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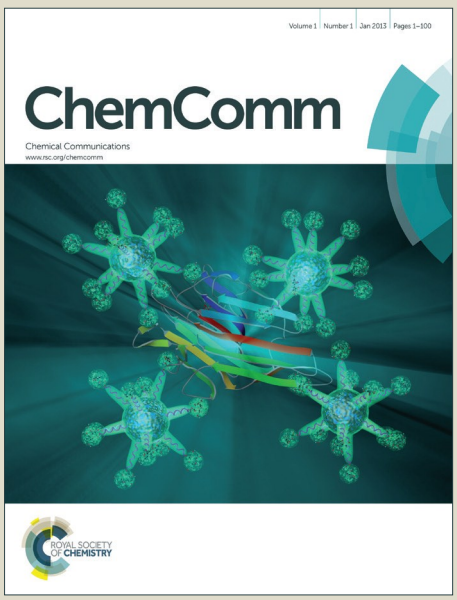

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Surface engineering of macrophages with nucleic acid aptamers for circulating tumor cell capture

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In order to enhance the interactions between macrophages and cancer cells, thiol-terminated nucleic acid aptamers were immobilized on methacryloyl-functionalised carbohydrates of macrophages. The adhesion of cancer cells on the surface modified macrophages was significantly accelerated.

Metastasis is the dominant trigger of cancer death.[1] The mechanistic systems of metastasis are very complex, and the process of metastatic pathogenicity is still largely unclarified. Chaffer et al. simply defined metastasis as occurring in two phases: (i) the physical translocation of cancer cells from the primary tumor to the micro-environment of a distant tissue and (ii) colonisation.[2] Understanding the processes involved in the first phase is more critical for suppressing cancer metastasis. Cancer cells can spread through various routes including transcoelomic, lymphatic and haematogenous processes. Cancer cells that have broken away from the primary tumor, termed circulating tumor cells (CTCs), are the main components of metastasis. Therefore, a system to eliminate CTCs from the metastatic routes is required for the reduction of metastatic cancer.[3]

In the human body, certain mechanisms protect against cancer during the multistage process of carcinogenesis.[4] One of the most important forms of protection is the immune system activity in which several immune cells, cytokines and complementary factors participate in anticancer activities. Macrophages are innate immune cells that play a broad role in host defence and homeostasis maintenance.[5] Macrophage-mediated programmed cell removal (PrcR) is an important mechanism in diseased and damaged cells elimination before programmed cell death.[6] The induction of PrcR by 'eat-me' signals on tumor cells is countered by 'don't eat me' signals such as CD47. Blockade of CD47 leads to phagocytosis by macrophages.[6.] By enhancing selective adhesion of macrophage to cancer cells, macrophage-mediated therapeutics may be more effective.

It has been clarified that an immobilization of nucleic acids on cell surface is one of the most reliable techniques to control cell-cell contacts because the sequences could be freely designed while avoiding cytotoxicity and activation of an innate immune response.[7] In the present study, surface modification of macrophages with nucleic acid aptamers, so-called "eat-you" motifs which can bind to membrane proteins of cancer cells, was performed to capture cancer cells. In order to immobilize nucleic acid aptamers on the living cell surface, synthetic reactive groups were metabolically delivered to the macrophages. The metabolic labelling process of sialic acid is robust for the surface engineering of living cells because this process can be applied for various mammalian cells.[8] *N*-Acetyl mannosamine (ManNAc) analogues intercept sialic acid biosynthesis and deliver bio-orthogonal reactive sites to the cell surface. Recently, we synthesised a methacryloyl-modified ManNAc analogue, termed *N*-methacryloyl mannosamine (ManM), and succeeded in delivering methacryloyl groups to sialic acid residues of mammalian cells.[9] The methacryloyl groups delivered on the carbohydrates of mammalian cells could be applied for a thiol-ene reaction and free radical polymerization.[9]

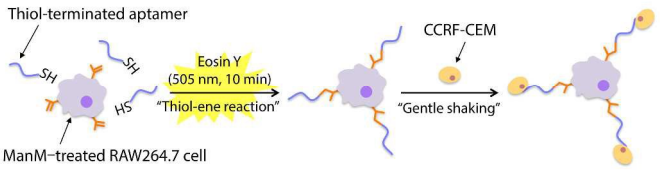

Figure 1 Schematic representation of surface modification of a macrophage (RAW264.7) with nucleic acid aptamers and the capture of the engineered cancer cell (CCRF-CEM).

