Characterizing the Attachment and Proliferation of Human Dermal Fibroblast (HDF) In Vitro

By XXX

Objectives

- Assess the Impact of surface treatments (i.e. Tissue-Culture, Fibronectin, Untreated) on cell attachment
- Quantitatively characterize the effects of serum-to-media concentrations on cell proliferation

Evaluating Cell Attachment With a Quantitative Cell Attachment Assay

- Objective Tested: Cell Attachment
- Parameter Tested: Surface Treatment: Tissue Culture (TC) treated, Untreated, Fibronectin Treated.
- Method: 12 wells of each surface condition were seeded with 10,000 HDF cells. The cells were seeded in 1mL Dulbecco's Modified Eagle Medium (DMEM) with 10% Serum & 1% antibodies. At times 30min, 1hr15min, 2hr30min, and 4hrs post seeding, 3 wells of each treatment are rinsed (media aspirated and a Phosphate Buffer Saline (PBS) ran over cells) of media and the unattached cells. After being rinse, the well's confluency, cell density, and cell morphology was observed using the 10x objective of the light microscope.

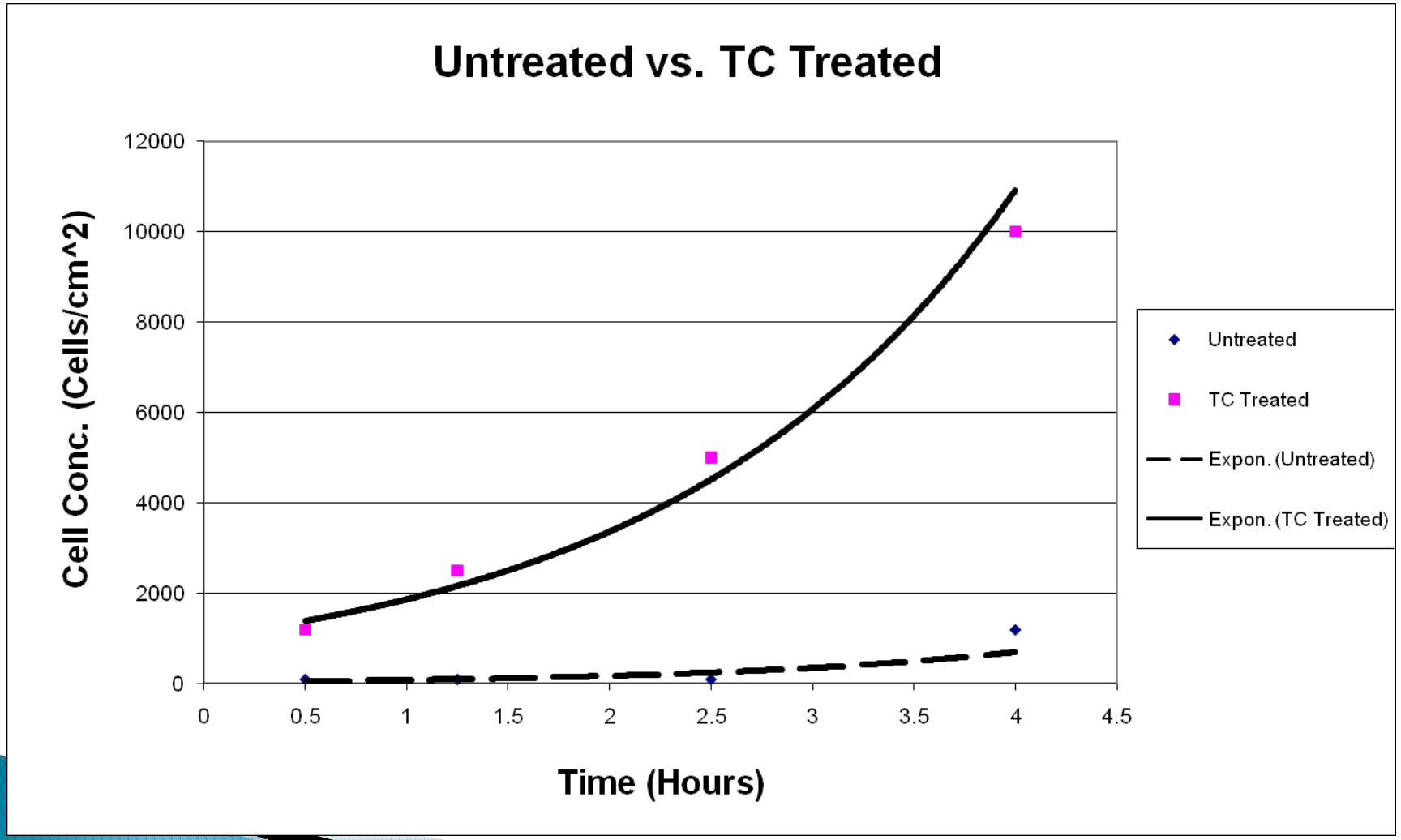
Evaluating Cell Attachment With a Fibronectin (Fn) Attachment Assay

