

Cell Adhesion

Application of Localized
Forces to Cells

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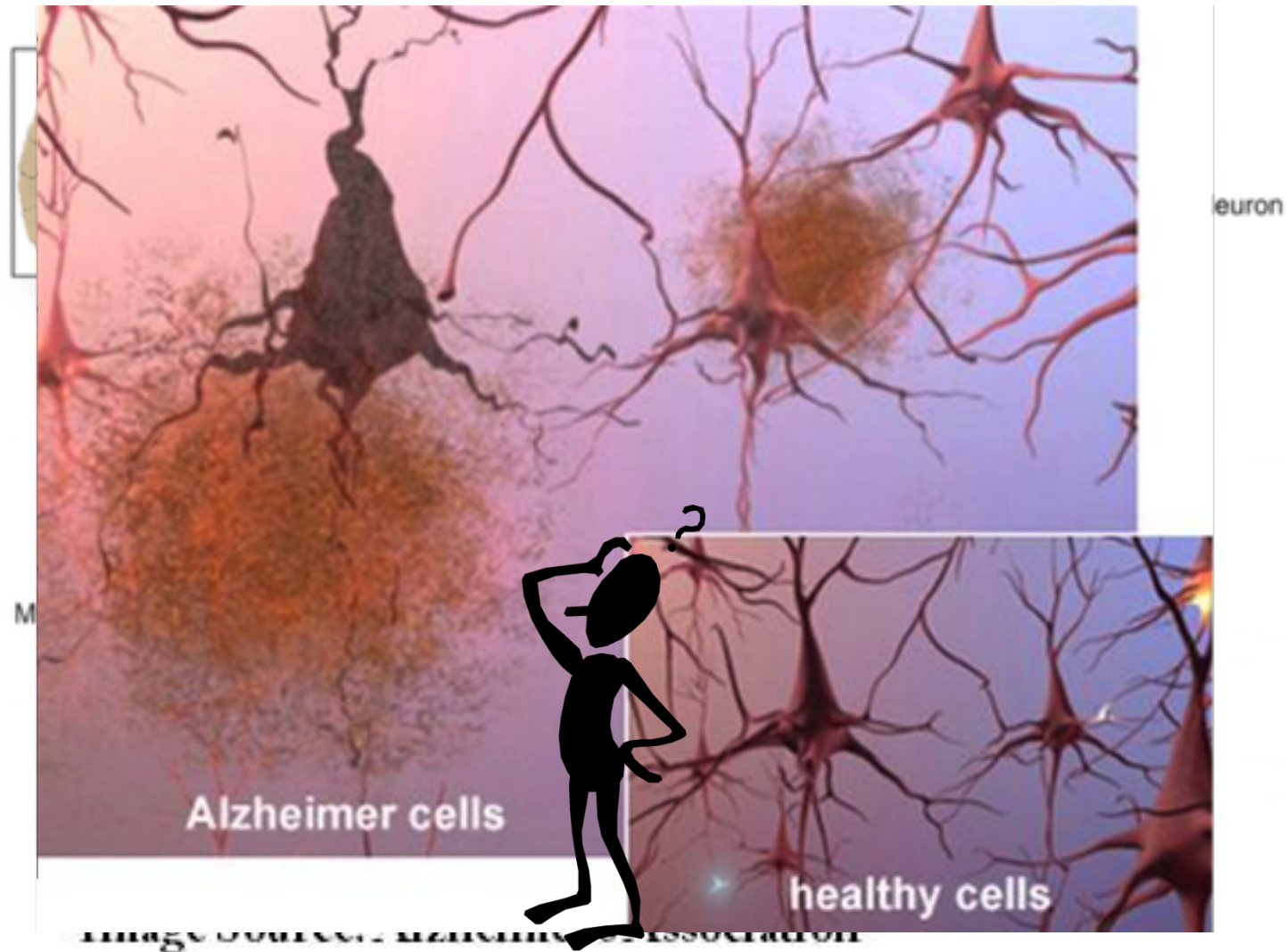
Funding Source: National Science Foundation



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The Importance of Cell Adhesion