Figure 1 shows a schematic representation of this reaction. A thiol-terminated nucleic acid aptamer (sgc8-SH), which can bind to protein

tyrosine kinase-7 (PTK7), was immobilized on macrophages through visible light-assisted thiol-ene reactions[10] and the success of cancer cell capture by these macrophages was investigated. The sequence of the sgc8-SH and its complementary DNA bearing a fluorophore are shown in supporting Table S1.

Murine macrophage-like cells (RAW264.7) were used as a model for macrophages. In order to deliver methacryloyl groups onto the carbohydrates of RAW267.4 cells, the cells were incubated with ManM. The optimal concentration of ManM was determined through a WST-8 cytotoxicity test and the result is shown in supporting Figure S1. No adverse effect of <20 mM of ManM was observed on the viability of RAW264.7 cells. Consequently, these cells were incubated with 20 mM of ManM for 24 h.

Nucleic acid aptamers are robust for binding to specific target molecules, which range from small molecules to macromolecules, such as proteins.[11] Aptamers immobilization can be applied in bio-sensing, diagnostics and therapeutics.[12] The surface modification of living cells using nucleic acid aptamers was originally performed by Tokunaga et al.[13] They succeeded in immobilizing nucleic acid aptamers on living cell surfaces through both covalent conjugation and direct anchoring techniques. Furthermore, the release of adenine compounds as gliotransmitters were monitored on the cell surface. Aptamers are nontoxic to the host cell and useful for the engineering of living cell surfaces.

Sgc8, selected as a nucleic acid aptamer in the present study, has affinity to PTK7, which is overexpressed on the cancer cell surface. [14] Sgc8-SH was immobilized by a visible light (505 nm)-assisted thiol-ene reaction. Eosin Y (EY) was used as a photosensitiser because it excites in visible light. Xu et al have recently studied the cytotoxicity of EY[15] and clarified that the toxicity of EY at ≤100 μg/mL was negligible. Similar result was obtained for RAW264.7 cells and the concentration of EY was subsequently adjusted for surface modification at 5 μg/mL. Viability of RAW264.7 was not adversely affected after photo irradiation for <20 min using 5 μg/mL of EY, as shown in supporting Figure S2.

Figure 2(a) shows differential interference contrast (DIC) and laser scanning confocal (LSC) micrographs of RAW264.7 cells treated using sgc8-SH and subsequently using Alexa Fluor 488 C_5-conjugated complementary DNA. On the DIC micrographs, each adherent RAW264.7 cell had a similar shape, and no effect of ManM treatment and surface modification with nucleic acid aptamers was observed on the cell shape. In contrast, a significantly different image caused by ManM treatment was observed in the LSC micrographs. The outline of the ManM-treated cells was clearly observed on LSC micrograph and the surfaces of the cells were homogeneously immobilized. The morphology of the cells in LSC micrograph matches well with that in DIC micrograph. However, no cells bordered with fluorescent probes were observed among non-treated cells. Figure 2(b) shows the flow cytometric data for the cells in contact with the fluorescent probes. Similar to the results of microscopic analyses, the fluorescence intensity of ManM-treated RAW264.7 cells, which were in contact with sgc8-SH and subsequently the complementary Alexa Fluor 488 C_5-immobilized DNA, was significantly higher than that of non-treated RAW264.7 cells. The fluorescence intensity of non-treated cells in contact with sgc8-SH and Alexa Fluor 488 C_5-immobilized complementary DNA was slightly increased due to nonspecific binding, but it was significantly lower than that of ManM-treated cells. Furthermore, the fluorescence intensity data for ManM-treated cells was dose-dependent; that is, the density of methacryloyl groups delivered on the cell surface could be controlled by altering the concentration of ManM in Dulbecco's Modified Eagle's Medium, as shown in supporting Figure S3. The results presented in Figures 2(a) and 2(b) indicate that immobilization of sgc8-SH with methacryloyl groups delivered on the cell surface was successful, and sgc8-SH immobilized on the cell surface forms a double-stranded structure with the complementary DNA.

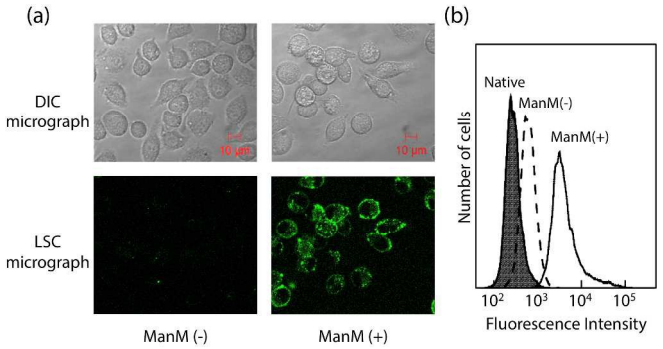

Figure 2 (a) Differential interference contrast (DIC) and laser scanning confocal (LSC) micrographs of RAW264.7 cells in DMEM with 10% foetal bovine serum and containing 20 mM ManM. The cells were treated after contact with sgc8-SH and Alexa Fluor 488 C_5-immobilized complementary DNA. (b) Fluorescence intensity determined by flow cytometry.