- Objective Tested: Cell Attachment
- Parameter Tested: Surface Treatment: Untreated and Fibronectin Treated.
- Method: Using a Non-TC-Treated 24 Well plate, 3 control wells were coated with .5mL of PBS. 3 "Half n Half" wells had half their surface coated with fibronectin. 3 "Design" wells had an 'X' painted with fibronectin, and 3 wells had 0.3mL of fibronectin deposited in them. After initial set up the well plates were allowed to incubate for 30 minutes, then they were rinsed (using PBS with 10mg/mL Bovine Serum Albumin). The 12 wells of were seeded with 50,000 HDF cells suspended in 1mL DMEM with 10% Serum & 1% antibodies. 2 hrs post being seeding, The wells were rinsed, (regular PBS rinse). The cell adhesion & morphology is checked pre- and post- this 2nd rinse

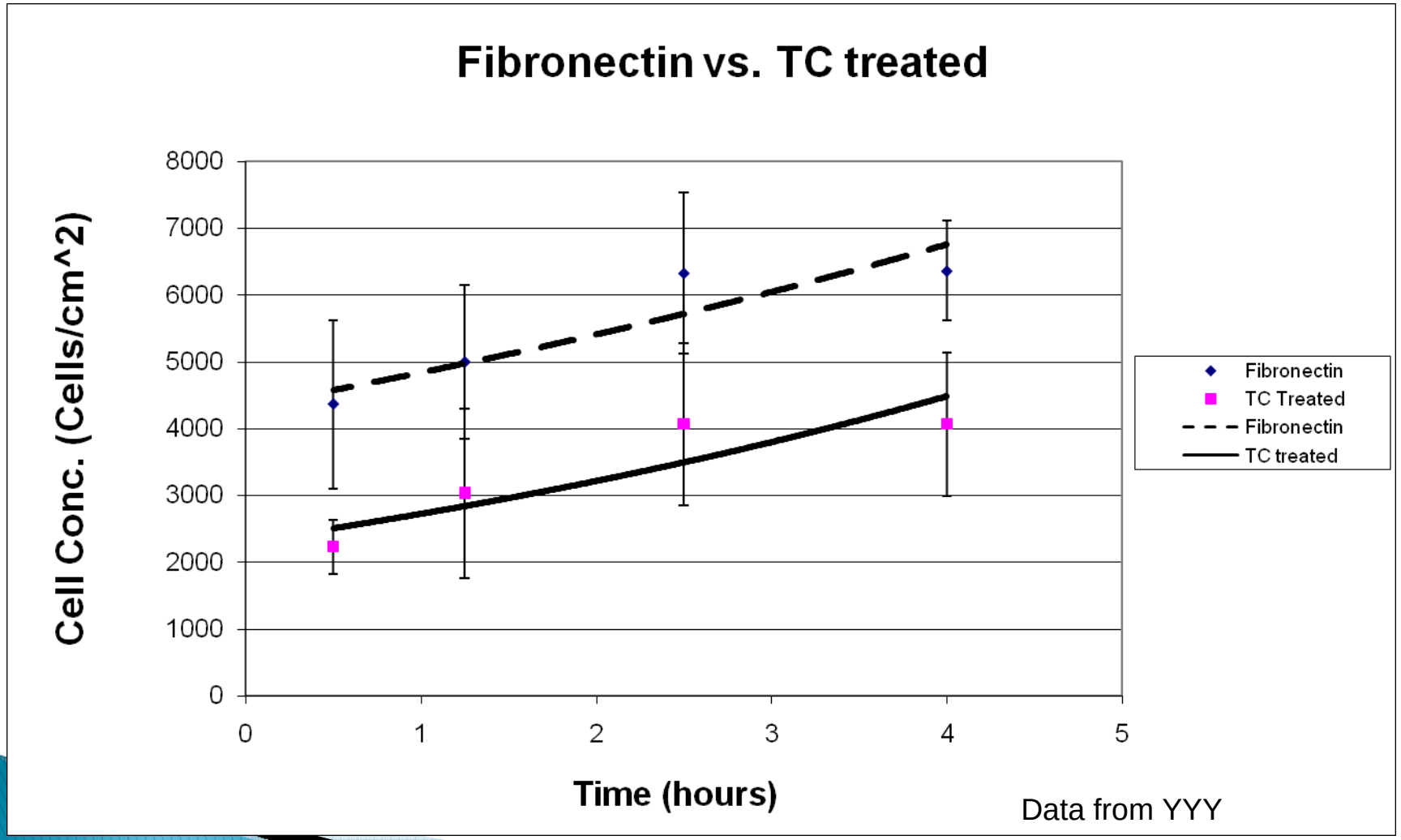
Evaluating Cell Proliferation With a Cell Proliferation Assay

