TAML Degraded Estrogen Effects on Cell Behavior

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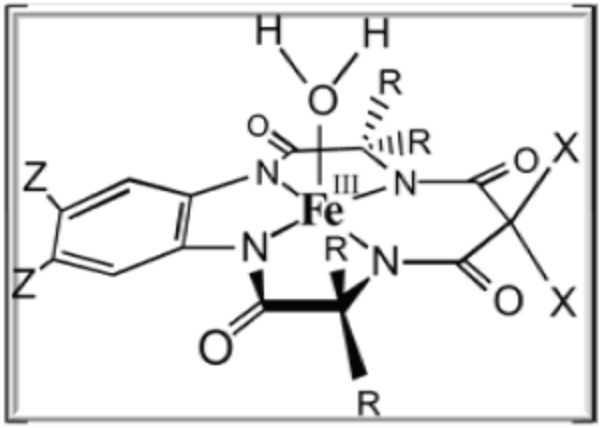
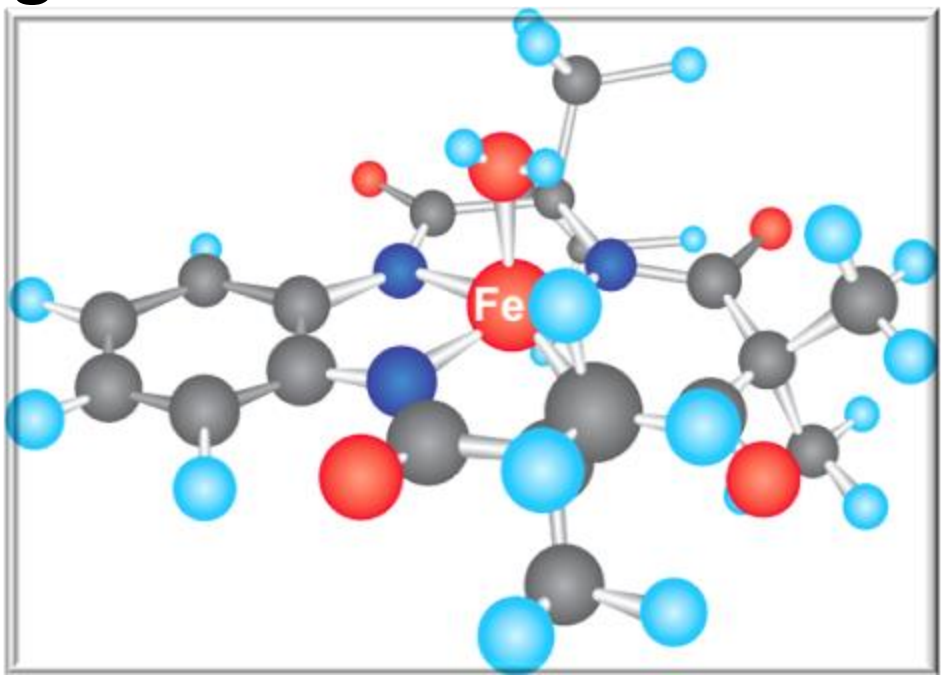
TAML

- Tetra-Amido Macrocyclic Ligands
- Catalyst engineered by Dr. Terry Collins of Carnegie Mellon University
- Accelerates the H2O2 oxidation reaction
- Made of common elements (C, H, O, N, Fe)



Structure of TAMLs

- Central iron atom contains specific location of reactivity. H2O2 bonds to this site
- Iron atom attached to ringed carbon structures by 4 nitrogen atoms



Overview of Cancer Cells

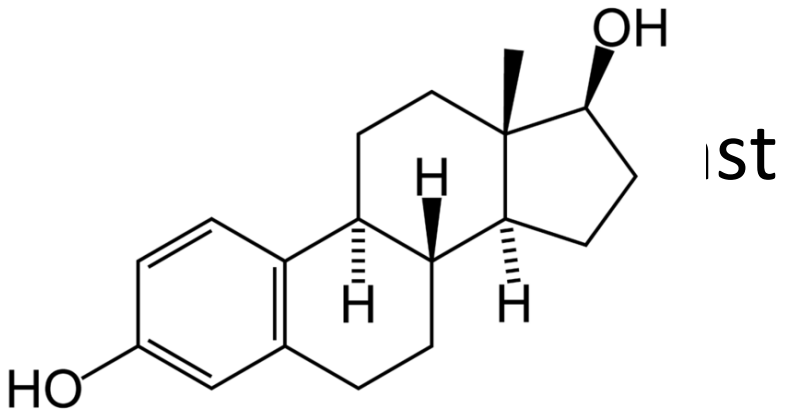
- Cancer cells are cells that grow and divide at an irregular, unregulated pace.
- Apoptosis does not occur in cancerous cells; their mutations are passed on to the second generation, eventually clustering and forming tumors.
- Tumors can be malignant (aggressive) or benign.

