Development of Improved Assays for Cell Adhesion through Local Application of Forces to Cells

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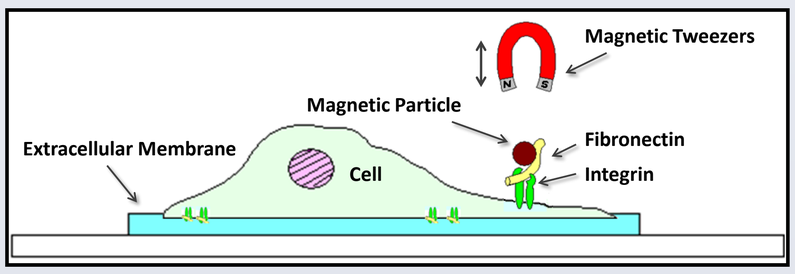
Abstract

In this project, we are developing new methods to investigate if and how cell adhesion strength changes in diseased cells. In neurons, cell adhesion is particularly important, as cell-cell contacts at the synapse are required for electrical signal transmission. In Alzheimer's and other neurological diseases, neuron signaling networks fail by cell retraction, but it is not known if retraction arises from loss of adhesion, or by another pathway. To test this, we are developing improved methods to apply forces directly to cells using optical traps or magnetic tweezers, custom-built tools that manipulate small dielectric or magnetic particles that are coupled to the cell surface. In our preliminary work we are using both a synthetic system of engineered hydrogels to test adhesion strength of known cellular proteins, and to optimize our binding and pulling protocols, as well as living cells. Our cellular work, we will start with a non-neuronal cell line (Cos 7 cells), which has the advantage of fast proliferation, ease of transfection with proteins of interest, and ease of imaging with microscopy. By applying forces directly to the cells we can measure detachment forces, and in the future will also visualize cell deformation under loading. In addition to investigating the role of adhesion in neurological disease, the ability to apply forces to neurons would also allow us to investigate the effects of blunt force trauma, with importance to understanding traumatic brain injury.

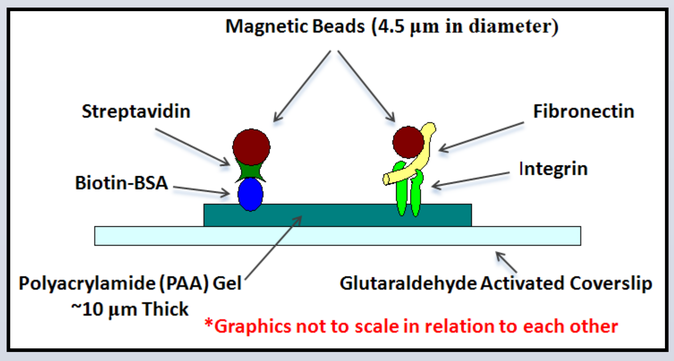
Objectives

Our goal is to create methods to apply localized forces to cells. In order to do so, we must first create and optimize binding and force detachment protocols with known cellular proteins on synthetic engineered hydrogels.



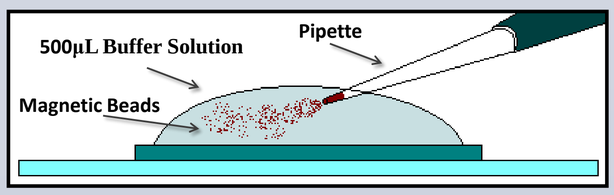
Binding Protocol

- Activated 22x40 mm (1.5 mm) Coverslip with glutaraldehyde.
- Coupled Streptavidin and Integrin proteins, respectively, to 4.5µm Magnetic Bead (Dynabeads® M-450 Tosylactivated) in x1 PBS + BSA.
- Created a ~10µm thick Polyacrylamide (PAA) gel with a shear modulus of 230 Pa. Biotin-BSA or Integrin protein was mixed in the Acrylamide solution before polymerization. Gel thickness obtained by placing 10µL Polyacrylamide solution on a hydrophobic glass slide (3" x 1" x 1mm) surface and placing a Glutaraldehyde Activated Coverslip on top to flatten out the solution and allowing it to polymerize for >40 minutes.



