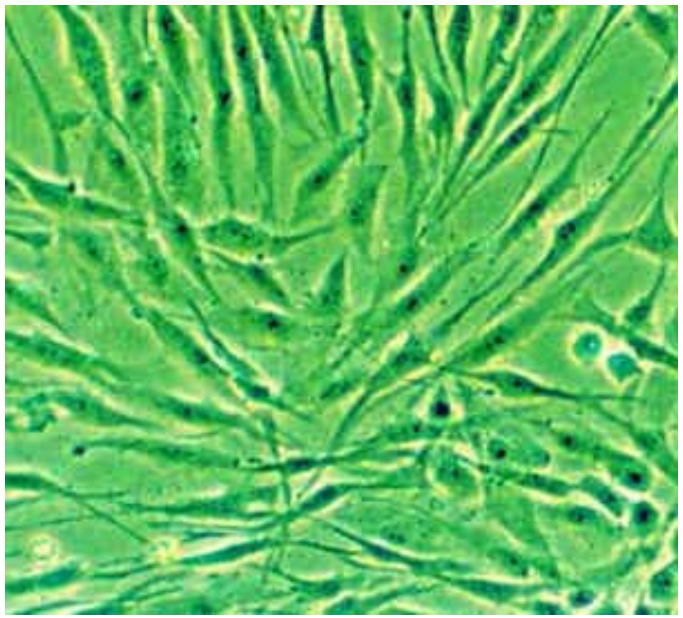
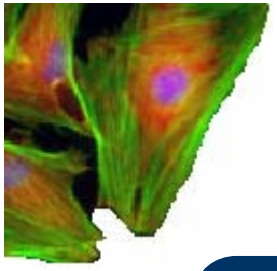
Human Dermal Fibroblast (HDF) Viability and Proliferation in Culture

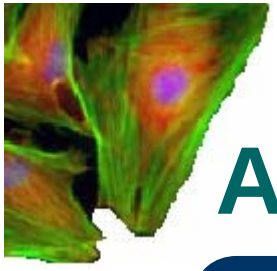


April 8, 2008



Objective

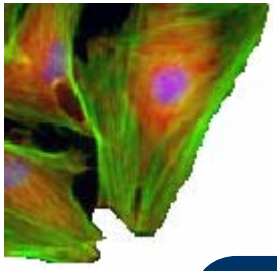
- To determine viability of HDF
  - qualitatively by fluorescent microscopy when subjected to various treatments
  - indirectly through metabolic assay
- To measure cell proliferation in media with differing concentrations of fetal bovine serum (FBS)



Assessing Viability Fluorescently

Live/Dead Fluorescence Assay

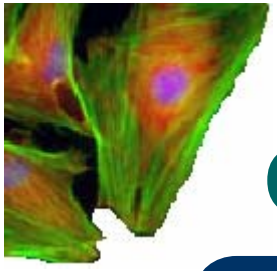
- Cells were seeded and incubated 2 days.
- Treatment (PBS, ethanol, PBS+2 drops ethanol) and dye were added (n=2).
- After 30 min, cells were visualized by fluorescent microscopy.
  - EthD-1 stained dead cell nuclei red.
  - Calcein AM stained live cell cytoplasm green.



Measuring Viability via Metabolic Assay

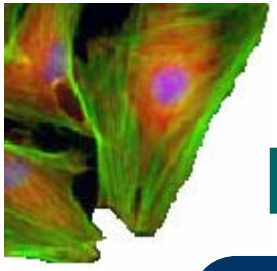
MTT Viability Assay

- Cells were seeded at 6 concentrations in duplicate and incubated 2 days.
- One set trypsinized, counted by coulter counter.
- Yellow dye solution added and incubated 2 hours; metabolites turned dye purple
- Solubilization/Stop solution added, pause 45 min
- Absorbances read with spectrophotometer at 570 nm and plotted against cell concentration.

