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

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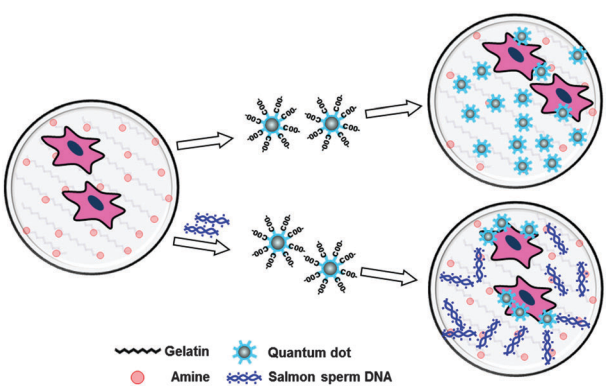
Salmon sperm DNA was used as a blocking agent to reduce background fluorescence signals from gelatin-coated cell culture dishes.

Nanoparticles (NPs) have been widely used to study mammalian cells, tissues, and stem cells.[1] Functionalization of NPs with a targeting probe and fluorescent dye allows them to be delivered to cells and organisms and monitored non-invasively.[2] Semiconductor quantum dots (QDs) are widely used fluorescent nanoparticles for imaging mammalian cells and multiplexed assays[3] because they have broad absorption with narrow photoluminescence spectra, high quantum yield, low photobleaching, and resistance to chemical degradation.[2] Moreover, the QD surface can be functionalized easily, and several methods have been developed.[4]

To study primary cells isolated from tissue in *ex vivo* culture, choosing a desirable extracellular matrix (ECM) substrate is important. The ECM comprises various fibrous proteins and glycosaminoglycans secreted by exocytosis from resident cells. It provides mechanical support to cells, regulates cell activities, and affects proliferation and differentiation of primary cells.[5] An ECM protein, gelatin, derived from collagen, resembles the chemical composition of collagen, and is applied for primary culture of embryonic stem (ES) cells, capillary endothelial cells,[6] and myoblasts,[7] because of its low cost and high efficiency for cellular attachment. The amine group of gelatin facilitates cell attachment to the tissue culture plate through electrostatic interactions with the cell membrane.[8] However, gelatin can generate undesirable background signals during immunocytostaining for fluorescence imaging, because of its electrostatic interactions with NPs. Gelatin consists of 20 amino acids containing several amine functional groups. The positive charge of the amine groups in gelatin easily interacts with negatively charged NPs through electrostatic interaction.

In this work, to reduce background signals from gelatin-coated cell culture dishes, salmon sperm DNA (SSDNA) was selected as a blocking agent. Salmon sperm DNA has been widely used as a blocking agent to reduce non-specific binding in southern and northern blotting.[9] When the cells were treated with SSDNA before adding QDs, SSDNA bound to the positively charged, gelatin-coated surface and, thereby, decreased nonspecific background fluorescence signals (Scheme 1).

To examine the ability of SSDNA to reduce background signals, gelatin, fibronectin (FBN), poly-D-lysine (PDL), laminin, and poly-L-ornithine (PLO), which are routinely used in primary



Scheme 1 Schematic diagram of the decrease in background fluorescence caused by salmon sperm DNA in a gelatin-coated cell culture plate.

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or stem cell culture, were used to coat cell culture plates. The concentration of each coating reagent was adjusted according to the manufacturer's instructions. Various concentrations of SSDNA (0, 0.01, 0.1, 1, 10, and 100 μg) were then added to the culture plates at 37 °C for 2 h. After the SSDNA incubation, two different carboxyl-functionalized QDs, QD 525 (1 pmol) and QD 705 (5 pmol), were added to the culture plate and washed out. Fluorescence from the attached coating reagent was eluted with radioimmunoprecipitation assay (RIPA) buffer, and the released background signals from coating reagent were measured using a fluorometer. The background fluorescence intensity of QDs was reduced efficiently on five types of coating reagents in a SSDNA concentration-dependent manner (Fig. 1 and Fig. S1, ESI†). Non-specific binding of QD 525 was reduced by 64% in the gelatin-coated plates and 44% in the laminin-coated plates at 10 μg of SSDNA. Non-specific binding of QD 705 also decreased in coating reagents. There were no significant differences among plates treated with 0.1 to 100 μg of SSDNA. Moreover, the PDL-coated plate had an obvious and gradual decrease in background fluorescence for both QDs in an SSDNA dose-dependent manner (Fig. S1c and f, ESI†).