No adverse effect of the cell surface modification with aptamers was observed on the proliferation of RAW264.7 cells (supporting Figure S4). Thus, we showed that visible light-assisted surface immobilization is a cytocompatible process.

Human lymphoblasts (CCRF-CEM) were used as a model for CTCs and incubated with sgc8-immobilized RAW264.7 cells under gentle shaking. It is well-known that PTK7 is abundantly expressed on the surface of CCRF-CEM cells.[16] RAW264.7 and CCRF-CEM cells were stained with PKH67GL (green) and PKH26RE (red), respectively. Figure 3(a) shows the fluorescence micrographs of RAW264.7 cells after 30 min incubation. The adherent CCRF-CEM cells were hardly observed on native and non-treated cells. In contrast, CCRF-CEM cells were clearly adhered on sgc8-immobilized RAW264.7 cells. The density of adherent CCRF-CEM cells on RAW264.7 cells is summarised in Figure 3(b). On sgc8-immobilized RAW264.7 cells, we observed a significantly large number of adherent CCRF-CEM cells compared with controls. The number of adherent CCR-CEM cells on RAW264.7 cells increased with an increase in the incubation time as shown in supporting Figure S5. The specific cell adhesion was not obstructed even in the presence of serum proteins. Moreover, an inhibitory test for the adhesion of CCRF-CEM cells to sgc8-immobilized RAW264.7 cells was also performed. When CCRF-CEM cells were pre-treated with sgc8, the adhesion of CCRF-CEM cells on sgc8-immobilized RAW264.7 cells was completely reduced, as shown in supporting Figure S6. These results indicate that the nucleic acid aptamer was quite effective, enabling RAW264.7 cells to capture CCRF-CEM cells. Zhao et al. reported the elimination of cancer cells from human whole blood using a platform bearing nucleic aptamers having a similar sequence to that used in the present study.[14]

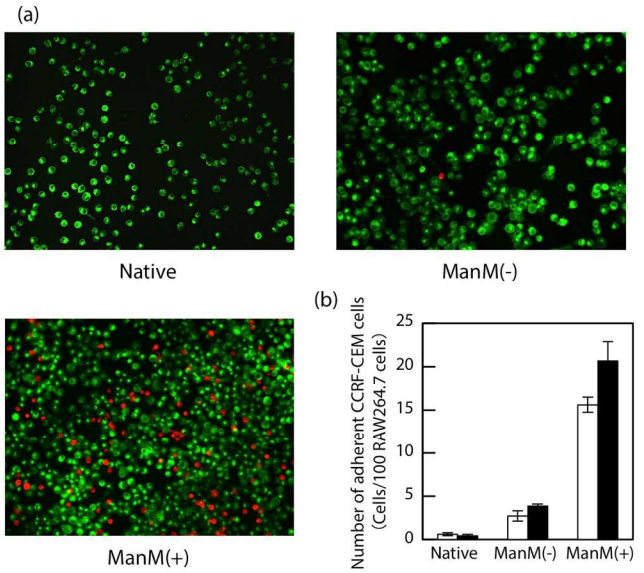

Figure 3 (a) Fluorescence micrographs of murine macrophages (RAW264.7, green) in contact with human lymphoblasts (CCRF-CEM, red) in culture medium for 30 min. (b) Number of adherent CCRF-CEM cells on 100 RAW264.7 cells. □: in cell culture medium; ■: in phosphate buffered saline.

In conclusion, the surface modification of RAW264.7 cells with nucleic acid aptamers was achieved through a visible light-assisted thiol-ene reaction. The reaction conditions were optimised, and the surface modification did not show any adverse effect on the viability of RAW264.7 cells. CCRF-CEM cell capture by RAW264.7 cells was significantly induced by the nucleic acid aptamer immobilization on RAW264.7 cells. Nucleic acid aptamers can be freely designed for targeting specific cell, protein or other bio substance.[17] Therefore, various 'eat-you' motifs could be immobilized on macrophages thorough the bio-orthogonal process established in the current study. This process would induce PrcR and be a useful technology for the elimination of CTCs and other pathogenic factors.

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Notes and references

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† Electronic Supplementary Information (ESI) available: For all experimental procedures and additional data. See DOI: 10.1039/c000000x/

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