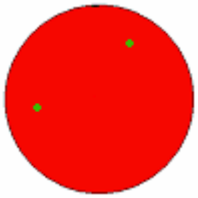
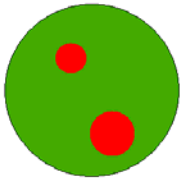


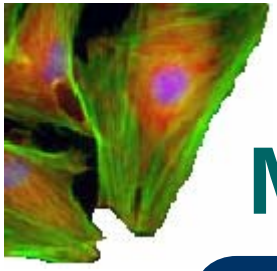
Quantifying Cell Proliferation

- 7000 cells/well were seeded in 1% FBS and incubated 4 hours.
- Cells trypsinized and counted by coulter counter for day 0 control (n=6).
- Media with 1% ,5% and 10% FBS were added (n=3) for days 2, 5 and 7.
- Each day's cells were trypsinized and counted by coulter counter; media was changed.



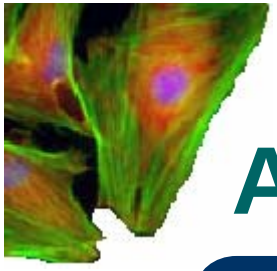
HDF Viable in PBS not Ethanol

Treatment	Result	Conclusion
250 µl PBS	 >99% of cells stained green	PBS allows for viability of HDF.
250 µl ethanol	 >99% of cell nuclei stained red	Ethanol kills HDF.
250 µl PBS + 2 drops ethanol	 ~75% of cells stained green; drops obvious	High concentrations of ethanol kill HDF upon contact.

