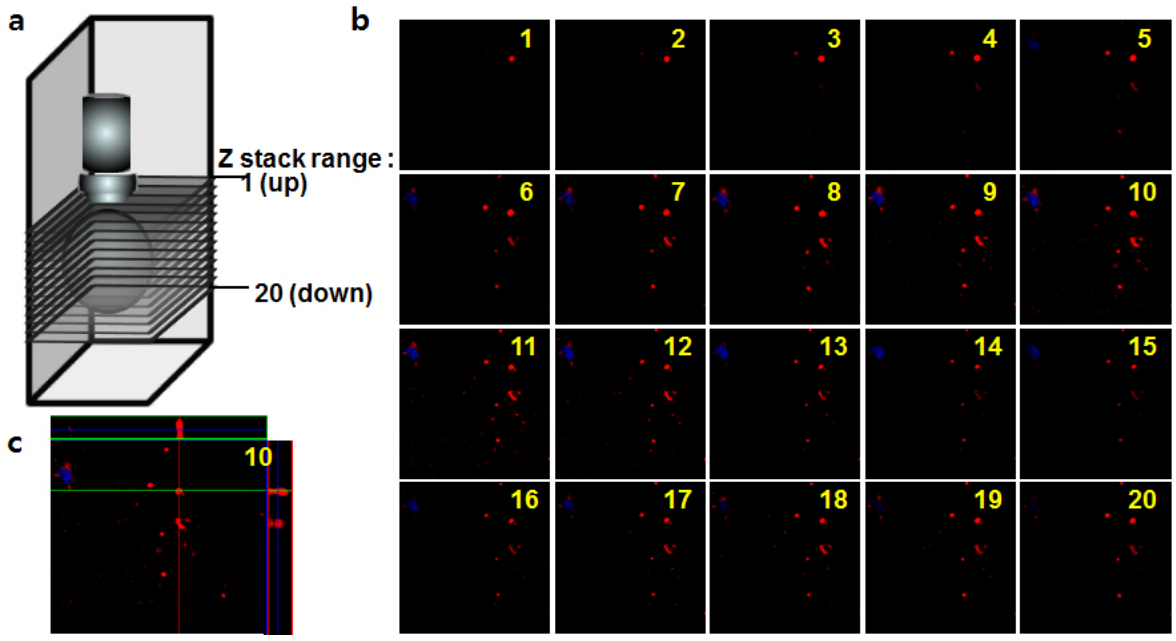

Figure S4. (a) Serial scanning of an optical Z-section image with a red channel of QD 705. Scanning in the Z direction at 1-µm intervals from the bottom of the cell to the bottom of cover slide. (b) The number represents the QD 705 fluorescence intensity from section '1' (top) to '20' (bottom). Red fluorescence signals were more intense on the bottom of the plate (image 20), which had bounded QD particles, and the background signal was prolonged on the top of the slide. This image indicates that QD particles are present on the surface of the coated plate. (c) Orthogonal view of spots in the *x, y*, and *z* axes clearly indicate that QD particles were stuck to the coated surface.