EFFECTS OF CULTURE CONDITONS ON HDF CELL BEHAVIOUR

YYY

BIOE 342:TISSUE CULTURE

OBJECTIVES

- Qualitative and quantitative assessment of HDF (human dermal fibroblast) cell attachment to different surfaces
- Comparison of different HDF cell viability assessment methods
- Effects of different media conditions of HDF cell proliferation

METHODS: Cell Attachment Assays

- Effects of fibronectin (fn) on HDF attachment to untreated polystyrene plates
  - Control: PBS with 10mg/mL BSA (bovine serum albumin)
  - Partial fn- coated and part control + fn-design wells
  - Fully fn-coated wells
  - 30min plate incubation and 2hr cell incubation
  - Cells rinsed then observed under light microscope

- Test HDF attachment to different polystyrene plate surfaces over 4hrs
  - Untreated
  - TC-treated
  - Fn-treated
  - Cell densities determined using light microscope
  - Time points at .5, 1.25, 2.5 and 4hrs

METHODS: Cell Viability Assessment

- MTT Assay
  - Culture several known cell concentrations on TC-treated plates over 2 days
  - 2hr incubation with MTT dye and 45min with stop solution
  - Obtain absorbance values using spectrophotometer

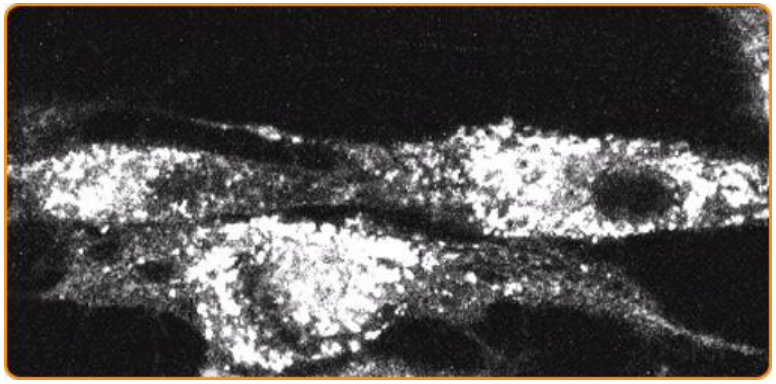
- Live/Dead Fluorescence Assay
  - Culture same cell concentrations over 2days
  - Test 3 culture conditions
    - PBS
    - Ethanol
    - PBS and 2 drops ethanol

- Cell counts and concentrations determined using Coulter Counter

METHODS: Cell Proliferation

- Culture same cell concentrations on TC-treated plates over 6 days
- Culture in different DMEM serum concentrations
  - 1, 5 and 10% FBS (serum) concentrations
  - 6 wells with 1% FBS for Day 0
  - 3 wells each for the 3 serum concentrations for days 1, 3 and 6
- Cell counts and concentrations determined using Colture Counter

RESULTS: Fibronectin Attachment Assay



[1]

- ❑Morphology of attached cells: grouped and elongated with pseudopodia
  - ❑White/bright: cytoplasm
  - ❑Dark/hollow: nuclei
  - ❑Gray: pseudopodia

- No cells on wells not fn-coated
- Cells attached only on half of well coated with fn
- Cells attached in shape of fn-coated design (x-shape fn-coat)
- Cells attached all over surface coated with fn

RESULTS: Quantitative Cell Attachment Assay Variances

- Two way ANOVA (Analysis of Variance) used to test variances in cell densities based on incubation time and cell plate condition

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Time Points	4.48E+08	3	1.49E+08	17.1	0.00242	4.76
Plate Conditions	4.36E+07	2	2.18E+07	2.49	0.163	5.14
Error	5.24E+07	6	8.74E+06			
Total	5.44E+08	11				

- α = .05

[3]