Bead Deposition

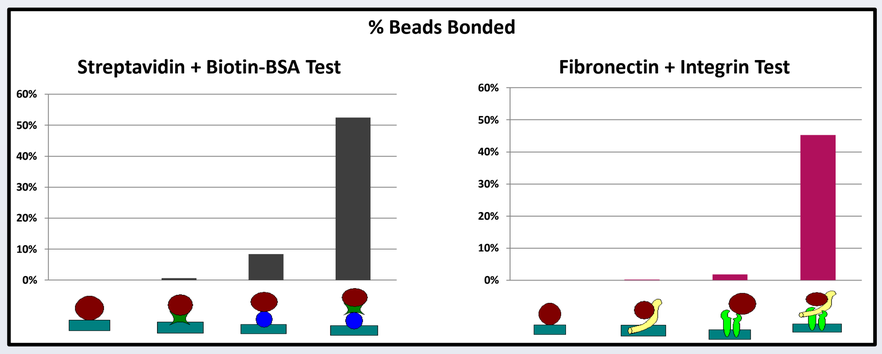
- Drop deposition was done on a 500µL x1PBS+BSA buffer surface on gel.
- Used bead concentration of 1 x 10^8 / 1µL in x1 PBS.
- Allowed protein coupled beads to incubate for 1 hr.



Binding Results

- Optimized Binding Protocol by imaging gel using microscopy before and after a standardized wash to observe percentage of beads bonded.
- Created four conditions to observe protein binding as well as non-specific binding.
  - Beads with and without protein.
  - Gels with and without protein.

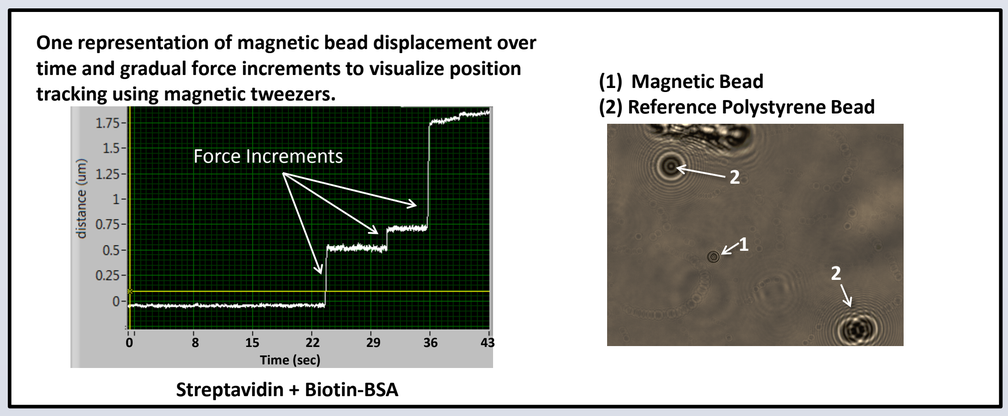
After troubleshooting and applying improvements to protocol we accomplished significant reduction in non-specific binding and maintained specific protein binding.



Force Detachment Protocol

- Used magnetic tweezers, custom-built tool used to track and manipulate magnetic beads. Placed Polystyrene reference beads on coverslip surface to compare diffraction changes of moving magnetic bead, then used that data to calculate detachment force.
- From current pull observations:

-Fibronectin beads detach from Integrin gel at **54 pN**, others detached at **406 pN**, the beads that remained attached were displaced **0.5-1 µm** towards magnet at **406 pN**.
-Streptavidin beads did not detach, they displaced **2 µm** towards magnet at **406 pN**.



Conclusions

We have created and optimized binding protocols and have obtained a desirable specific binding percentage and significantly reduced non-specific binding through troubleshooting. Initial force detachment analysis indicates expected stronger Streptavidin + Biotin-BSA bonds over Fibronectin + Integrin bonds. Further work on force detachment protocol, using magnetic tweezers, will be necessary to obtain more precise protein detachment force measurements. After these force measurements have been further investigated, we will then be able to apply both protocols to investigate protein detachment forces on Cos 7 cells.

References

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