Bioconjugate Chemistry

Controlled Detachment of Chemically Glued Cells

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Supporting Information

ABSTRACT: We demonstrate a chemically detachable cell– glue system based on linkers containing disulfide bonds as well as functional groups for metabolic glycoengineering and bioorthogonal click chemistry. Azide groups are generated on the cell surface by metabolic glycoengineering, and they are further modified into tetrazine (Tz) or trans-cyclooctene (TCO) using rationally designed cross-linkers. When the Tzmodified and TCO-modified cells are mixed together, cell gluing between these two cell groups is established by Tz-TCO click chemistry. This artificial cell–cell adhesion can be broken by the administration of glutathione (5 mM), which triggers the degradation of disulfide bonds. Both the gluing and detachment processes are rapid (<10 min) and minimally cytotoxic.

Cell A Cell A Cell pair Cell gluing Cell gluing (1) Cell gluing (2) Cell glu

1. INTRODUCTION

Adhesion between cells is a fundamental process enabling direct cell-cell interaction and tissue formation.^{1,2} Cell-cell adhesion in vivo involves complex cell signaling and specific adhesion molecules.³ Recently, there has been interest in developing artificial methods to form cell-to-cell adhesion for biomedical applications. These methods are based on various artificial binding molecules on the cell surface, such as avidinbiotin pairs,⁴ nucleotide with complementary sequences,⁵ aptamers,⁶ and antibody dimers.⁷ Recently, we developed a robust cell-gluing system based on click chemistry and metabolic glycoengineering.⁸ The bioorthogonality, specificity, and fast reaction rate of click chemistry based on tetrazine (Tz) and trans-cyclooctene (TCO) allowed the gluing process to be completed in vitro in 10 min and remain stable in vivo in mice. Here, we describe an extension of this method to incorporate a mechanism to break the bonds between glued cells by simple administration of a chemical agent.

Two different approaches for detachable cell glue systems have been previously reported. Yousaf's group used oximehydroquinone chemistry that can reversibly degrade via electrochemical stimuli.⁹ Although this technique is useful for two-dimensional cell patterning, the electronic signal requires special plates, such as gold surfaces, and cannot be easily applied in vivo. Wagner's group developed a genetic engineering-based method that forms cell–cell assembly by recombinant fusion protein of dihydrofolate reductase inhibitor methotrexate and disintegrates by treatment of trimethoprim, a bacterial dihydrofolate reductase inhibitor.¹⁰ By comparison, our chemical-based technique is simpler and applicable to general situations including in vivo environments.

As before,⁸ we used metabolic glycoengineering to introduce chemical functional groups to the cell surface with minimum perturbation on cellular viability and functions.¹¹ After treatment of tetraacetylated N-azidoacetyl-D-mannosamine (Ac₄ManNAz) to A549 cells, azide groups are generated on the cell surface (Figure S3 (SI)).¹² In this work, we used novel rationally designed cross-linkers, dibenzocyclootyne disulfide tetrazine (DBCO-SS-Tz) and dibenzocyclootyne disulfide trans-cyclooctene (DBCO-SS-TCO). They have two functional groups for click chemistry-DBCO and Tz/TCO-and degradable disulfide (SS) bonds in their backbone (Figure S1 and S2 (SI)). The azide-modified cells are treated with these cross-linkers, so that the cross-linkers are conjugated to the azide groups by azide-DBCO click chemistry, and Tz- or TCOmodified cells are prepared;¹³ When the Tz-modified and TCO-modified cells are mixed together, cell gluing between these two cell groups is established by Tz-TCO click chemistry (Scheme 1).¹⁴ The disulfide bonds in the backbone are cleaved by glutathione (GSH).¹⁵ Therefore, the detachment of the glued cells can be achieved simply by administration of GSH (Scheme 1).

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Scheme 1. Illustration of the Chemically Detachable Cellular Glue System Based on Click Chemistry Linkers with Degradable Disulfide Bonds



2. RESULTS AND DISCUSSION

To determine the optimum concentration of GSH, we first evaluated the intrinsic toxicity of GSH on cells. A549 cells were treated with various concentrations of GSH for 10 min and a cell viability assay (Prestoblue) was performed. We found that GSH at concentrations of 10 and 20 mM decreased cell viability significantly, but concentrations below 5 mM had negligible effects on cell viability (Figure 1a). Based on this result, we determine 5 mM to be the maximum concentration for nontoxic detachment. In this method, we used GSH as reducing agent because it is an already existing chemical in body and thus may be more appropriate to use for in vivo applications. We expect that another reducing agent including



Figure 1. Chemical modification of cells for gluing and detachment. (a) Cell viability after treatment of glutathione (GSH) using Prestoblue assay. (b) Detachment of Tz groups from cell surface after treatment of GSH. Tz-modified A549 cells were labeled with TCO-Cy3 (red), and treated with GSH (blue = Hoechst 33342). Scale bar, 50 μ m. (c) Magnitude of fluorescence from the data in (b).

dithiothreitol (DTT) may also can cleave the disulfide bond and detach the glued cells in our system.

To evaluate the efficiency for cleaving linkers, we added Cy3-TCO conjugates to the culture media of Tz-modified cells. The fluorescent probes are bound to the Tz groups on the cell surface, and the fluorescence intensity from Cy3 bound on the cell surface indicates the amount of Tz groups. We treated these cells with GSH at three different concentrations, 0.1, 1, and 5 mM for 10 min. After washing the cells, the Cy3 fluorescence was measured. As expected, the fluorescence intensity decreased with the concentration of GSH (Figure 1b). This supports the mechanism that the administered GSH breaks disulfide bonds and thus reduce the total amount of linkers on the cell surface. At a concentration of 5 mM, the Cv3 fluorescence intensity decreased to about 16.4% compared to the control group without GSH treatment (0 mM). This indicates that at this condition the glue strength between cells would be degraded by a factor of 6. We obtained similar results with TCO-modified cells by using DBCO-SS-TCO crosslinkers and Cy3-Tz as fluorescent probes (Figure S4 (SI)).