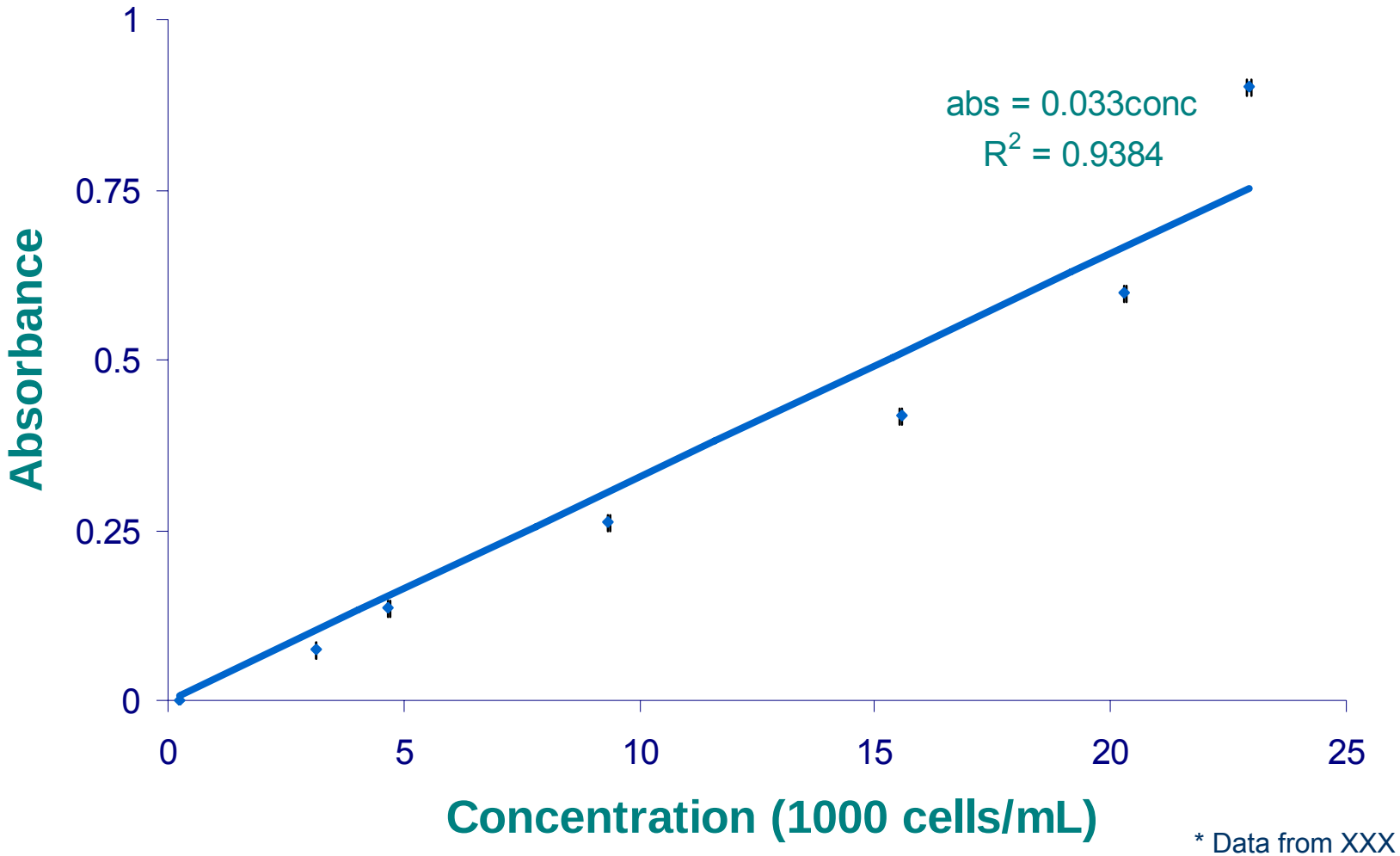


Metabolites suggest active HDF

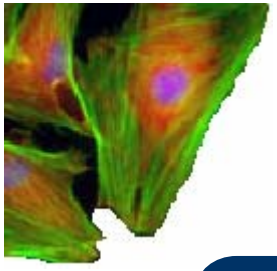
- Increased concentration of cells turned the dye more purple with their metabolites, increasing absorbance.
- Higher absorbance corresponded to increased metabolic activity of the cells.
- Absorbance and concentration relationship established standard metabolic activity levels for comparisons of unknowns.