To investigate the influence of SSDNA on cell viability, cellular metabolism was assessed using the Cell Counting Kit-8 (CCK-8) assay. U343 glioma cells were incubated with 0.01, 0.1, 1, 10, and 100 μg of SSDNA for 2 h. After incubating with water-soluble tetrazolium salt (WST-8) for 2 h, the formazan product was quantified at 450 nm using a microplate reader. As expected, cell viability in the control and SSDNA-treated groups did not differ (Fig. S2, ESI†). In addition, about 20% enhanced cell viability was detected after 48 h. These results indicate that SSDNA had no adverse effect on cell viability or proliferation.

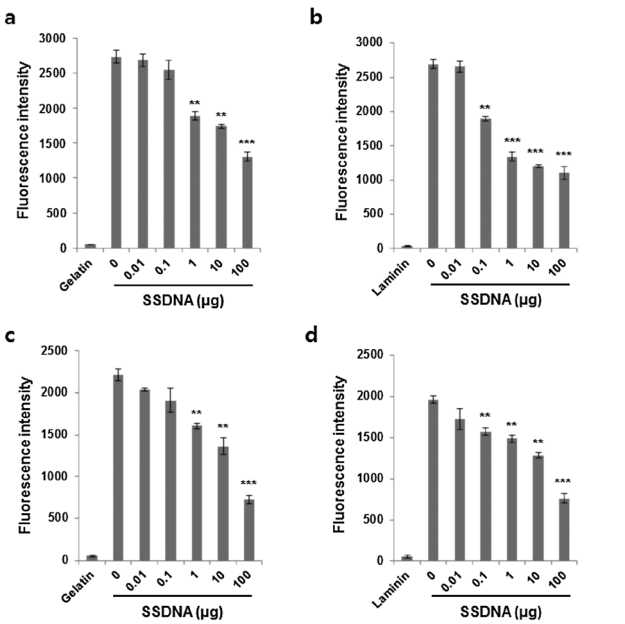

Fig. 1 Decreased background fluorescence signal caused by SSDNA treatment. Decreased QD 525 signal on (a) gelatin- and (b) laminin-coated surfaces and QD 705 on (c) gelatin- and (d) laminin-coated surfaces. Data are represented as a mean ± standard error of triplicated samples (* $P < 0.05$, ** $P < 0.01$, and ** $P < 0.001$).

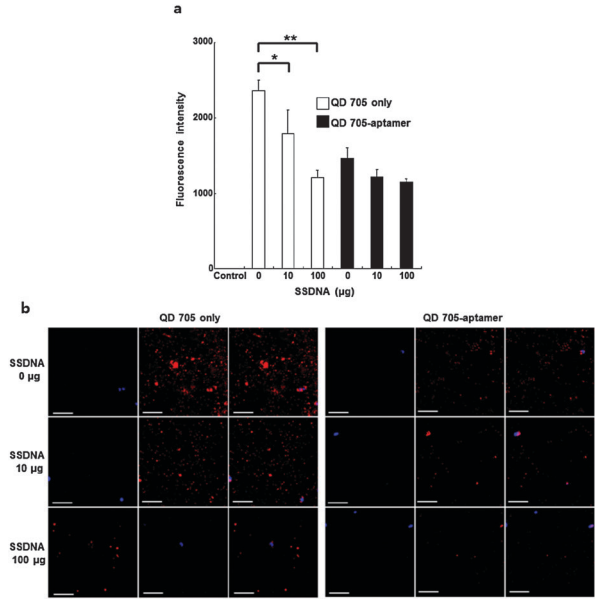

Fig. 2 (a) Relative background fluorescence intensity of QD 705 and QD 705-aptamers on SSDNA-treated gelatin surfaces. Data are presented as a mean ± standard error of triplicated samples (* $P < 0.05$ and ** $P < 0.01$). (b) Confocal microscopy images of QD 705 and QD 705-aptamers in U343 cells on SSDNA-treated gelatin surfaces. Column 1: DAPI (blue); column 2: QDs (red); column 3: merged image of DAPI and QDs. Scale bars, 100 μm.

