

# DNA-Mediated Cellular Adhesion on a Polyacrylamide-Based Hydrogel Substrate

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## Introduction

Hydrogels are synthetic, hydrophilic, polymeric networks composed of one or more cross-linked monomers. Due to their biocompatibility, hydrogels have extensive applications as biomaterials. In order to closely mimic biological tissues, hydrogels must exhibit similar stiffness and adhesive moieties. In past work, we have studied the tunable mechanical properties of polyacrylamide hydrogels, altering hydrogel composition and volume fraction to tune the shear storage modulus, or “stiffness.” This study explores a DNA-mediated approach to functionalizing the hydrogel surface with the adhesive peptide necessary to support cell adhesion and spreading.

The use of DNA as an assembly tool was pioneered by Mirkin<sup>1</sup>, who used DNA cross-linking strands to assemble gold nanoparticle aggregates. Recently, Milam<sup>2</sup> investigated DNA-mediated assembly of polystyrene microspheres, replacing the cross-linking strand with direct hybridization. This project will expand on these studies by applying the concept of DNA-mediated assembly to a cellular adhesion system.

DNA presents several advantages over alternative assembly methods. First, specificity of design allows for a finely tuned adhesion system. Each DNA strand can be precisely designed with a specific length and number of complimentary base pairs to its counterpart. In addition, as illustrated in Figure 1, competitive strand hybridization provides a disassembly mechanism.

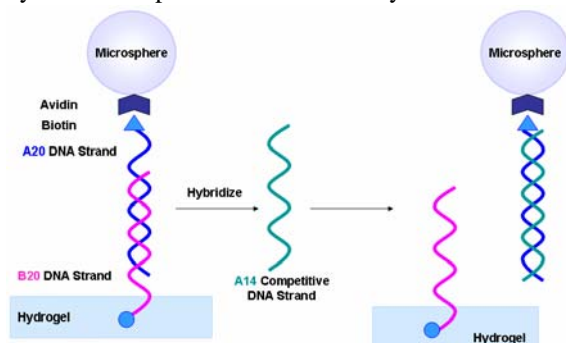


Figure 1: DNA-mediated assembly and disassembly of the cellular adhesion system. The A14 strand is introduced to competitively hybridize to the A20 strand, thus breaking the linkage between the microsphere and hydrogel.

## Experimental Design

The DNA scheme designed for the assembly and disassembly of the adhesive system is displayed in Figure 2 (below). Two strands compose the initial adhesive complex; the B20 strand, 20 base pairs long, will be immobilized on the hydrogel surface at the 5', acrydite-modified end. The biotin-modified A20, also 20 base pairs long, will be hybridized to B20 with a 10 base-pair overlap. Finally, A14 will be introduced to the system to competitively hybridize to A20 with a 14 base pair overlap, reversing adhesion of the microsphere to the hydrogel substrate.

**B20:** 3' - **ACGCCGACTA**TTTTTTTTTTT - **Acrydite** - 5'  
**A20:** 5' - **Biotin** - TTTTTTGGATT**GCGGCTGAT**-3'  
**A14:** 3' - **CCTAACGCCGACTA** - 5'

Figure 2: DNA strand sequences for A20, B20, and A14.

This study is composed of three separate experimental systems; a cellular mimic model system, a cellular mimic RGD system, and a fibroblast adhesion system. Each system utilizes the previously described DNA assembly sequences, and a polyacrylamide hydrogel substrate.

The cellular mimic model system will demonstrate DNA-mediated adhesion to a hydrogel substrate. An avidin-coated 10 μm microsphere will be used as a cellular mimic; biotin will be immobilized on the hydrogel surface using DNA-mediated assembly. Because the avidin/biotin linkage is a known, highly specific linkage, adhesion will depend on the success of the DNA-mediated step of the assembly.

The cellular mimic RGD adhesive peptide system replaces the known avidin/biotin linkage of the previous system with a specific RGD peptide/RGD antibody linkage. RGD (R: arginine; G: glycine; D: aspartic acid) is an adhesive peptide motif that has been studied extensively, and has a strong binding affinity for fibroblasts. The cellular mimic RGD system will demonstrate DNA-mediated immobilization of the RGD adhesive peptide to the hydrogel substrate. An antibody-coated microsphere will act as the cellular mimic.

Finally, the fibroblast adhesion system (Figure 4) will demonstrate successful DNA-mediated fibroblast adhesion to the hydrogel substrate.

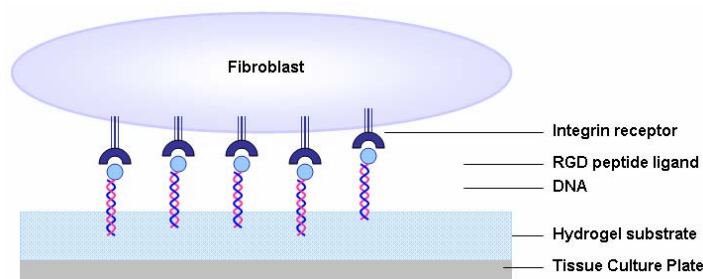


Figure 4: Schematic of the fibroblast adhesion system.

## Expected Results

In each system, DNA-mediated adhesion will be observed using microscopy. Fluorescently labeled microsphere cellular mimics may also be used to better visualize successful adhesion. In the final system, fibroblasts should exhibit a spread morphology after successful adhesion to the hydrogel substrate, but should revert to a spherical morphology once released from the substrate.

## References

1. Mirkin, C, Letsinger, R., Mucic, R., and Storhoff, J. A DNA-based method for rationally assembling nanoparticles into macroscopic materials. 1996. *Nature*. **382**: 607-609.
2. Zhang, Y., Milam V., Graves D., Hammer D. 2006. Differential adhesion of microspheres mediated by DNA hybridization I: experiment. *Biophys. J.* **90**: 4128-4136