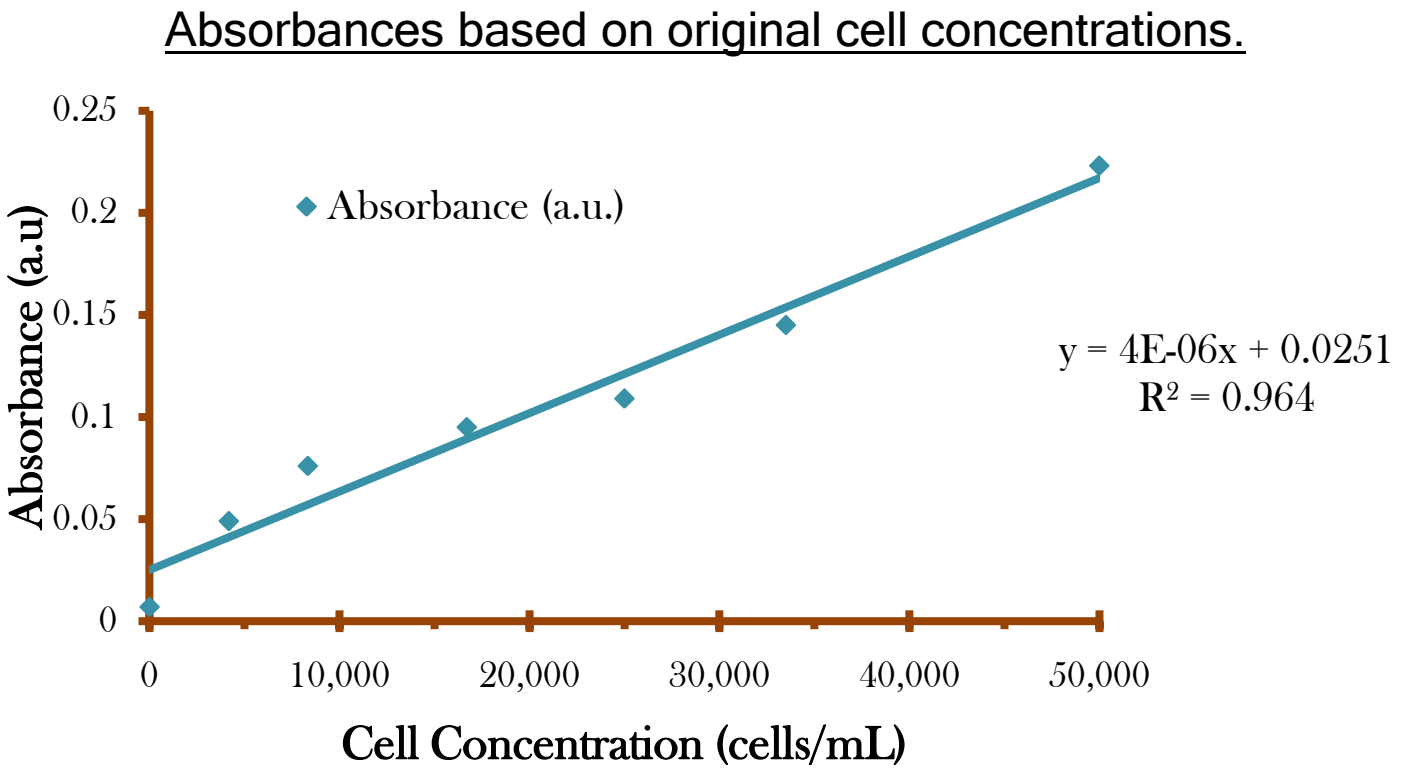
- p-value for time points = .00242 < .05
  - Differences in incubation time don't significantly affect changes in cell densities
- p-value for plate conditions = .163 > .05
  - Differences in plate conditions (i.e. treated and fn-coated) significantly affect changes in cell densities among wells