We prepared aptamer-conjugated QD 705 to further evaluate background fluorescence induced by electrostatic interactions. Aptamer-conjugated QD 705 lowered the basal fluorescence level of the untreated plate (Fig. 2a). The basal fluorescence intensity of the plate treated with non-conjugated QD 705 without SSDNA was 2369.75 ± 137.48. That of the plate treated with aptamer-conjugated QD 705 without SSDNA was 1460.75 ± 138.45. These results indicate that the carboxy-functionalized surface of QD 705 was almost completely covered by aptamers, and it reduced the number of carboxyl groups available to interact with the amine groups of gelatin. Aptamer-conjugation resulted in the decrease in the number of surface attached QDs through a covalent bond. Even when the SSDNA concentration increased, the fluorescence decrease was not significant because of a low amount of initially attached aptamer-conjugated QD 705. It strongly demonstrated that the cause of background fluorescence signals in this system was electrostatic interaction between charged coating material and QDs, and the reduction in background signal can be induced by treatment of SSDNA.

To quantify the decrease in background fluorescence signal caused by SSDNA, fluorescence analysis was performed with U343 cells seeded on 0.1% gelatin-coated plates. U343 cells cultured with SSDNA before treatment with 5 pmol of non-conjugated or aptamer conjugated QD 705 also had a dose-dependent decrease in background fluorescence (Fig. 2b and Fig. S3, ESI†). SSDNA treatment significantly reduced nonspecific surface attachment of two types of QDs. Under the SSDNA untreated conditions, the amount of non-conjugated QD 705 attached to the surface was significantly higher than that of aptamer-conjugated QD 705. A Z-stack confocal image further confirmed that the QDs were attached to the top of

the gelatin surface coating (Fig. S4, ESI†). The red QD fluorescence signals were more frequently detected on the bottom of the slide (Fig. S4, ESI† image 20), and the fluorescence signal was prolonged on the top of the slide. An orthogonal view of the spots in the *x*, *y*, and *z* axes further demonstrated that QDs were attached to the surface of the gelatin-coated plate (Fig. S4c, ESI†).

To improve the sensitivity of optical imaging, a highly sensitive and adequate photo-stable fluorescent probe is needed. Compared with conventional organic fluorophores, semiconductor QDs have many advantages, including resistance to photobleaching and chemical degradation, functional moieties for conjugation with various molecules, size-dependent emission wavelengths, wide adsorption spectra, high quantum yields, and high molar extinction coefficients. These advantages have extended the application of QDs for the detection of intra- and extracellular molecules in a wide variety of cell types. Moreover, a carboxyl- or amine-functionalized QD moiety is a prerequisite for conjugating a protein or peptide. However, the amine and carboxyl moiety can induce nonspecific interactions with plate coating reagents, which are derived from ECM components or charged polypeptides. Electrostatic interactions between a negatively charged QD and a positively charged plate coating reagent could be a main cause for undesirable background signals.

In this study, we reduced nonspecific background fluorescence caused by electrostatic interactions between the carboxyl-functionalized QD and the coating reagent by using SSDNA. Treating FBN-, gelatin-, PDL-, laminin-, and PLO-coated culture plates with SSDNA reduced the background signals from QD 525 and QD 705. PDL-coated plates showed a significant decrease in background QD 705 signal. Because of the positive charge on poly-D-lysine, the decrease of background signal was larger than that with other coating reagents. Confocal microscopy further confirmed the effectiveness of SSDNA in reducing background signals from nonspecifically attached QDs. The initial decrease in background signal from cell culture plates treated with aptamer-conjugated QD 705 was higher than that with nonconjugated QD 705. This result demonstrates that covering the QD 705 surface with immobilized aptamers decreased the carboxyl moiety of QD 705, which reduced the background signal.

In conclusion, we showed a simple, efficient, and inexpensive method to reduce background fluorescence for *in situ* fluorescence assays using functionalized QDs by treating plates with SSDNA. The SSDNA worked to neutralize the positive charge of the gelatin polypeptide. Our method could facilitate the application of functionalized NPs to many targeting and imaging studies.

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