Supporting Information

Surface engineering of macrophages with nucleic acid aptamers for circulating tumor cell capture

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Materials: N-Methacryloyl mannosamine (ManM) was synthesized using a previously described method.[1] Eosin-Y disodium salt was purchased from Thermo Fisher Scientific, Inc., Waltham, MA, USA. All DNA shown in supporting Table S1 were procured from Gene Design, Inc., Osaka, Japan. Cell Counting Kit-8 was purchased from Dojindo Molecular Technologies, Inc., Kuamamoto, Japan. PKH26 Red Fluorescent Cell Linker Kit and the PKH67 Green Fluorescent Cell Linker Kit were purchased from Sigma-Aldrich, St Louis, MO, USA. Dulbecco's phosphate buffered saline (PBS) was purchased from Nissui Pharmaceutical Co., Ltd., Tokyo, Japan.

Cell culture experiment: Human T-cell lymphoblast-like cell line (CCRF-CEM) was purchased from the Japanese Collection of Research Biosources Cell Bank and maintained in GIBCO® RPMI 1640 medium with 20% foetal bovine serum (FBS; Biowest), 100 unit/mL penicillin (GIBCO®), 100 μg/mL streptomycin (GIBCO®) and 4.0 μg/mL amphotericin B (GIBCO®), and was stored at 37 °C in a humidified atmosphere containing 5% CO_2. Murine macrophages (RAW264.7) were purchased

from DS Pharma Biomedical, Co., Ltd. and maintained in GIBCO® Dulbecco's Modified Eagle Medium (DMEM) with 10% FBS, 100 unit/mL penicillin, 100 μg/mL streptomycin and 4.0 μg/mL amphotericin B, and kept at 37 °C in a humidified atmosphere containing 5% CO_2.

Cytotoxicity test: A suspension of RAW264.7 cells (1000 μL; 2 × 10^5 cell/mL) in DMEM was introduced into a 24-well tissue culture plate (Thermo Scientific, Rochester, U.S.A.) and incubated in DMEM at 37 ºC with 5% CO_2. After 24 h of cultivation, PBS containing ManM or Eosin-Y was separately added to the well and the cells were further incubated for 24 h. The viability of the RAW264.7 cells in each well was evaluated using a WST-8 Cell Counting Kit (Dojindo, Kumamoto, Japan). The effect of visible-light irradiation on cell viability was also determined.

The cell viability was determined using the following equation (1):

$$\text{Cell viability (\%)} = \frac{\text{Absorbance of WST - 8 formazan reduced with sample cells}}{\text{Absorbance of WST - 8 formazan reduced with native cells}} \times 100 \quad \cdots\cdots(1)$$

Surface modification of macrophages using nucleic acid aptamers: After 1 day old culture of RAW264.7 cells was rinsed three times with PBS containing 100 μg/mL sgc8-SH in presence of 5 μg/mL Eosin-Y. The cells were then exposed to visible light for 10 min at room temperature. The cells were again rinsed three times with PBS to remove any unreacted sgc8-SH. In order to confirm immobilization of sgc8 on RAW264.7 cells, the cells were subsequently treated with 100 μg/mL complementary-sgc8-Alexa Fluor 488 for 1 min.

After rinsing with the culture medium, morphological characterization of the cells was performed using a confocal laser scanning microscope (LSM 5 PASCAL, Carl Zeiss Microscopy, Jena, Germany). The fluorescence intensity of the surface-immobilized cells was evaluated by flow cytometry (EPICS XL, Beckman Coulter, Brea, USA). For this measurement, the adherent cells on temperature-responsive culture dishes (RepCell®, CellSeed Inc., Tokyo, Japan) were detached by lowering the temperature, and a cell suspension containing 3.0×10^5 cells/mL in DMEM was prepared. Histogram data of flow cytometry was obtained by counting 5000 cells for each of the analysed samples. The viability of aptamer-immobilized RAW264.7 was also investigated using a WST-8 test as described above.