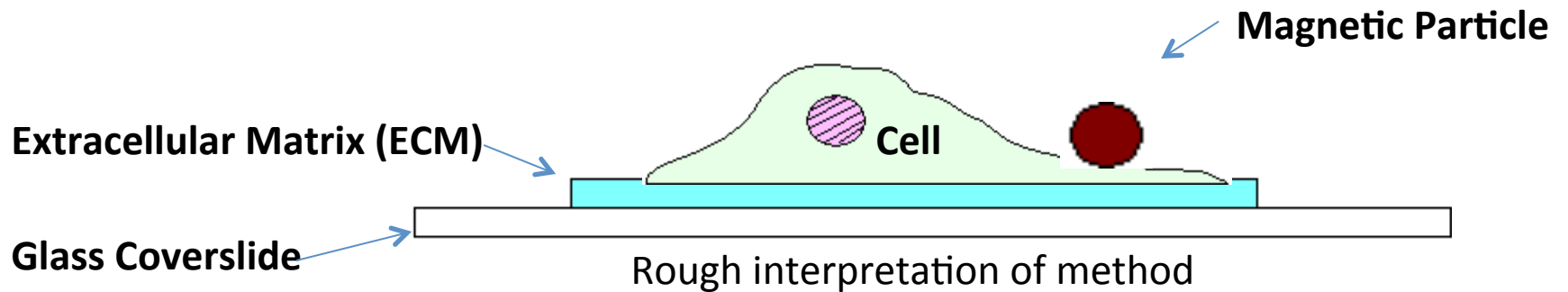


Goal: Investigate If and How Cell Adhesion Strength Changes in Diseased Cells.

- Develop methods to apply forces to cells.
- Must first create and optimize protocols using gels.

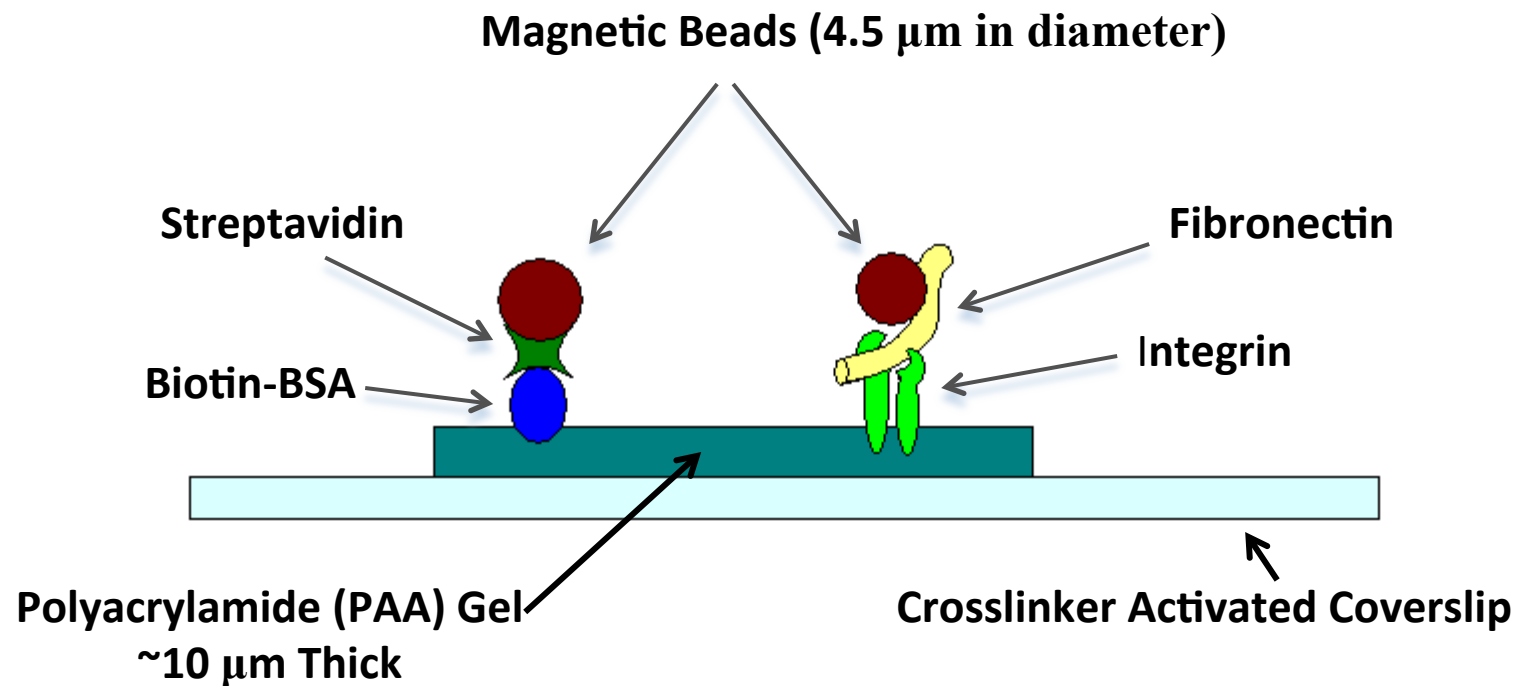


Magnetic Tweezers



Working on Protocols

- Using synthetic gels to optimize binding and pulling protocols of known cellular proteins.

