Effects on Human Dermal Fibroblasts in Different Culture Conditions

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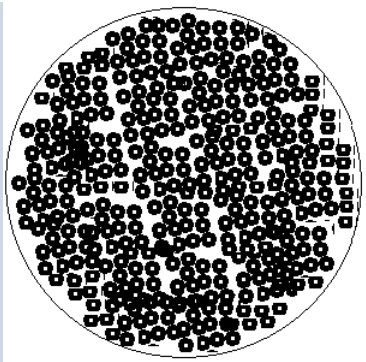
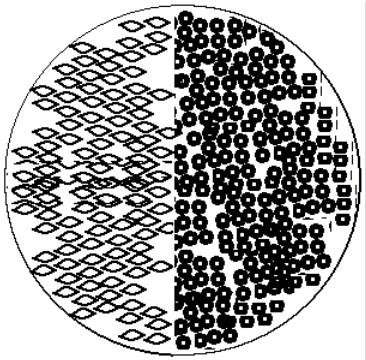
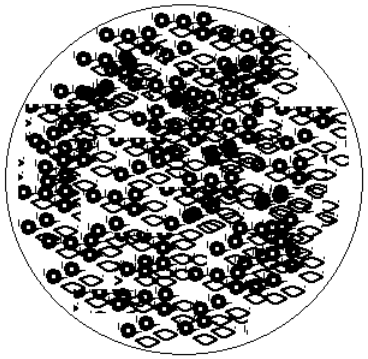
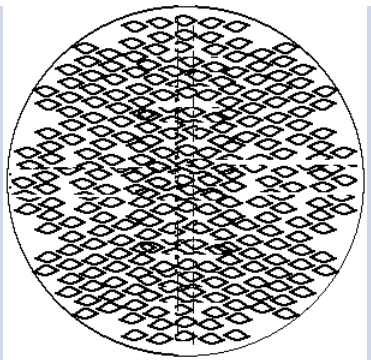
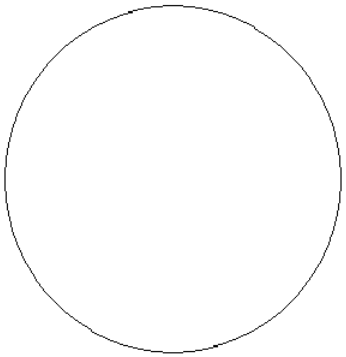
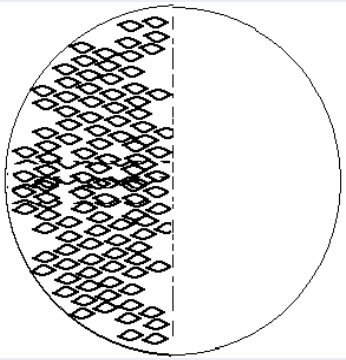
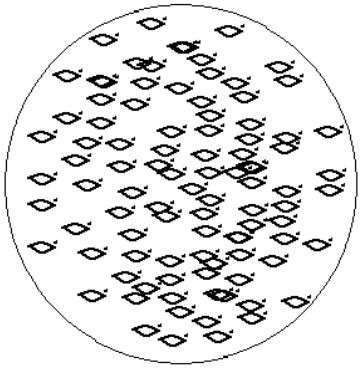
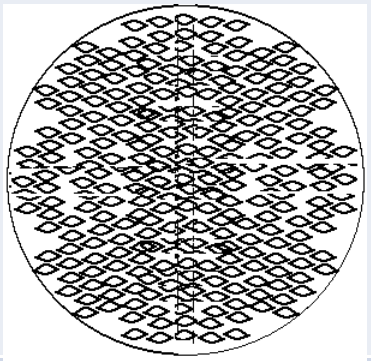
Objective

- To assess the attachment of Human Dermal Fibroblasts (HDF) cells to varying conditions
- To measure cell viability using different methods in different test conditions