MG-63 Cell Line

- Human Cancer Cell Line
- Osteosarcoma cancer cells
- Useful model to test the effects of variables on cancer cell proliferation

Estradiol

- Major sex hormone in females, and present in low amounts in males
- Accounts for female secondary sex characteristics
- Steroid Hormone
- Associated with activat
  oncogenes, often prom
  cancer



Purpose

- The purpose is to observe the effect of TAML degraded estradiol on the cell behavior of MG-63 cancer cells.

Hypothesis

- Null Hypothesis: TAML WILL NOT significantly degrade estradiol's effects on MG-63 cancer cells
- Alternate Hypotheses:
  - TAML WILL significantly degrade the effects of estradiol on MG-63 cancer cells.
  - The addition of the estradiol WILL significantly affect the proliferation of MG-63 cancer cells.

Materials

- Cryotank
- 3 75mm^2 tissue culture treated flasks
- 18 25 mm^2 tissue culture treated flasks
- Fetal bovine serum (FBS)
- MG-63 Osteosarcoma Cancer Cell Line
- Trypsin-EDTA
- Pen/strep
- Macropipette + sterile macropipette tips (1 mL, 5 mL, 10, mL, 20 mL)
- Micropipettes + sterile tips
- DMEM Serum - 1% and Complete Media (4 mM L-glutamine, 4500 mg/L glucose, 1 mM sodium pyruvate, and 1500 mg/L sodium bicarbonate + [10% fetal bovine serum for complete])
- Estradiol (powder)
- 75 mL culture flask
- Incubator
- Nikon Inverted Microscope
- Aspirating Vacuum Line
- Labeling Tape
- Hemocytometer
- Sterile PBS
- Ethanol (70% and 100%)
- Distilled water
- Fe TAML
- Hydrogen Peroxide
- Catalase
- Sodium Bicarbonate

Procedure

- A 1 mL aliquot of MG-63 cells from a Cryotank was used to inoculate 30 mL of 10% serum DMEM media in a 75mm^2 culture flask yielding a cell density of approximately 10^6 to 2x10^6 cells.
- The media was replaced with 15 mL of fresh media to remove cryo-freezing fluid and incubated (37° C, 5% CO2) for 2 days until a cell density of approximately 4x10^6 to 5x10^6 cells/mL was reached.
- The culture was passed into two sets of 3 flasks in preparation for experiment and incubated for 2 days at 37° C, 5% CO2.

Procedure: Addition of Variable- Day 0

- After trypsinization, cells from all of the flasks were pooled into 3 75mm^2 flasks (cell density of approximately 1 million cells/mL).
- 0.1 mL of the cell suspension was added to eighteen 25 mm^2 tissue culture treated flasks containing 5 mL of DMEM (com) media, creating a cell density of approximately 10^5 cells per flask.
- Two stock solutions of estradiol were created, 10^-4 and 10^-3
- The TAML oxidation reaction was performed on a stock of estradiol
- The following concentrations of variable (next page) were added to the flasks
- The cells were incubated at 37°C, 5% CO2 for the remainder of the study.

Concentrations of Variable

Final Concentration of Estradiol	Stock of Estradiol	Sterile Water	Total
0	0	5 ml	5 ml
10^-6	10 µl of 10^-3 stock	4.9 ml	5 ml
10^-8	10 µl of 10^-4 stock	4.9 ml	5 ml