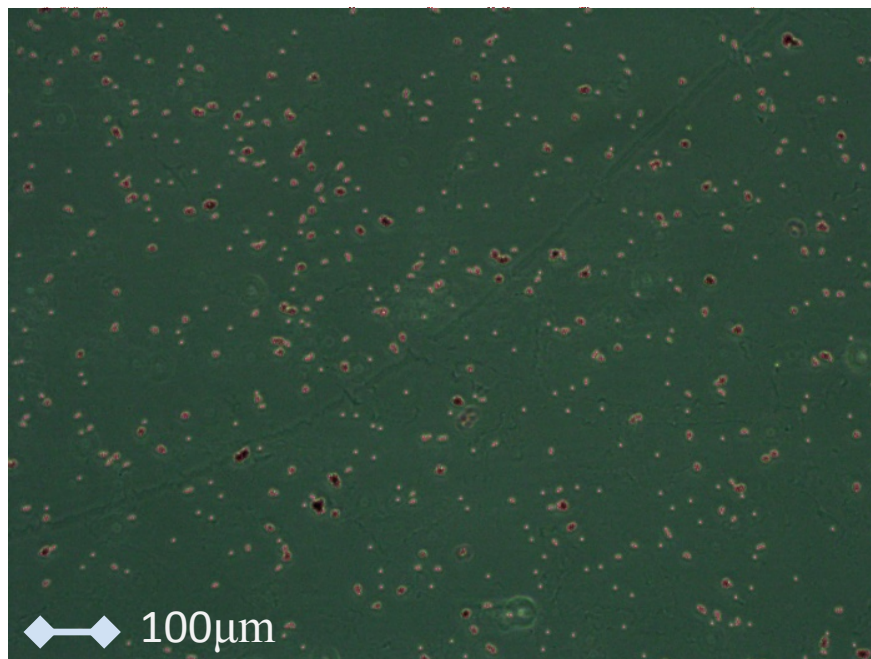


***Graphics not to scale in relation to each other**

Optimizing Binding Protocol

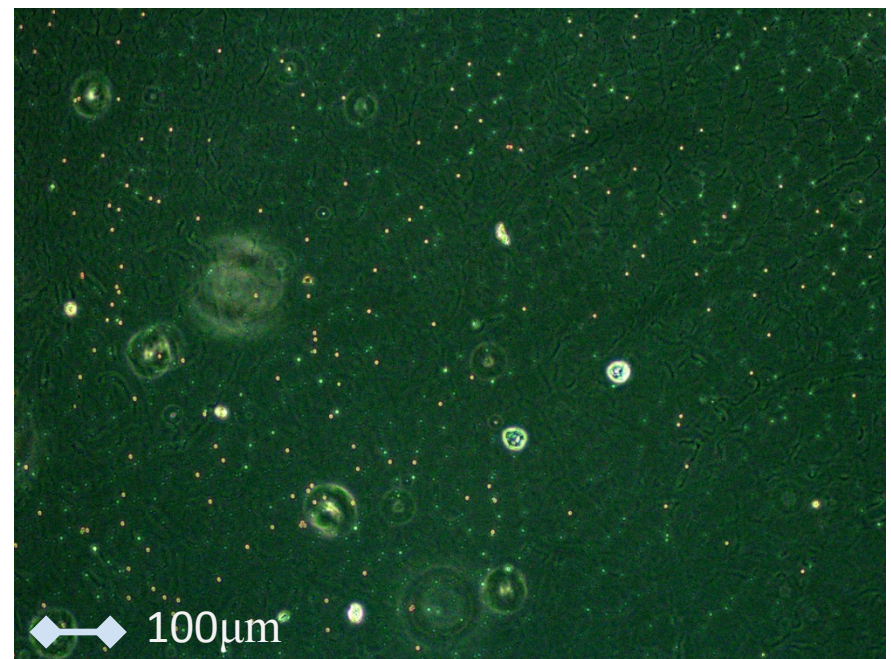
- Created four conditions to observe protein binding as well as non-selective binding.
 - Beads with and without protein.
 - Gels with and without protein.