RESULTS: Cell Attachment Assays

- Fibronectin attachment
  - Fn enhances ability of cells to adhere and attach to plate surfaces
  - Cells adhere over time, even to non-treated surfaces
  - Qualitative assessment using morphology and confluence observations
- Quantitative attachment
  - Fn-coat more effective than TC-treatment for cell adhesion to plates
  - Cell adhesion dependent on surface treatment
  - Determination of cell densities allows for mathematical analysis

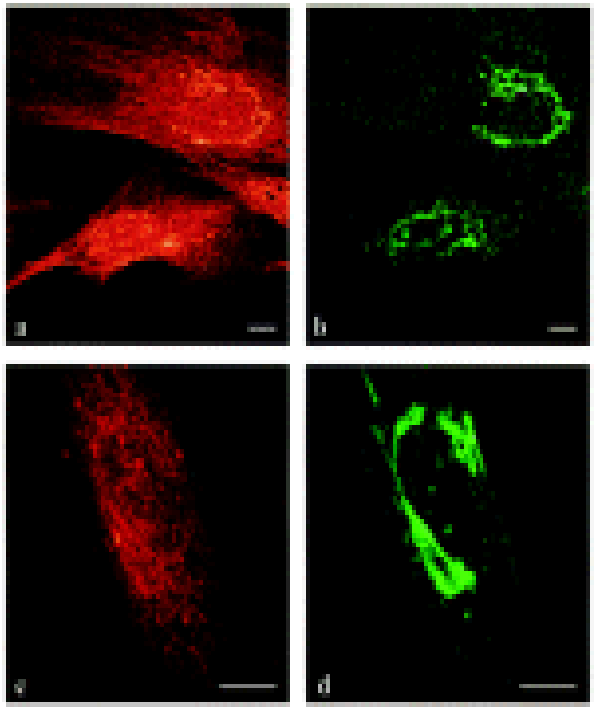
RESULTS: MTT Viability Assays

- Linear relationship observed between absorbance values and original cell concentrations



RESULTS: Live/Dead Assay

- Ethanol kills cells
  - .25mL vs. 2 drops
- Live cells stained green
  - 100% in well with PBS
  - ~70% in well with 2 drops of ethanol
- Dead cells stained red
  - 100% in well with .25mL ethanol
  - ~30% in well with 2 drops of ethanol