Procedure- Day 1 and Day 3

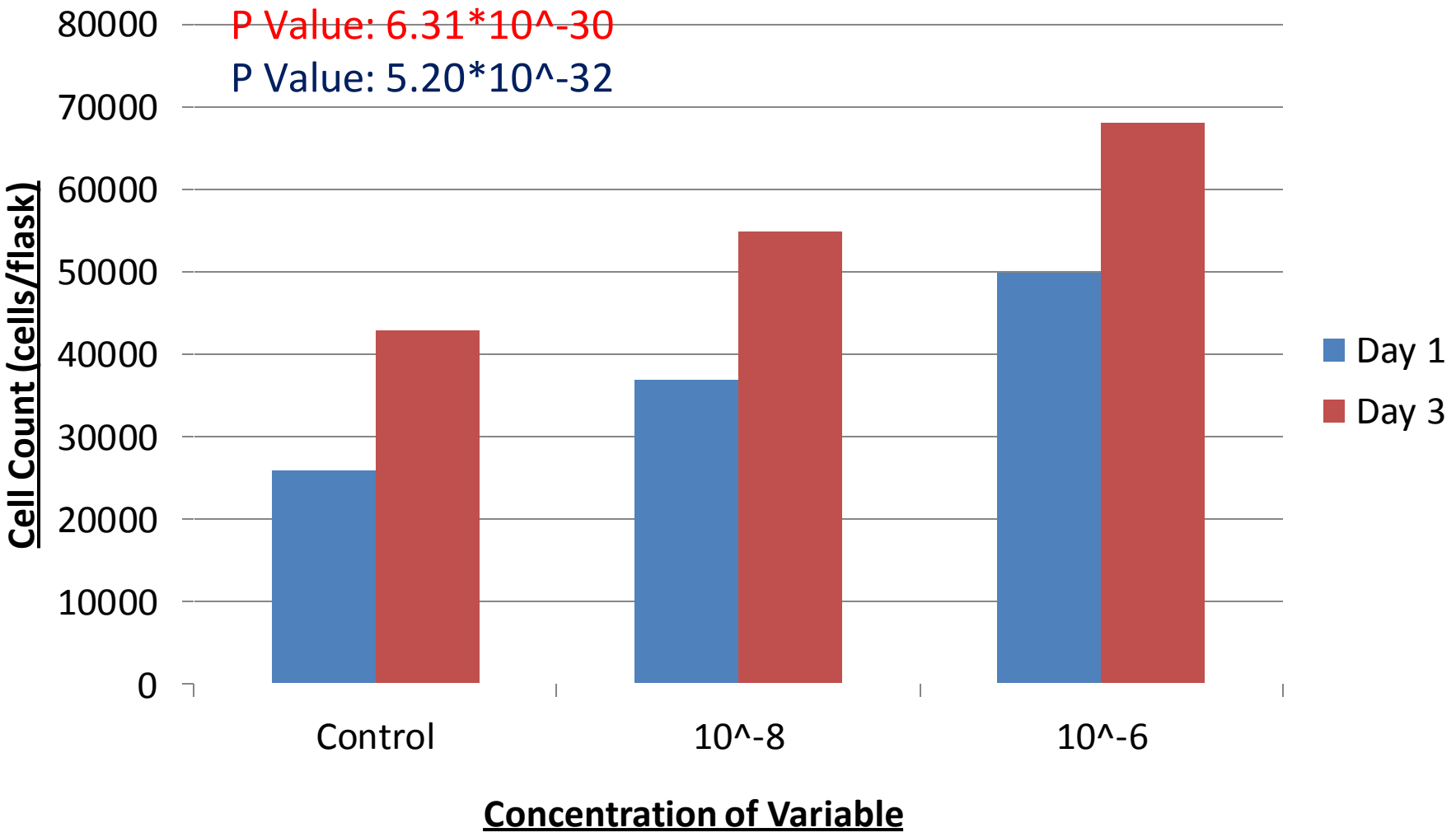
- Day 1 and Day 3
  – Cell densities were determined as follows:
- The cells were trypsinized and collected into cell suspension.
- 25 µl aliquots were transferred to a Hemacytometer for quantification (3 counts per flask).
- Day 1 and Day 3
  – Nikon Inverted Microscope was used to take images of representative areas of each flask.

Statistical Analyses

- ANOVA
  - Compares variation within groups to variation between groups.
  - Using the ANOVA, a *p*-value less than the alpha of .05 was gathered (significant variation).
  - Reject the null hypothesis.