Beads on gel before wash



Used ImageJ program to count beads.

Beads on gel after wash



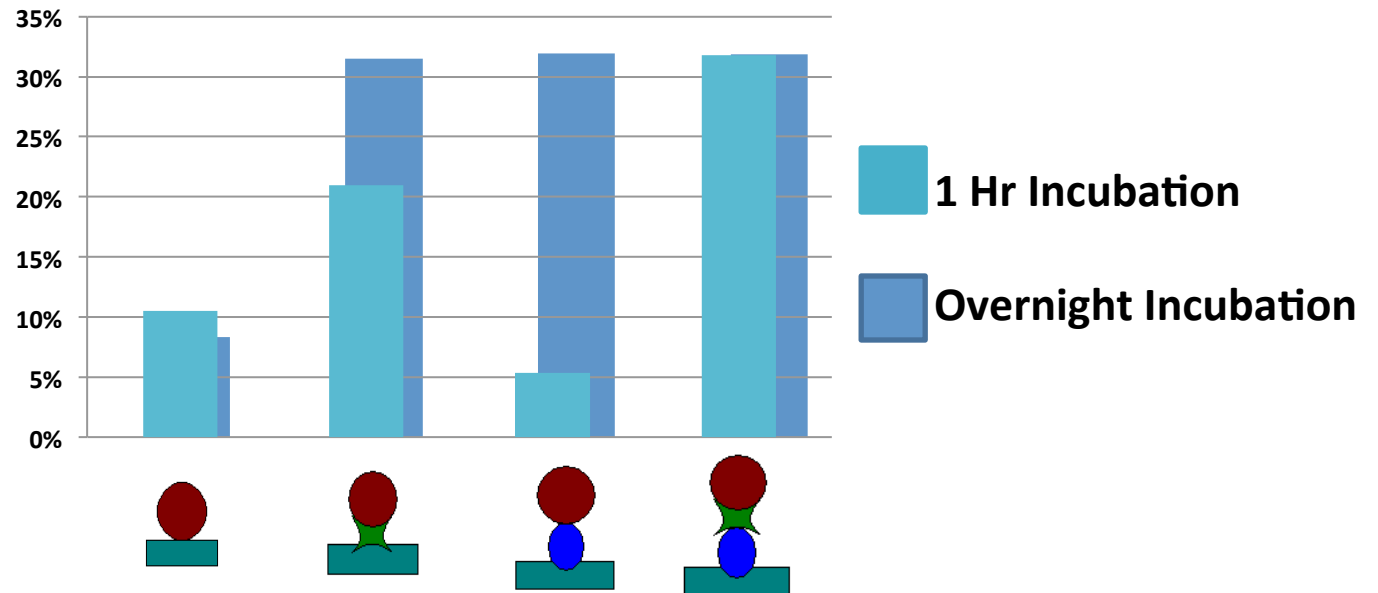
Area= 1.4 mm^2 (1mm * 1.4mm)

Troubleshooting Protocols

Biotin-BSA coated gels with Streptavidin coupled beads.

- Used Buffer solution for bead application (PEM 80), screens out charges.
- Lower binding in absence of protein in gel.
- 1 hr incubation is favorable.
- Beads bind well when either protein is present, especially Streptavidin.