- Objective Tested: Cell Proliferation
- Parameter Tested: Serum Concentration in 1% antibody DMEM: 10% Serum, 5% Serum, and 1% Serum
- Method: 33 TC-Treated wells were seeded with 5000 HDF cells. The cells were seeded in 1mL of the 1%-DMEM. After 4hrs, 6 of the wells are rinsed, trypsinized (250 uL trypsin for 5-10min, then 750uL media), and their cells were counted via Coulter Counter (1mL of suspended Cells+9mL Isoton placed in Coulter Counter). If this count confirms that cell attachment is >60%, the other 27 wells were rinsed (using aspiration and a PBS rinse). 9 of the wells were re-suspended in 1%-DMEM, 5%-DMEM & 10%-DMEM. At times 2days, 5days, and 7day post seeding, 3 wells of each concentration are rinsed, trypsonized, and counted using Coulter Counter.

Effects of Surface Treatment on Cell Attachment

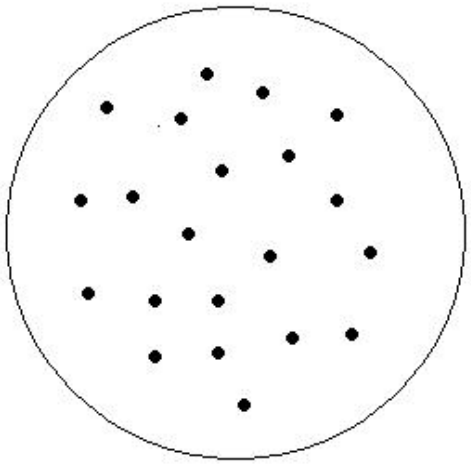


Effects of Surface Treatment on Cell Attachment

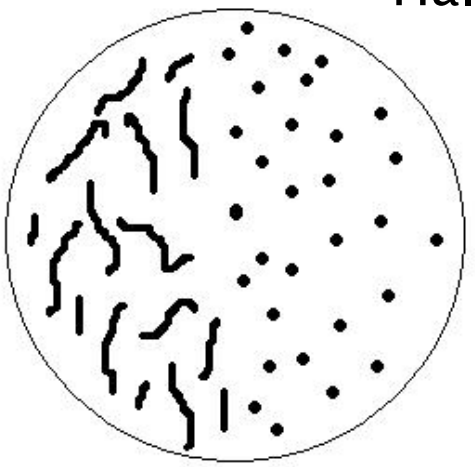


Pictures of Fn Attachment Well Patterns: Pre-Rinse

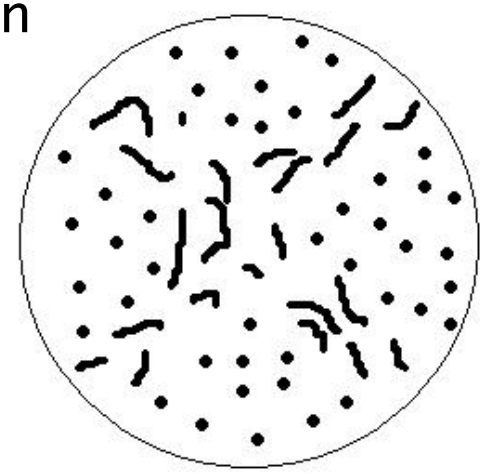
Untreated Only



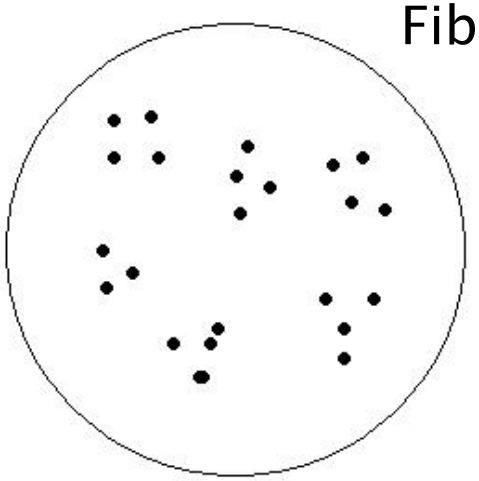
Half n Half



Design



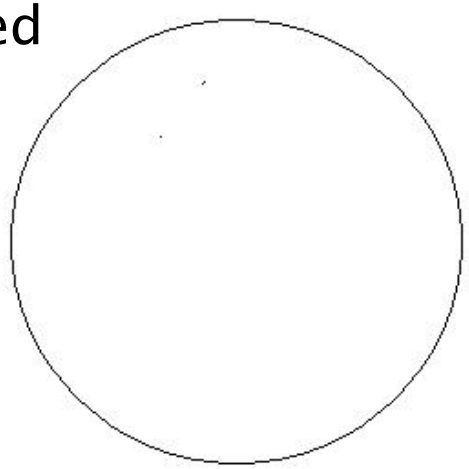
Fibronectin



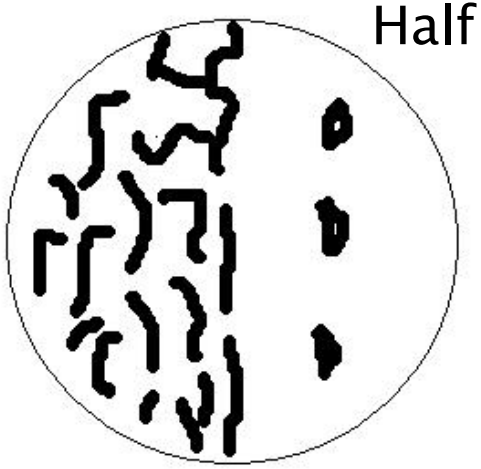
Note: This slide is covered with cells but you can not see them below the unattached cells.

Pictures of Fn Attachment Well Patterns: Post-Rinse

Untreated Only



Half n Half



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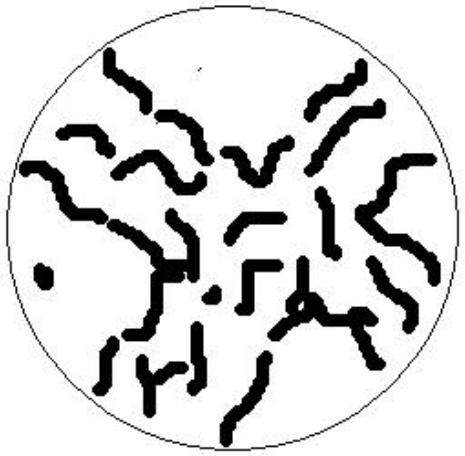
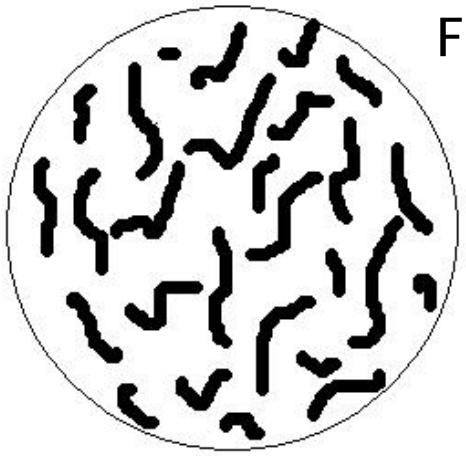