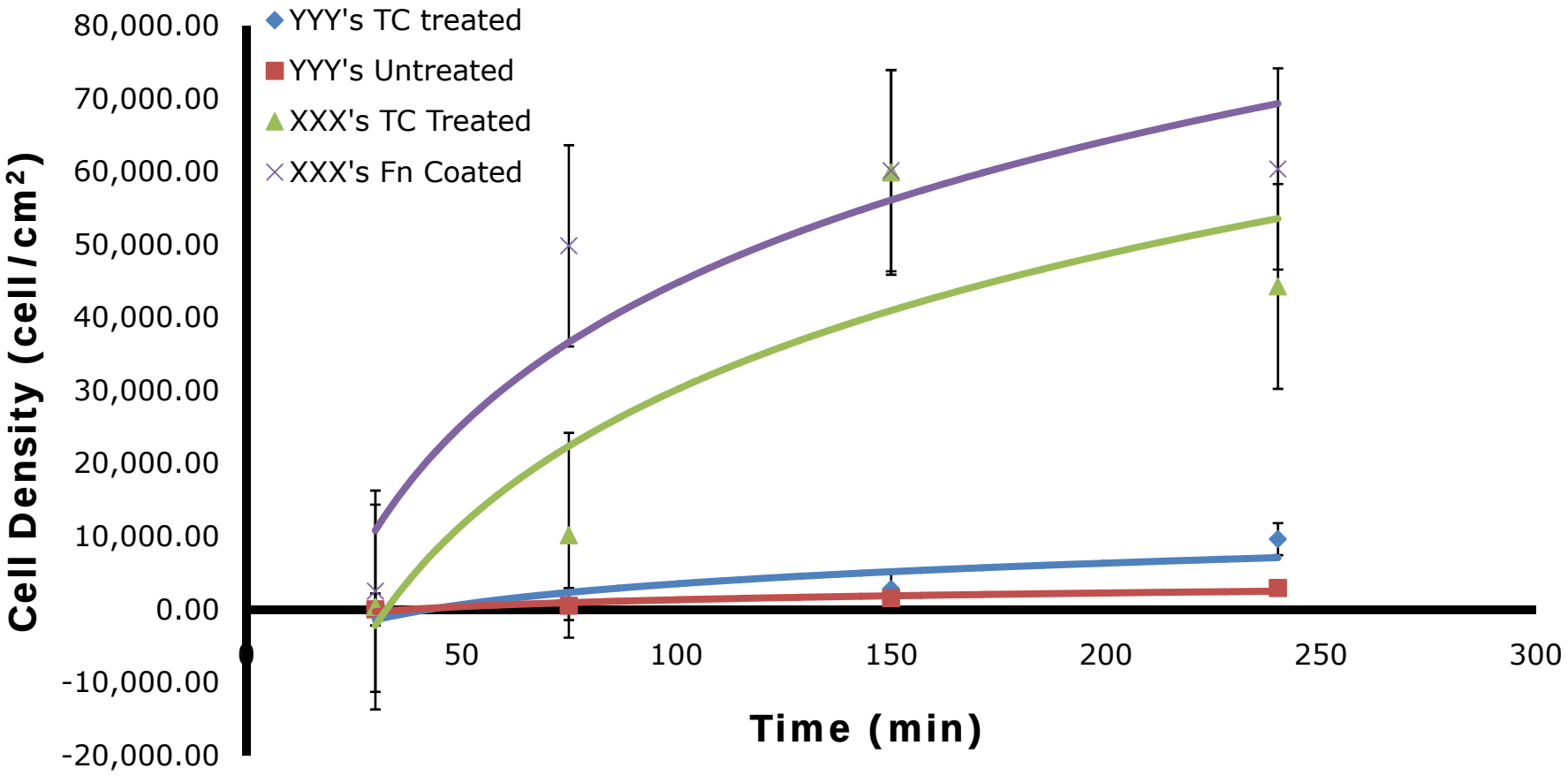
Assessing Attachment

Fibronectin (Fn) Attachment Assay	Quantitative Cell Attachment Assay
One 24-well plate with 4 different conditions:	Two 24-well plates with different conditions (each partner does 2 conditions):
• Control (no Fn coat) [A] • Half Fn / Half Control [B] • Fn X Design [C] • Full Fn-coat [D]	• Tissue Culture (TC)-treated • Untreated • Fn-coated
HDF cells seated in wells and incubated for 2 hours.	HDF cells seated in wells and incubated for varying times.
Cells examined under a microscope after:	Cells examined under a microscope after:
• 2 hour incubation • 2 hour incubation and washed with PBS	• 30 minutes • 1 hour 15 minutes • 2 hours 30 minutes • 4 hours

Fn Attachment Assay Results

Condition	A	B	C	D
After 2 hour incubation				
After 2 hour incubation + PBS wash				
Brief Summary	All the HDF cells were washed away from the PBS wash because none were attached.	The side that was Fn-coated had cells attach. The rounded, free-floating cells washed off.	The original X pattern brushed on was not seen. There were some attached cells left over.	The entire well was coated with Fn and thus many elongated and stretched cells attached.