% Beads Bonded

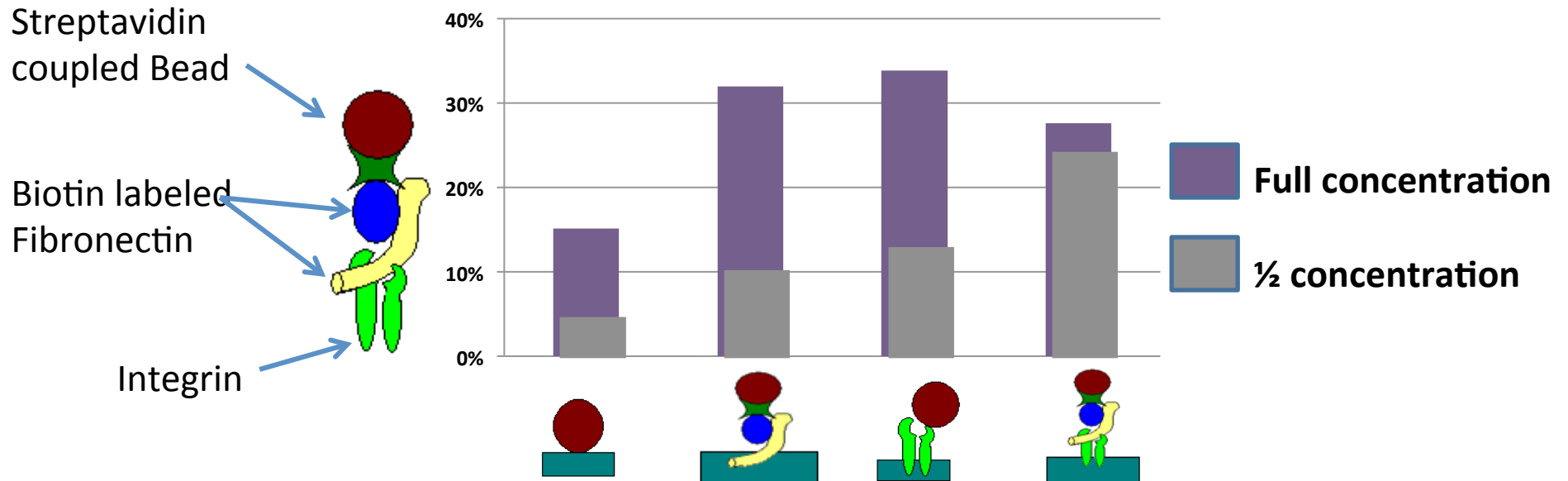


Troubleshooting Protocol

Streptavidin coupled beads with Biotin labeled Fibronectin on Integrin coated gel.

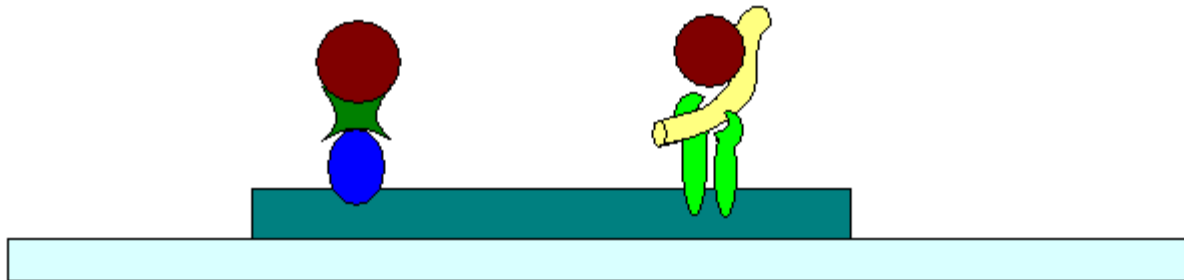
- Reducing bead concentration is favorable.
- Presence of Streptavidin might be increasing non-specific binding.
- Began with concentration of 200,000 Beads / 1 μ L in x1 PBS + BSA.

% Beads Bonded



Improvements to Protocol

- Used Bovine Serum Albumin (BSA) solution to suspend and apply beads.
 - BSA strongly inhibits non-specific binding.
 - Has stability and lack of interference within biological reactions.
- Used ½ the previous bead concentration.
- Maintained favorable 1 hr incubation period.