Adhesion of cancer cells with macrophages modified with nucleic acid aptamers: For visualisation under a fluorescence microscope, CCRF-CEM cells were labelled with PKH26 Red, according to the manufacturer's instructions, and suspended in PBS or DMEM (3.0×10^5 cells/mL) with 10% FBS. ManM-treated RAW264.7 cells in culture dishes were similarly stained with PKH67 Green, and surface modification with sgc8-SH was performed as described above. One mL of CCRF-CEM-suspended solution (3.0×10^5 cells/mL) was subsequently added to the plastic-bottom dish, and the cells were co-cultured by gently shaking for 30 min. After rinsing with PBS or DMEM with 10% FBS for three times, the adherent cells on the plastic-bottom dish were observed by fluorescence microscope. An inhibitory assay for the aptamer-mediated cell adhesion in DMEM with 10% FBS was also performed. CCRF-CEM cells were pre-treated with or without sgc8 before contact with sgc8-immobilized RAW264.7 cells.

References

1. Y. Iwasaki and H. Matsuno, *Macromol. Biosci.* 2011, **11**, 1478.

Table S1 DNA sequences used in the present study

Abbreviation	Sequence (5′ → 3′)
sgc8-SH	Thiol-TTT TTT TTT TAT CTA ACT GCT GCG CCG CCG GGA AAA TAC TGT ACG GTT AGA
C-DNA-dye	Alexa Fluor® 488-TCT AAC CGT ACA GTA TTT TCC CGG CGG CGC-3′

The underscored sequence represents the CCRF-CEM-unit aptamer.

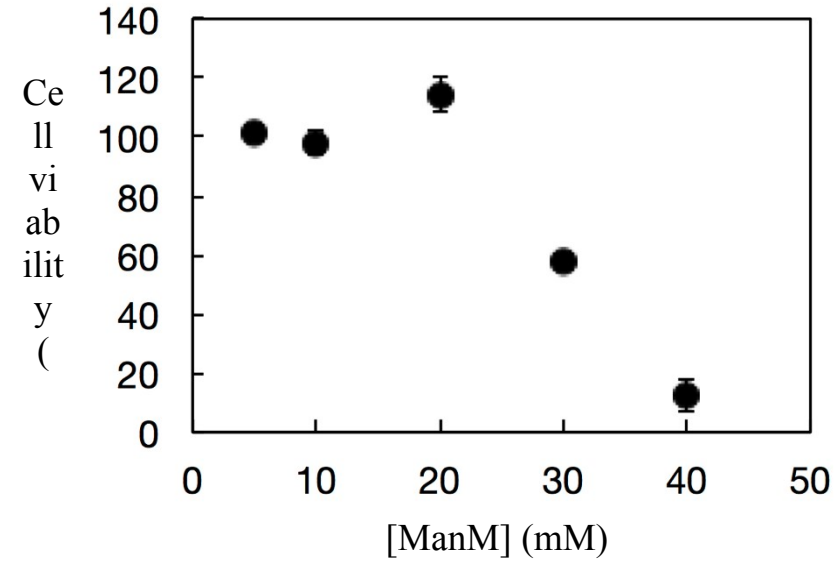

Figure S1 Influence of ManM on the viability of RAW264.7 cells.