Quantitatve Cell Attachment Assay Results



Brief Summary
Cell attachment increases over time. The rate of cell attachment is fastest with Fn-coated wells and the slowest with untreated wells.

Attachment Result Summary

Fibronectin (Fn) Attachment Assay	Quantitative Cell Attachment Assay
After a 2 hour incubation, cells attached specifically to wells that were coated with Fn.	Cell concentration increased with time as cells attached to the wells.
The density of cells that attached to the wells were proportional to the amount of Fn coated.	Wells coated with Fn had the highest rate of cell attachment, while untreated wells had the slowest rate.
	Discrepancies between partners in cell concentration of similar conditions is due to cell clumping in one partner's wells.

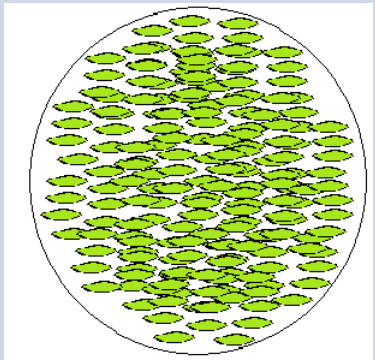
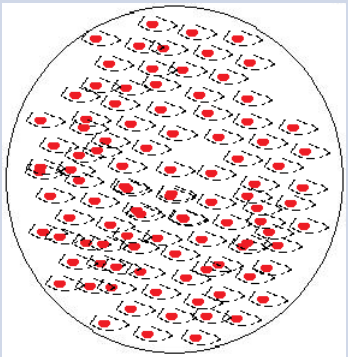
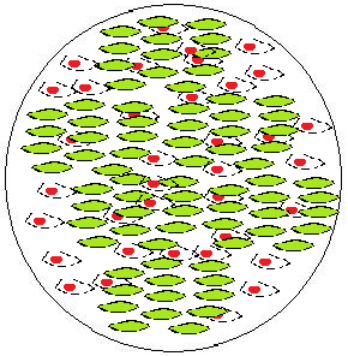
Summary and Comparison

Surfaces coated with fibronectin causes HDF cells to readily attach. Wells untreated or TC-treated do cause HDF cells to attach, but cells attach unspecifically and at a much slower rate.

