Surface Polymerization of Oligonucleotide Aptamers for the Enhanced Capture and Release of Target Cells

Erin Richards, Niancao Chen, Shihui Li, Mark Battig, and Yong Wang*
Department of Biomedical Engineering, Pennsylvania State University, University Park, PA 16802

Statement of Purpose: The functionalization of surfaces with affinity ligands is inherently constrained by available surface area and viable reaction sites. Inspired by the jellyfish, which uses flexible tentacles to hunt and grip prey, we aimed to develop a new and enhanced surface functionalization method through the synthesis of surface-anchored polyvalent ligands. These multiple ligands can act collectively to bind target receptors with strengths that are orders of magnitude higher than a corresponding monovalent interaction. Additionally, this method affords a higher density of ligands per unit available surface area, which may benefit applications in biosensing and on-chip diagnostics. In this study, an Sgc8c aptamer ligand was used as a model ligand to hybridize with surface-anchored DNA polymers[1] and bind target CCRF-CEM lymphoblast cells.[2] Following capture, cells may be released in a manner that is non-destructive to both cells and the affinity surface through the molecular competition. Thus, rare cell populations may be isolated for downstream analysis of characteristic or overexpressed surface proteins.

Methods: The presented work employs the principles of intermolecular hybridization and polyvalent interactions to enhance current surface-ligand immobilization techniques. Briefly, Acrydite-modified linear initiators were incorporated in a thin, polyacrylamide film as a model surface material. From these initiator "seeding points", two single-stranded DNA monomers were sequentially hybridized to form DNA polymers. As single-stranded branching regions extend from the linear DNA polymer backbone, any affinity sequence with a complementary binding region (in this case an Sgc8c aptamer) may hybridize with the DNA polymer, forming a polyvalent affinity structure. The formation of polyvalent aptamers was investigated via electrophoresis, surface plasmon resonance, and fluorescence microscopy techniques. Additionally, the direct conjugation of aptamers was compared with polyvalent aptamers in terms of cell capture in situations with equal aptamer content and equal reaction sites. Cell release was achieved by depolymerization of the polyvalent aptamer structure using a molecular trigger. The depolymerization process was evaluated using surface plasmon resonance, gel electrophoresis, and fluorescence microscopy.

Results: We first demonstrated that DNA polymers can be formed and hybridized with aptamers in aqueous solution via gel electrophoresis. Next, we analyzed the real-time synthesis of DNA polymers through SPR. The results show that the degree of DNA polymerization increased with time whereas aptamer hybridization was completed in less than 1 minute. Through fluorescence microscopy, we ensure that polyvalent aptamers could be formed from hydrogel surfaces. Additionally, we observed that spacing between the aptamer and the surface ranging, from 10 – 144 nucleotides, had little impact on cell adhesion. By comparing direct conjugation and polyvalent aptamer surfaces with equal amounts of aptamers or equal amounts of surface reaction sites, we found that polyvalent aptamers bound more cells than directly conjugated aptamers (*Figure 1*). We also found that the DNA polymers could be disassembled by molecular triggers for rapid cell release.

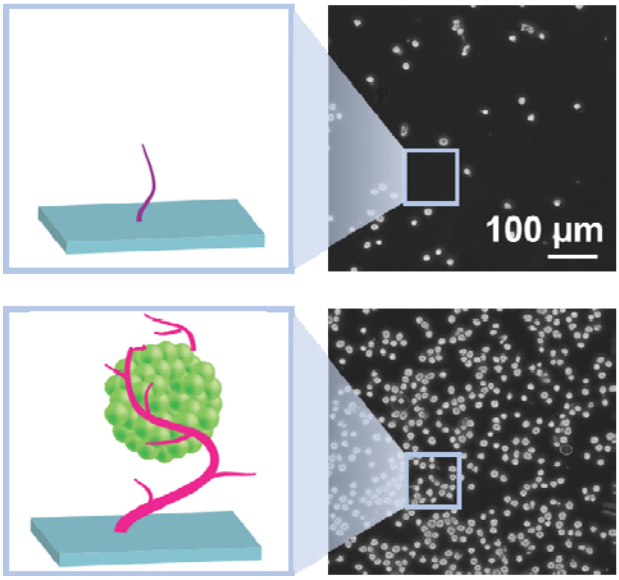

Figure 1. *Selective cell capture using directly conjugated monomeric aptamers (top panels) and polyvalent aptamers (bottom panels).*

Conclusions: We have demonstrated that polyvalent affinity ligands can be successfully functionalized on a surface. These polyvalent ligands prove more effective in target binding than directly conjugated single aptamers in situations with equal aptamer amounts and surface density. Therefore, this method can be extended toward the enhancement of binding affinity on inert surfaces or surfaces with limited available area. The potential hybridization of any affinity ligand to DNA polymers from the surface of theoretically any material highlights this method's versatility. Additionally, the nondestructive release of cells from the substrate allows for further downstream analysis of the captured cells or molecular regulation of cell-material interactions. We expect this immobilization advancement to impact diverse fields such as biosensing, biological separations, and regenerative medicine.

References:

[1] Chen, N., Li, S., Battig, M.R., and Wang, Y. Small. 2013; 9:3944-3949.

[2] Richards, E., Li, S., Chen, N., Battig, M.R., and Wang, Y. Biomacromolecules. 2014; DOI: 10.1021/bm501347s