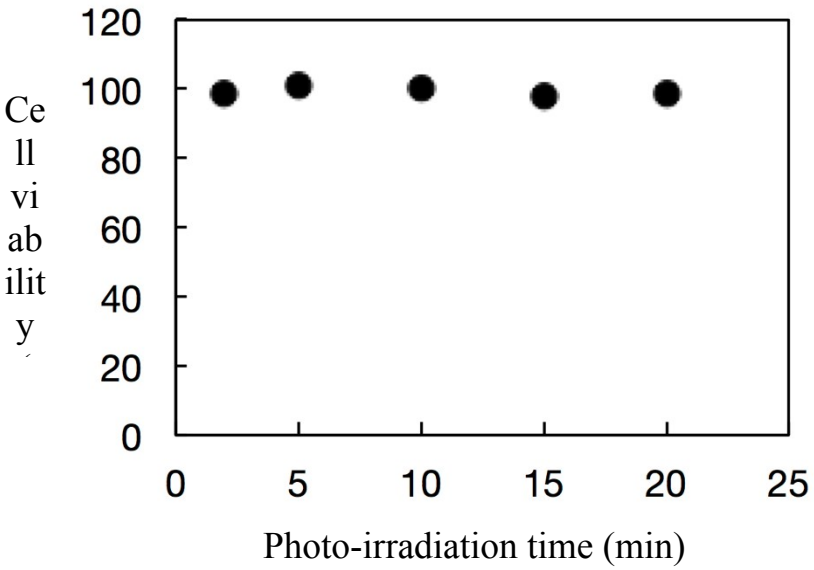

Figure S2 Effect of the duration of visible light-assisted reaction on the viability of RAW264.7 cells in the presence of 5 μg/mL Eosin-Y.

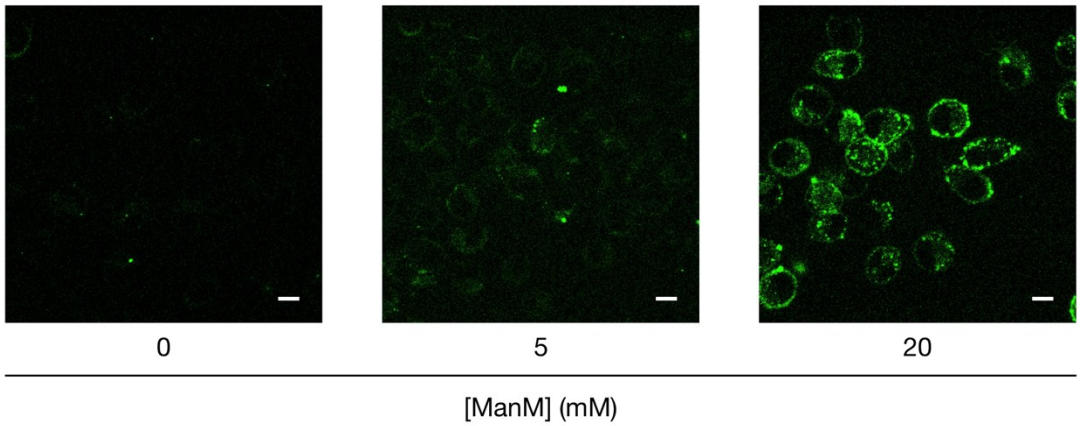

Figure S3 The effect of ManM dose on fluorescence intensity of nucleic acid aptamer-immobilized RAW264.7 cells after contact with Alexa Fluor 488 C5-immobilized complementary DNA. Bars represent 10 μm.

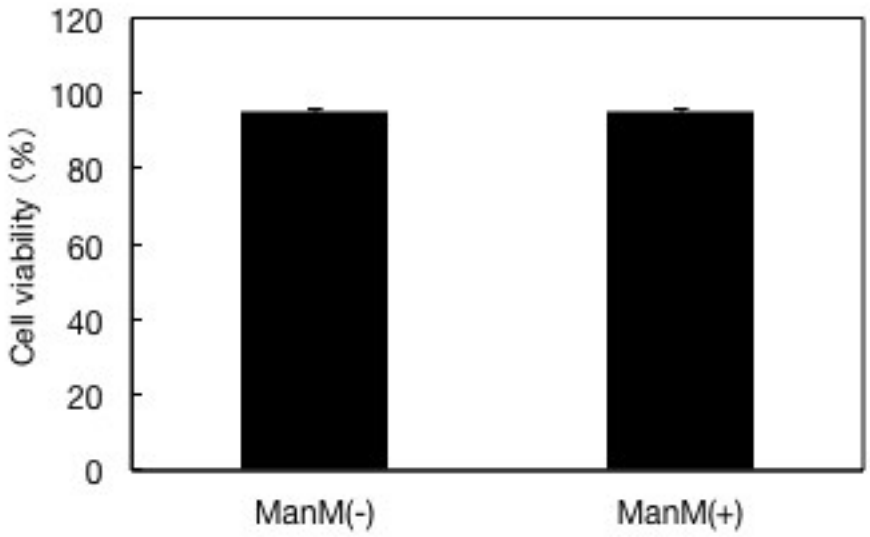

Figure S4 Viability of 24 h cultivated RAW264.7 cells after immobilization of nucleic acid aptamers. The percentage of viable cells is calculated by equation (1) mentioned in the text.