Measuring Viability

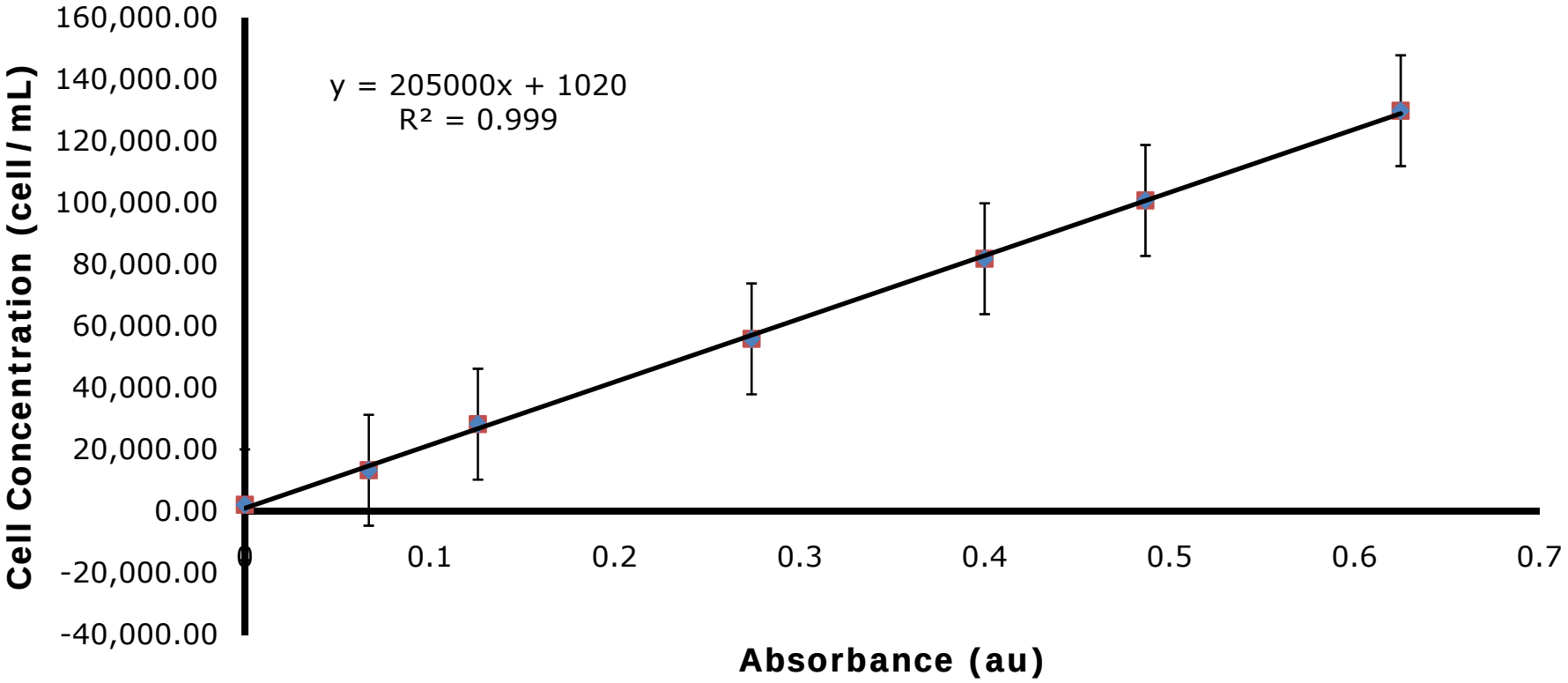
Live / Dead Assay	MTT Viability Test
One 24-well plate seated with:	Two 24-well plate each seated with:
3 different test conditions stained with dye:	7 different test concentrations in wells number 1 to 7 respectively:
• 250 µL of PBS [A] • 250 µL of ethanol [B] • 250 µL of PBS + 2 drops of ethanol [C]	• 50,000 cells/mL • 33,500 cells/mL • 25,000 cells/mL • 16,700 cells/mL • 8,330 cells/mL • 4,170 cells/mL • 0 cells/mL
Covered and incubated for 30 minutes	Incubated for 2 days
Observe cells under:	Counted cells with:
• light microscope • fluorescence microscope	• Coulter Counter • spectrophotometer

Live/Dead Assay Results

Conditions	A	B	C
Cells Seen Under a Fluorescence Microscope			
Brief Summary	All cells are stretched and elongated. All cells are alive and viable.	No spots of green can be seen; only small red dots are seen. All cells dead and unviable.	Some cells are green and alive while many seem to show up as red dots or dead.

Summary
Cell viability in this experiment is proportional to the amount of ethanol added to the well. Using a light microscope, one would be unable to differentiate between viable cells and unviable cells. By using the fluorescence microscope, one can tell between the unviable cells (red dots) compared to the viable cells (green elongations).

MTT Assay Results



Summary

HDF cellular concentration is proportional to absorbance taken by a spectrophotometer. A Coulter Counter does not give the amount of viable cells. Staining cells with a fluorescent dye and performing an MTT assay and comparing it with the above equation will give you cellular concentration.

HDF Attachment and Viability

- Fibronectin coated surfaces are ideal for allowing HDF cells to attach specifically.
  - Tissue Culture treated and untreated surfaces also allow for HDF cell attachment over longer period of time.

- Fluorescence microscopes and spectrophotometers are ideal tools to quantitatively and qualitatively assess cell viability.

- Methods are based from the BIOE 342 Lab Protocol book copyright Dr. Ann Saterbak, 2001-2009.
- Data taken from lab partner comes from XXX.