Abs. and Conc. Linearly Related

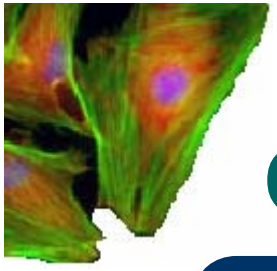


* Data from XXX

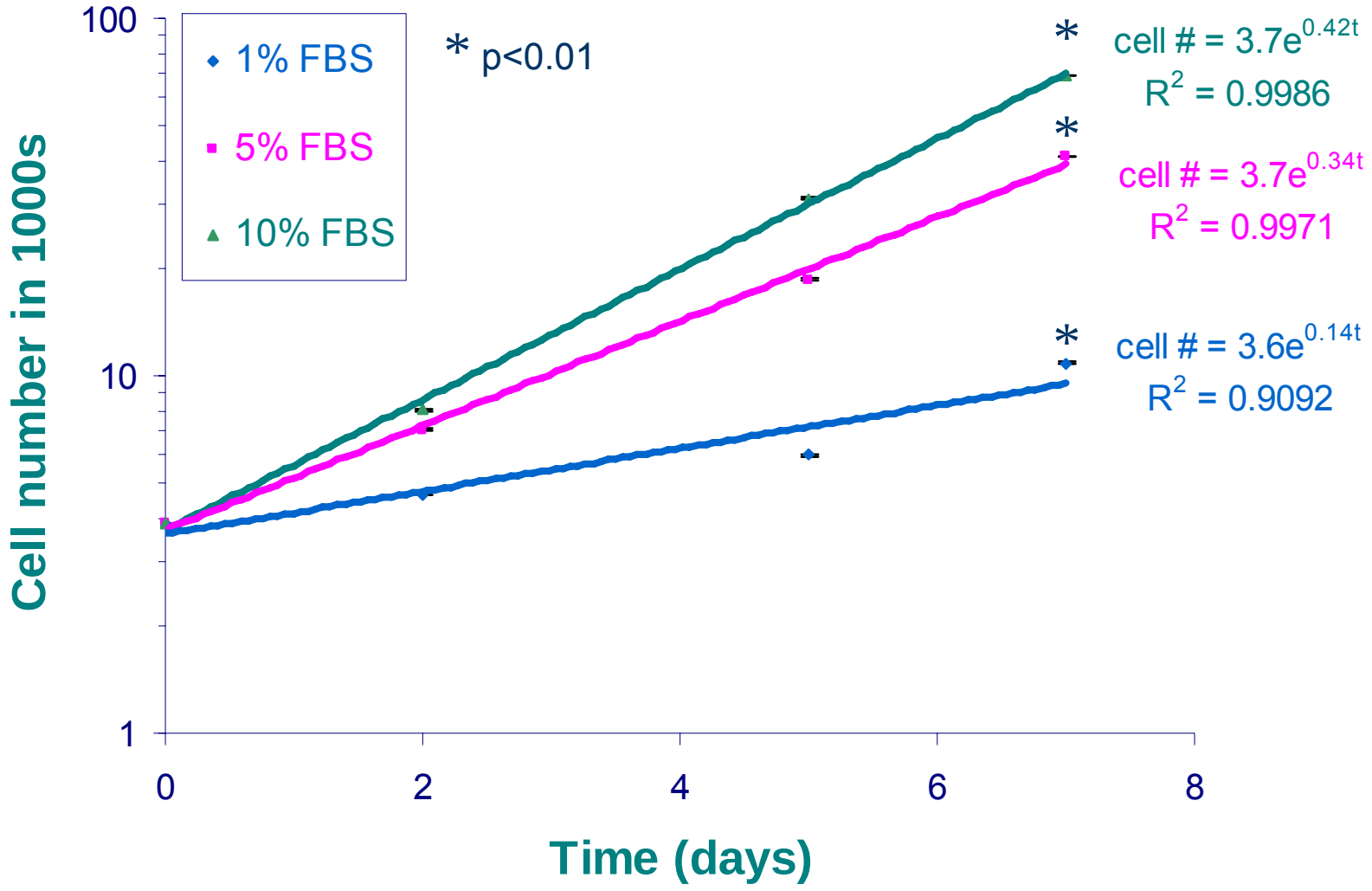


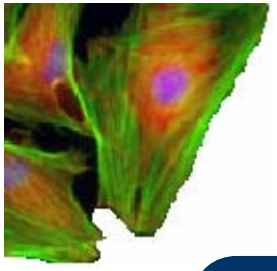
Live/Dead and MTT Complement Each Other

- Live/Dead: visual inspection method
  - Directly evaluates viability
  - Qualitative and subject to bias
- MTT: colorimetric metabolic assay
  - Measures amount of metabolite, not viable cells
  - Establishes level of activity for healthy HDF
- Combine techniques to:
  - Relate absorbance to living cell concentration
  - Assess metabolic activity of living cells



Cell Proliferation is Exponential





FBS Encourages Proliferation

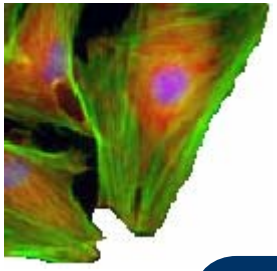
Increased FBS concentration resulted in:

→ Greater number of cells

Day 7: number of cells 10% > 5% > 1%
(ANOVA, Tukey $p<0.01$)

→ Faster doubling time

Percent FBS	Doubling Time
1%	5.0 days
5%	2.1 days
10%	1.6 days



Summary of Results

- Ethanol in high concentrations kills HDF on contact, while PBS sustains HDF for at least 30 minutes.
- More concentrated cells produce more metabolites: relationship between absorbance and concentration set standard activity.
- Greater percentages of FBS encourages faster and greater exponential proliferation of HDF.