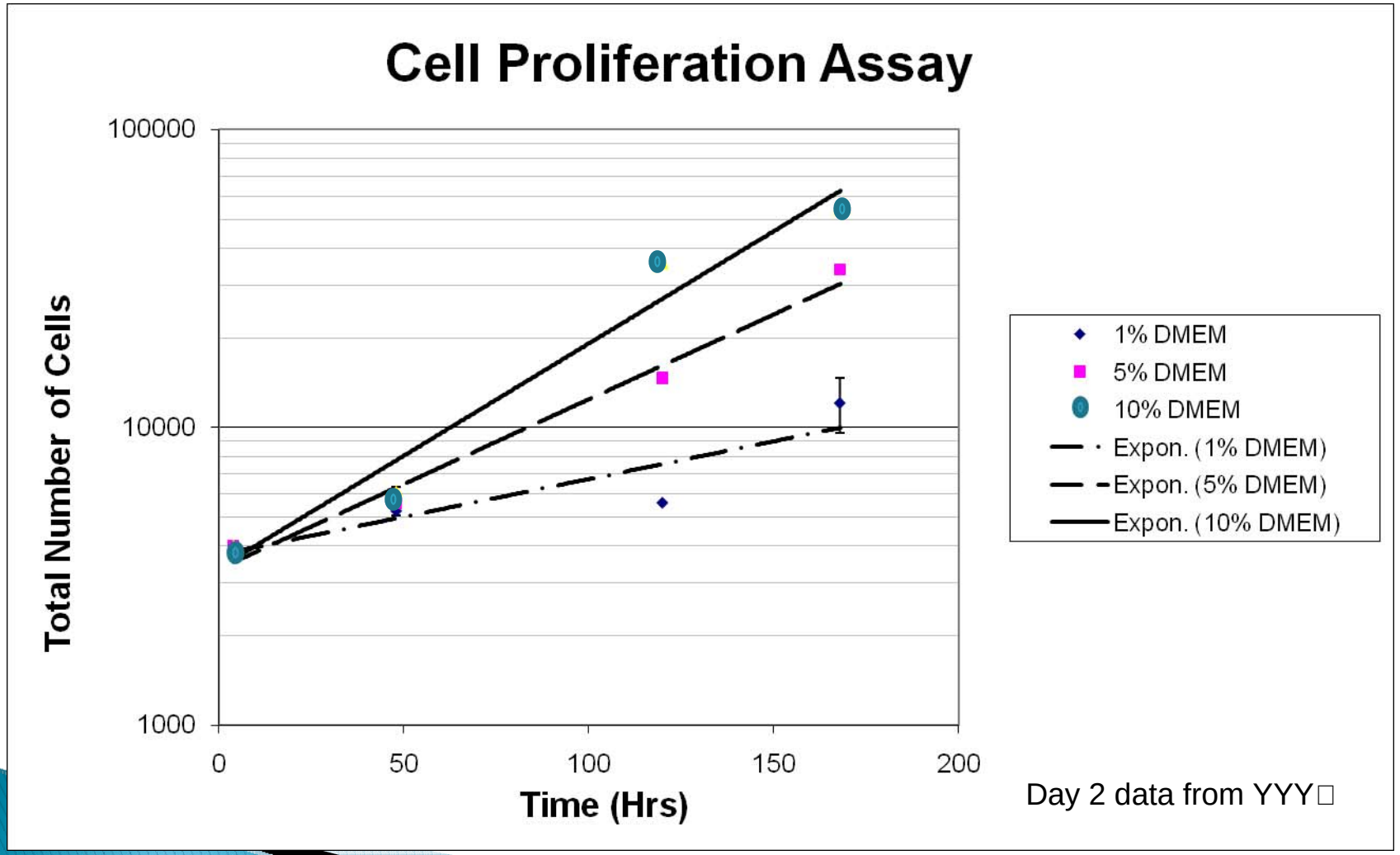
Fibronectin



Summary of Fn Attachment Assay

- Fibronectin (Fn) Treated Areas:
  - Increased cell Attachment
  - High Confluency
  - Greater Pseudopodia
  - Cells Completely Attached & Elongated.
- Untreated Areas:
  - Almost No Cells Attachment
  - Confluency Close to 0%
  - Little Noticeable Pseudopodia
  - Cells Loosely Attached & Rounded

Effects of Serum Concentration in DMEM on Cell Proliferation



Day 2 data from YYY□

Conclusion

- Objective 1: Cell Attachment
  - Having an untreated cell attachment surface has a profoundly negative impact on cell attachment. Both the Quantitative Cell Attachment (QCA) Assay and the Fn attachment Assay shows that an untreated surface takes a few hours before cells even begin to attach
  - Both the Fn attachment Assay and the QCA assay show that Fn treatment can significantly increase cell attachment rate from untreated. Statistically analysis (Paired T-test, $a = 0.05$) shows there is a statistically significant difference between the TC and Fn treated surface attachment at every time except 4 Hrs. Which shows that Fn attaches cells faster but TC treated is still comparably as effective.
- Objective 2: Cell Proliferation
  - An ANOVA and a Tukey Test showed that there was a statistically significant difference between the cell counts at the 3 different Serum Concentration. Therefore the Cell Proliferation Assay shows that increase in Serum Concentration of DMEM can increase cell growth rate.