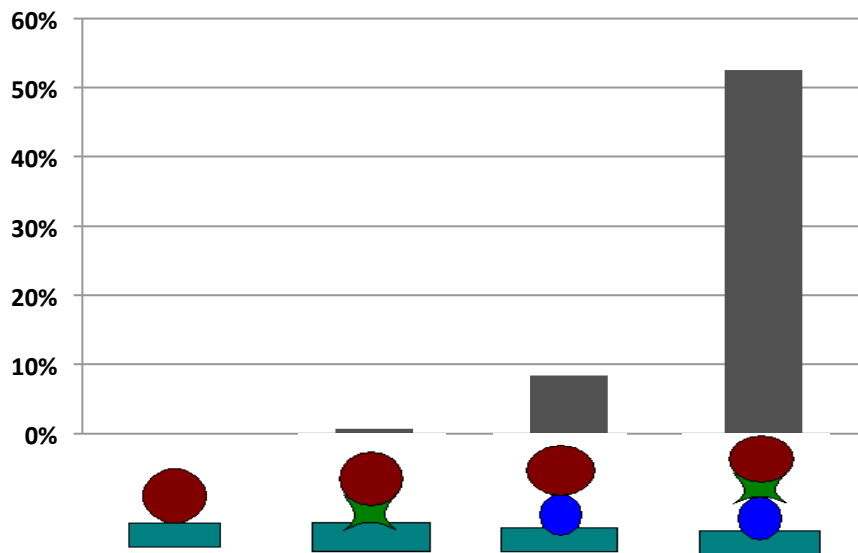


Successful Binding

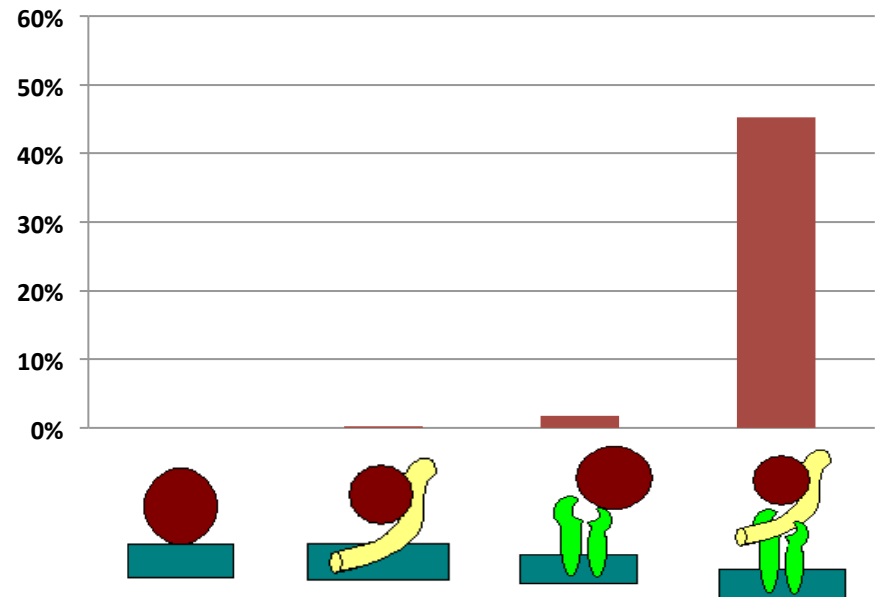
- Reduced non-specific binding and maintained specific binding.

