Engineering of cell surface with synthetic DNA using lipid insertion or click conjugation

Xuelin Wang, Connie Wen, Brandon Davis, Peng Shi, Lidya Abune, Kyungsene Lee, Cheng Dong and Yong Wang*
Department of Biomedical Engineering, The Pennsylvania State University, University Park, PA

Introduction: Engineering of cell surface with polyvalent DNA nanostructures is promising for various applications ranging from cancer immunotherapy to tissue engineering[1, 2]. The display of polyvalent DNA on the cell surface can be achieved using either click conjugation or lipid insertion. However, it is difficult to make a choice between the two methods as no study has been published to show their difference. The purpose of this study was to compare and evaluate these two methods in displaying polyvalent DNA on the surface of live cells. As the major difference between those methods is the way of engineering DNA initiator (DI) on cell surface, we firstly examined cell cytotoxicity, modification efficiency and DNA stability after DI modification using click conjugation and lipid insertion. Then we compared the effectiveness of lipid insertion and click conjugation in the in-situ synthesis of polyvalent DNA nanostructures for the promotion of cell-cell interaction.

Methods: For the lipid insertion method, cells were directly treated with the DI-Cholesterol (DI-Chol) conjugate. For the click conjugation method, cells were treated with N-azidoacetylmannosamine-tetraacylated (Ac_4ManNAz) in order to display azide sugar moieties on the cell surface. DI bearing a cyclooctyne group (DI-DBCO) would react with the azide groups and display DI on the cell surface. To evaluate the cytotoxicity, MTS assay was conducted. To examine the DNA modification efficiency, the fluorescence intensity, which reflected the DI intensity on cell surface, was measured via flow cytometry. To test the stability of DI on cell surface, the modified cells were incubated at 37 °C. At determined timepoints, the residual DNA on cell surface was measured via flow cytometry. The polyvalent DNA was formed through three steps: the display of a DI on the cell surface, the DI-triggered formation of a supramolecular DNA scaffold, and the scaffold-directed hybridization with multiple aptamers. The immune-cancer cell interactions were analyzed via flow cytometry.

Results: The cytotoxicity results showed that an increase in Ac_4ManNAz concentration led to a decrease in cell viability (**Figure 1a**). As cytotoxicity was relatively low at 50 μM Ac_4ManNAz, this concentration was used in further experiments. We also found that at relatively higher concentration (> 10 μM), DI-DBCO had less effect on cell viability compared to DI-Chol (**Figure 1b**). In addition, we compared modification efficiency. DI-Chol had a higher modification efficiency than DI-DBCO (**Figure 1c**). We further examined the stability of DI on the cell surface. The results revealed that DI molecules displayed using click conjugation persisted longer than those displayed using the lipid insertion (**Figure 1d**).

Conversely, the lipid insertion method enabled the modified cells to capture target cells more efficiently (**Figure 1e**). The representative flow cytometric cytograms showed the binding percentage of polyvalent DNA at 0 h (**Figure 1f**). These data suggest that while DNA molecules displayed on the cell surface using lipid insertion may not be stable in comparison to click conjugation, the former method is more effective in promotion cell-cell interaction due to high-efficiency of DNA display.

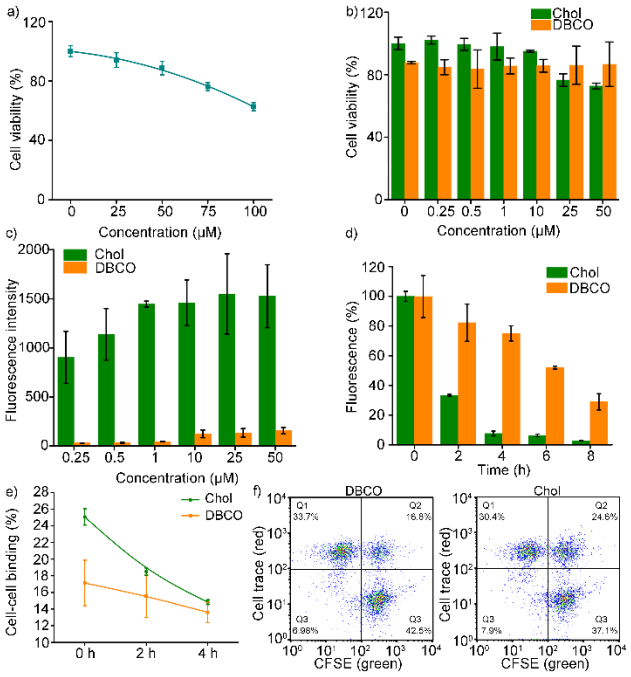

Figure 1: a) The cytotoxicity of different Ac_4ManNAz concentration. b) The cytotoxicity of different DI-DBCO/Chol concentration. c) The modification efficiency of DI-DBCO/Chol. d) The stability of DI-DBCO/Chol on cell surface. e) Analysis of captured CCRF-CEM cells at different time points. f) Representative flow cytometric cytograms showing cell-cell recognition at 0 h.

Conclusion: We compared click conjugation and lipid insertion in cell viability, engineering efficiency and displaying stability. Both methods have high biocompatibility without causing a significant decrease of cell viability when the concentrations of DI or sugar substrates are within a certain level. While DI molecules tethered on the cell surface using lipid insertion are less stable than click conjugation, lipid insertion has a higher modification efficiency. Resultantly, polyvalent DNA nanostructures formed on the cell surface with lipid insertion exhibits higher efficiency in recognizing target cells than those formed with click conjugation.

References:

1. Shi P. Angew. Chem., Int. Ed. 2018; 57:6800-6804.
2. Shi P. Angew. Chem., Int. Ed. 2020; 59:11892-11897.

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