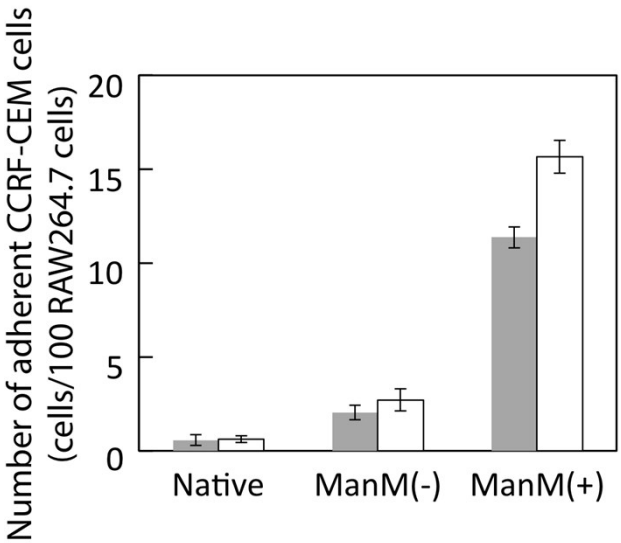

Figure S5 Number of adherent CCRF-CEM cells on 100 RAW264.7 cells in cell culture medium. ○: 15 min inculation; ●: 30 min inculation.

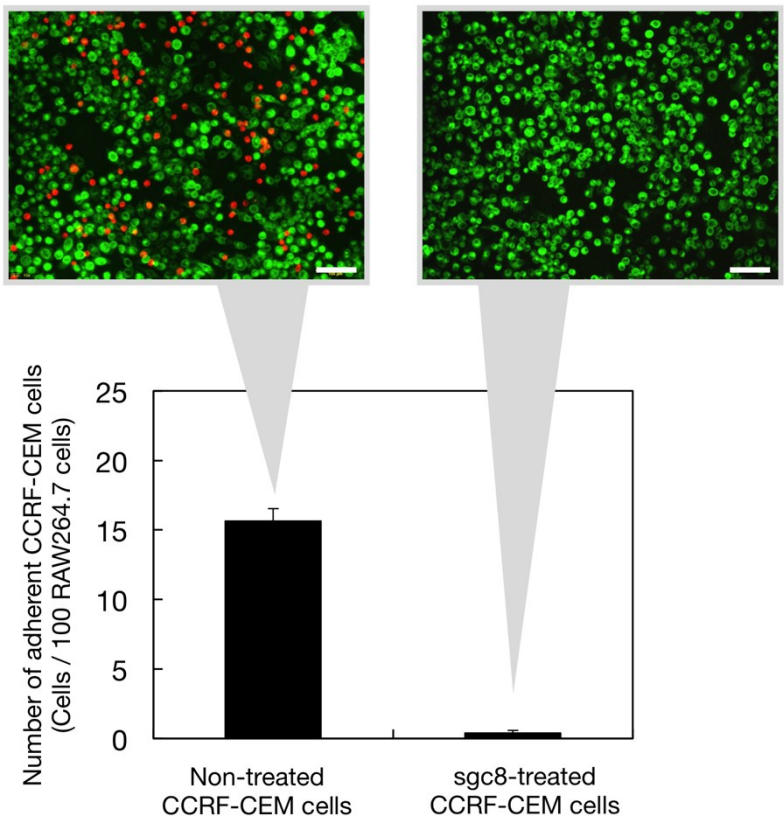

Figure S6 The inhibitory test for the binding aptamer-pre-treated CCRF-CEM cells on nucleic acid-immobilized RAW264.7 cells.