% Beads Bonded

Streptavidin & Biotin-BSA Test

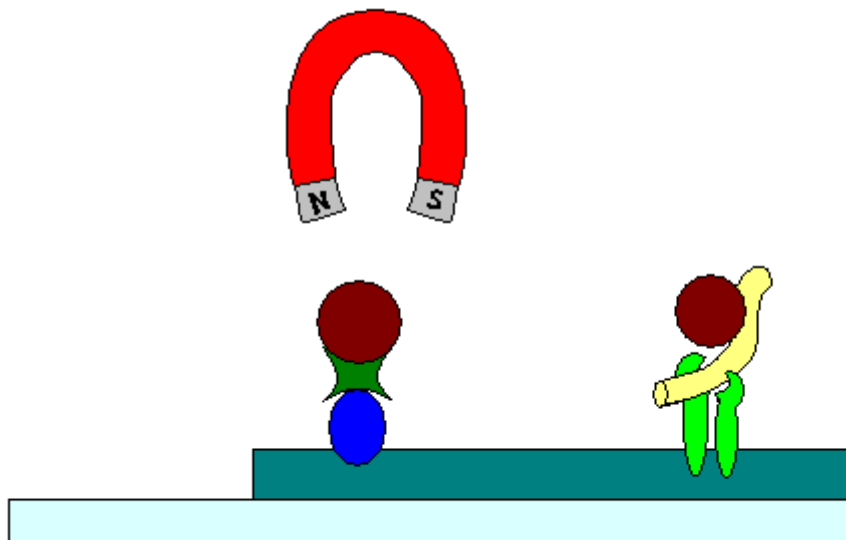


Fibronectin & Integrin Test

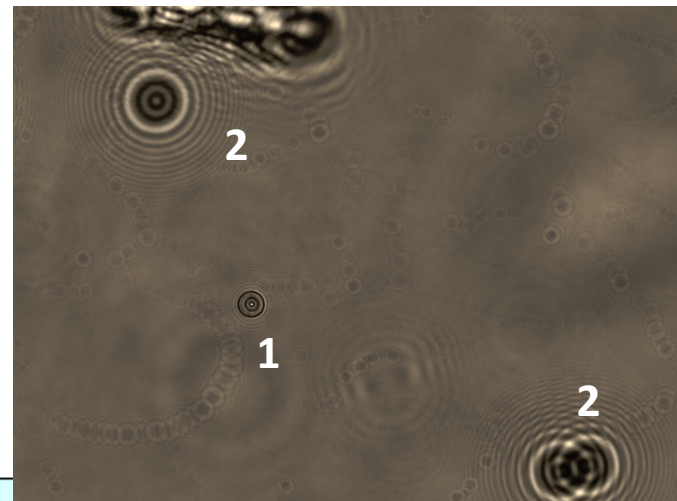


Pulling Protocol

- Used magnetic tweezers, custom-built tool used to manipulate beads and measure the detachment force.
- From current pull observations:
 - Some Fibronectin beads detach from Integrin gel at **54.65 pN**, others detached at **406.08pN**, the rest moved **0.5-1 μm** up at **406.08 pN**.
 - Streptavidin beads did not detach, they moved up **2 μm** at **406.08pN**

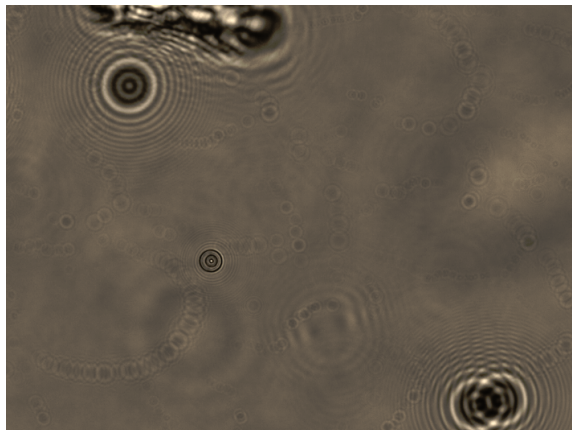


(1)Streptavidin Bead (2)Reference Beads



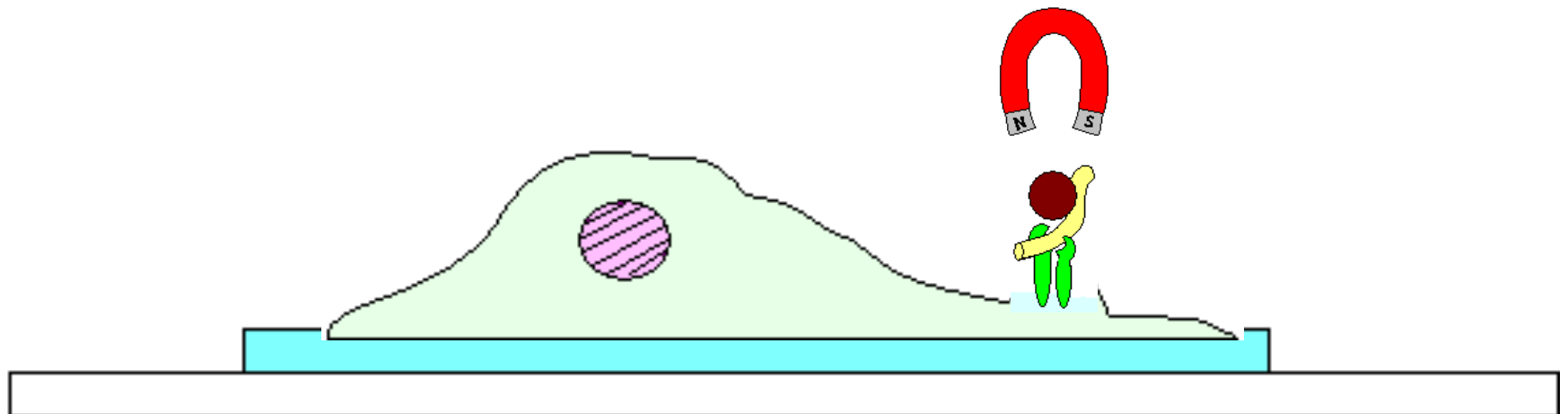
Summary

- Established protocols for binding beads to gels.
- BSA is a great relatively inert protein for use in adhesion studies.
- Through troubleshooting we obtained specific binding and reduced non-specific binding.
- Streptavidin + Biotin-BSA have stronger bonds than Fibronectin + Integrin.