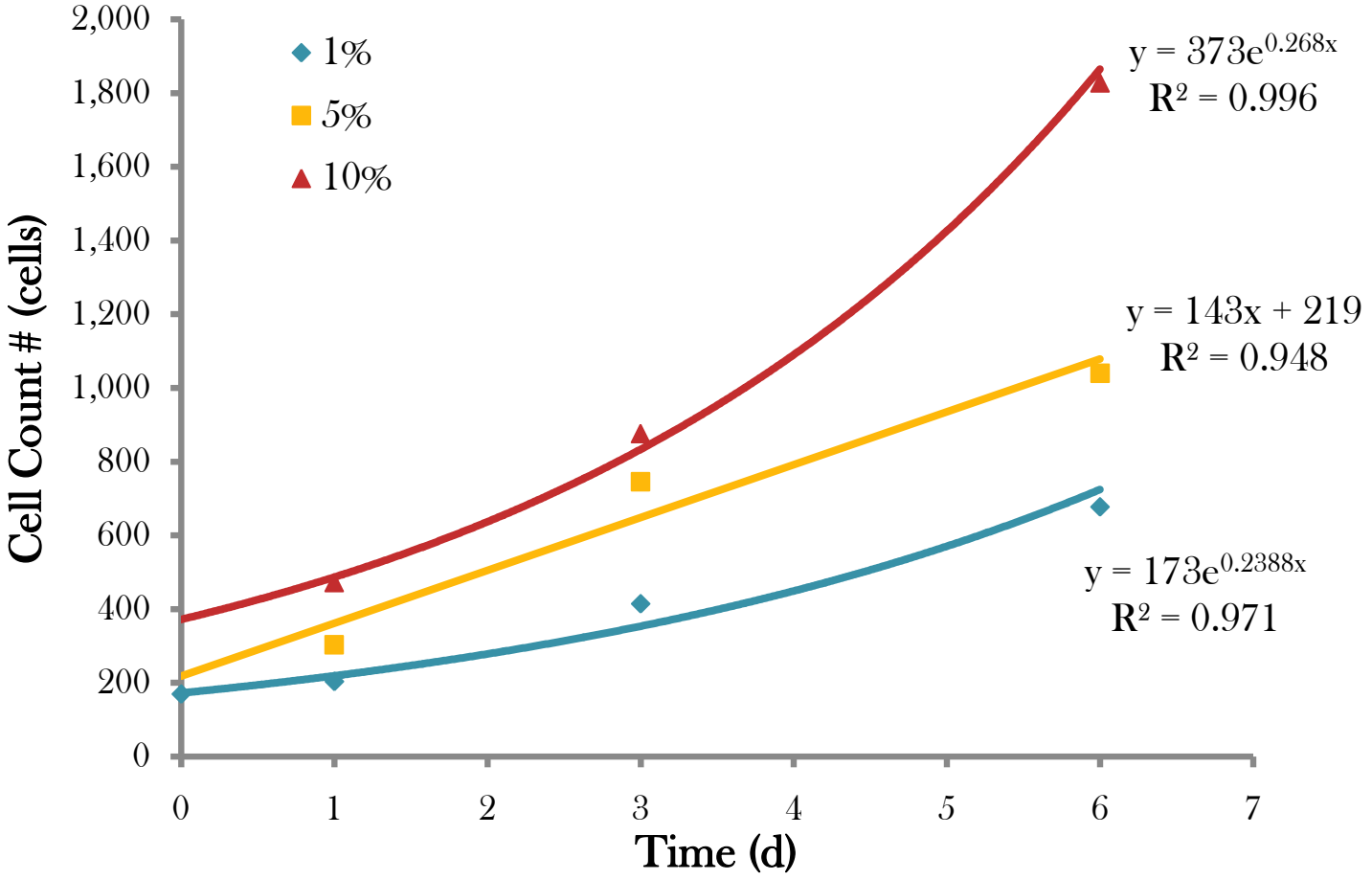


[2]

RESULTS: Comparison of the two Assays

ASSAY	METHOD	ADVANTAGES	DISADVANTAGES
MTT	Measures mitochondrial activity [4]	•Live/dead cell differentiation •Development of mathematical (linear) relationship	•Unable to reuse cell samples
Live/Dead	Measures via nuclear envelope integrity [4]	•Live/dead cell differentiation •Visual representation of data •Test effects of toxic substances	•Unable to differentiate under light microscope

RESULTS: Effects of Serum Concentrations on HDF Cell Proliferation



Cell proliferation is enhanced with the 5% and 10% serum concentrations when compared to the 1%

SUMMARY

- Different surfaces affect HDF cell attachment
  - TC-treated and fn-coated plates result in more cells attached
  - Fibronectin enhances HDF cell ability to attach to a surface
- Cell viability assessment methods
  - MTT: Mathematical relationship through spectroscopy
  - Live/Dead: Visual assessment through fluorescence
- Different DMEM serum concentrations affect HDF cell proliferation
  - Of the 3 tested, 10% allowed for most growth
  - Exponential relationships can be used to characterize cell growth

REFERENCES

- All methods taken from:
  - Dr. Saterbak and Dr. McHale. Tissue Culture Lab Module: Lab Protocols. Department of Bioengineering, Rice University. 2001-2009.

1. http://www.celluminate.com/assets/images/sct2.jpg
2. http://www.nature.com/jid/journal/v117/n2/thumbs/5601147f3th.gif
3. Values for untreated obtained from classmate XXX.
4. http://www.protocol-online.org/biologybjh