To analyze cell gluing strength, we cultured green fluorescence protein (GFP)-expressing A549 cells in a monolayer in a microfluidic chamber, and treated them to Tz-modified cells with the protocol described above. To produce glued cells, we added TCO-modified Jurkat T cells (labeled with Vybrant DiI Cell-Labeling Solution) in suspension into the chamber and incubated for 10 min. We injected PBS (phosphate-buffered saline) into the fluidic channels at different flow speeds for 1 min each and measured the number of TCO-modified Jurkat T cells that remained bound to the Tz-modified A549 cells adherent on the chamber under fluorescent microscopy. After applying flow at a speed of 60 mL/min, we found about equal number of T cells to be attached on the A549 cells (Figure 2a), whereas almost all nonmodified Jurkat T cells in a control group were washed away by the flow (Figure 2e).

We performed this flow assay on glued cells after GSH treatment at a concentration of 5 mM and found that the ratio



Figure 2. Gluing and detachment of cells in a microfluidic system. (a) Fluorescence images of Jurkat T cells (red) on A549 cells (green) in a microfluidic chamber after washing. Sham control sample without GSH treatment, flow rate = 60 mL/min. (b) Fluorescence image after a GSH treatment (5 mM) and washing at a flow rate of 60 mL/min. (c) GSH treatment and washing at a flow rate of 1 mL/min. (d) Control sample after gluing using DBCO-TCO instead of DBCO-SS-TCO so that each cell-connecting linker contains only a single S–S bond, GSH treatment, and washing at a flow rate of 60 mL/min. Scale bars, 50 μ m. (e) Ratio of glued cells (Jurkat-A549 pairs) remaining after flow tests.

of remaining Jurkat T cells to adherent A569 cells decreased significantly to about 10%; that is, about 90% of initially glued T cells were detached and washed away by the flow at a speed of 60 mL/min (Figure 2b). At a reduced flow speed of 1 mL/min, 70% of T cells were washed away (Figure 2c). These data show the effect of GSH on the degradation of the bonding strength of the glued cells. When the glued cells were treated with GSH longer than 10 min, up to 30 min, the ratio of the remaining glued cells did not changed significantly. It means that they were nonspecifically adhered to each other after chemical gluing and the mechanism needs to be determined by further studies.

To confirm that the mechanism of detachment is due to the GSH-induced breakage of the disulfide (S-S) bonds in the linkers, we prepared TCO-modified Jurkat T cells with DBCO-TCO (no disulfide bond) and glue the cells with A549 cells modified with DBCO-SS-Tz (Figures S5 and S6 (S1)). In this case, there is only one S–S cleavage site in each cell–cell linker, as opposed to the double S–S bonds in the previous linkers. After GSH treatment and flow at 60 mL/min, we measured a remaining cell ratio of 45%, indicating only ~55% of the glued Jurkat T cells were washed away (Figure 2d). It shows that two S–S bonds are advantageous for cleavage of the linkage and detachment of cells compared to one S–S bond. These results

also evidence that the detachment involves the degradation of S-S bonds by GSH.

To evaluate our system for suspension cells, we prepared two groups of Jurkat T cells labeled with CellTracker green CMFDA or Vybrant Dil Cell-Labeling Solution and modified them with DBCO-SS-Tz and DBCO-SS-TCO, respectively. After 10 min of incubation, fluorescence microscopy was used to confirm selective attachment of Tz- and TCO-modified Jurkat T cells (Figure 3a). In flow cytometry, the ratio of



Figure 3. Efficiency and viability of cell gluing and detachment. (a) Fluorescence images of the glued and detached Jurkat T cells in suspension. Scale bar, 30 μ m. (b) Flow cytometry data of the glued and detached Jurkat T cells. (c) Cell viability data after gluing and detachment (calcein AM staining and flow cytometry).

double-positive counts was measured to be 57% (Figure 3b). Scanning electron microscope (SEM) images of glued cells show that the cellular adherence is established over extensive contact areas in their cell membranes (Figure S7 (SI)). After incubating with GSH (5 mM), the ratio of glued cells decreased to 6.4%, indicating that nearly 90% (6.4/58) of the glued cells were dissociated by the GSH treatment. After incubating with a cell viability probe, calcein AM (acetoxymethyl), a cytometry analysis showed that 91% cells were alive after gluing, and that 87% cells remained vital after the gluing and degluing processes (Figure 3c). This data confirms the low cytotoxicity of the detachable cell glue system.

In summary, we have demonstrated a rapid, efficient, nontoxic, artificial gluing and controlled detachment of cells by click chemistry and chemically degradable bonds. In this study, we used disulfide bonds as the cleavable site in the cell–

Bioconjugate Chemistry

cell linkers and GSH as the chemical triggering agent. There are many other chemical bonds developed for degradation¹⁶ by various stimuli, such as chemicals,¹⁷ light,¹⁸ pH,¹⁹ or enzymes.²⁰ Our scheme may be extended to different embodiments optimized for specific applications.

ASSOCIATED CONTENT

S Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bioconj-chem.6b00546.

Characterization of chemicals, experimental details of gluing procedure, cell viability test, microscopic imaging, and flow cytometry (PDF)

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Notes

The authors declare no competing financial interest.

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