Future Goals

- Continue pulling protocol to obtain more data.
- Perform binding and pulling protocols with different size beads and on cells.
- Observing damage on cells after force is applied might also help us understand effects of blunt force trauma.



Acknowledgements

Thank you!

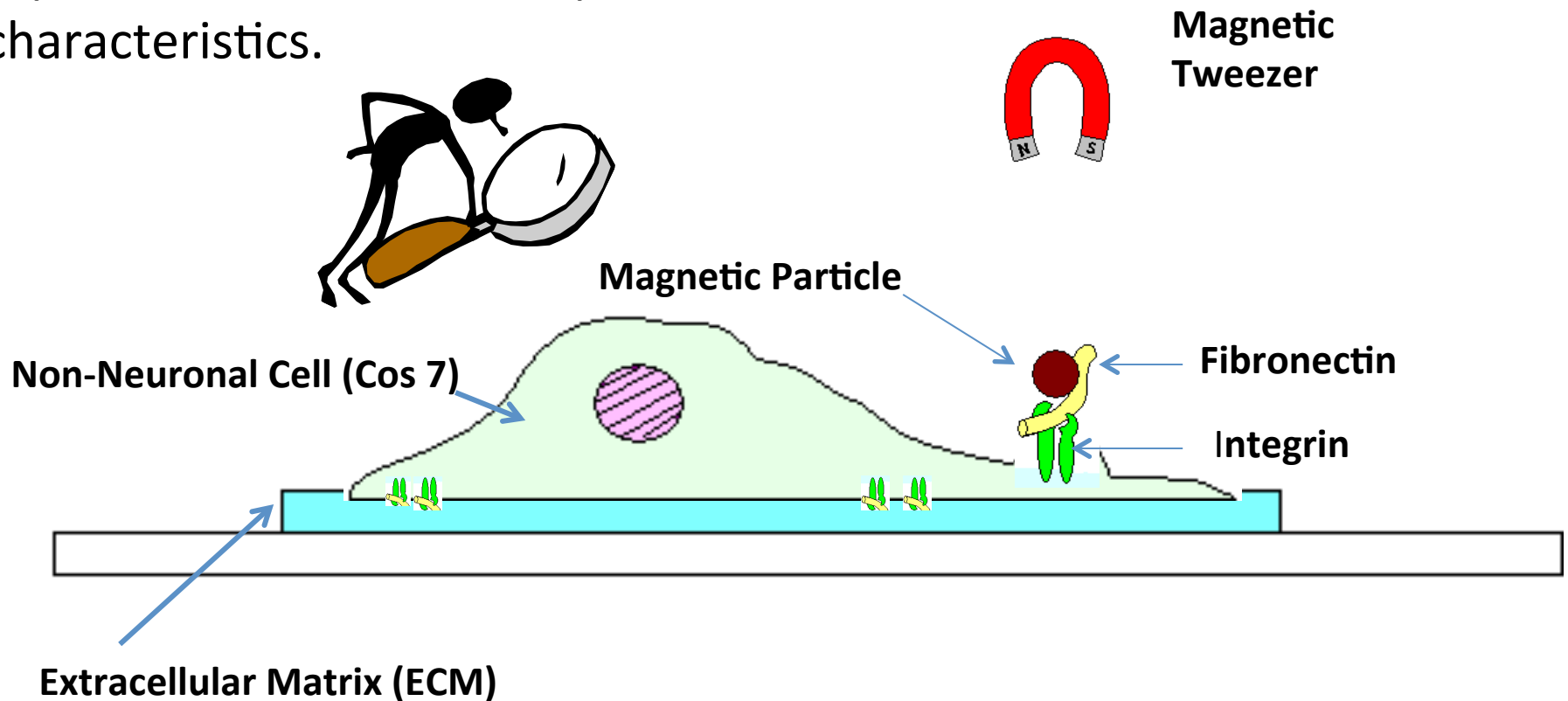
- Principal Investigator: Associate Professor Megan Valentine Ph.D.
- Mentor: Dezhi Yu, Ph.D. Candidate.
- Valentine Group

INSET Program



Cell Work

- Will be using Cos 7 cell, they have rapid reproduction and desired protein characteristics.



Obtaining Data