- Dunnett's test
  - Compares each experimental group to control individually.
  - 0.01 alpha was used, and the t-value compared to the t-critical value of 2.567

Proliferation Results of Un-Degraded Estradiol

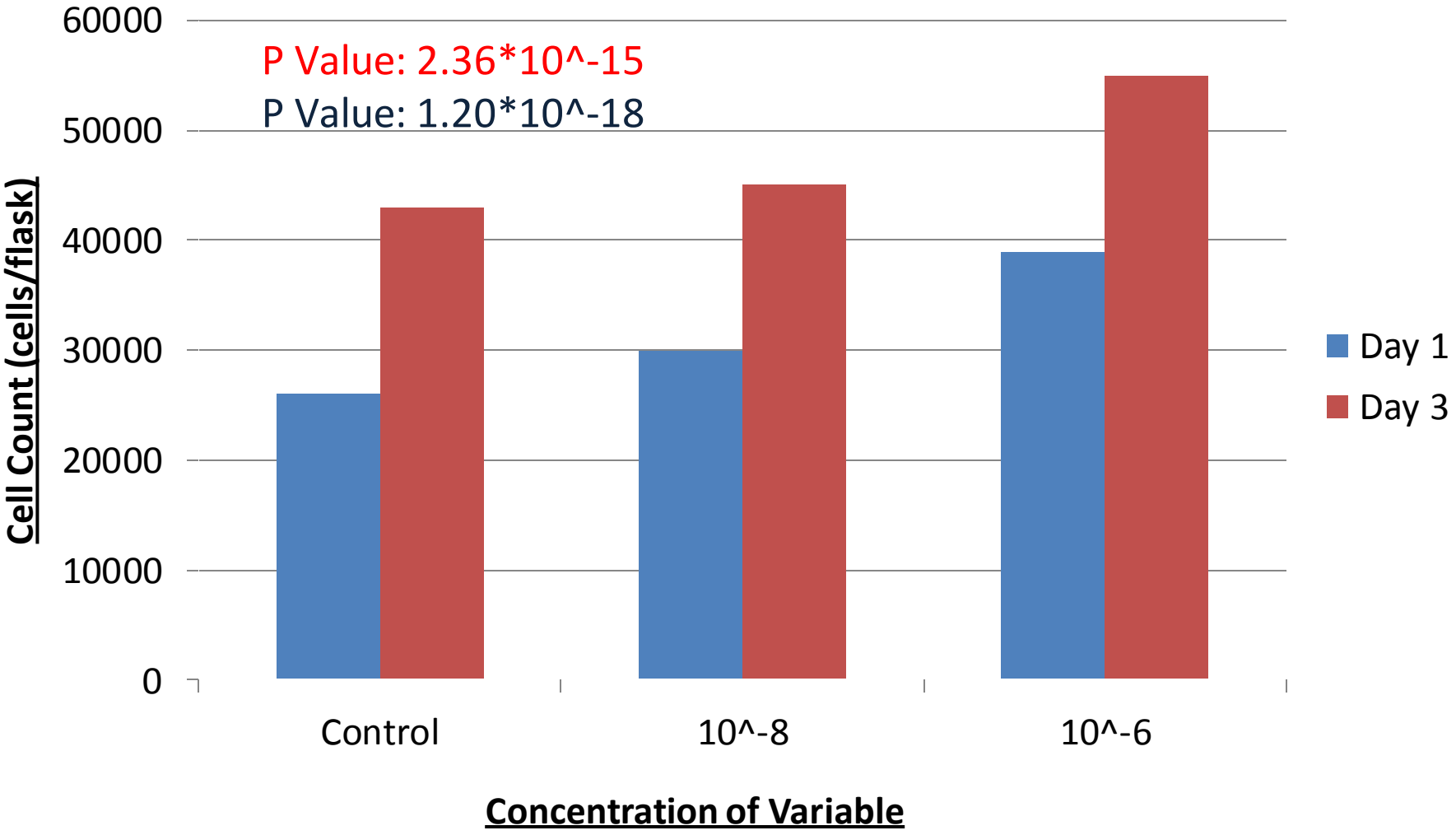


Dunnett's Test Undegraded

$$t_d = \frac{M_i - M_c}{\sqrt{\frac{2MSE}{n_h}}}$$

Concentration	t-value	t-critical (.05)	t-critical (.01)	Variation
10^{-6} Day 1	25.645	2.110	2.567	Significant
10^{-8} Day 1	11.189	2.110	2.567	Significant
10^{-6} Day 3	14.251	2.110	2.567	Significant
10^{-8} Day 3	28.439	2.110	2.567	Significant

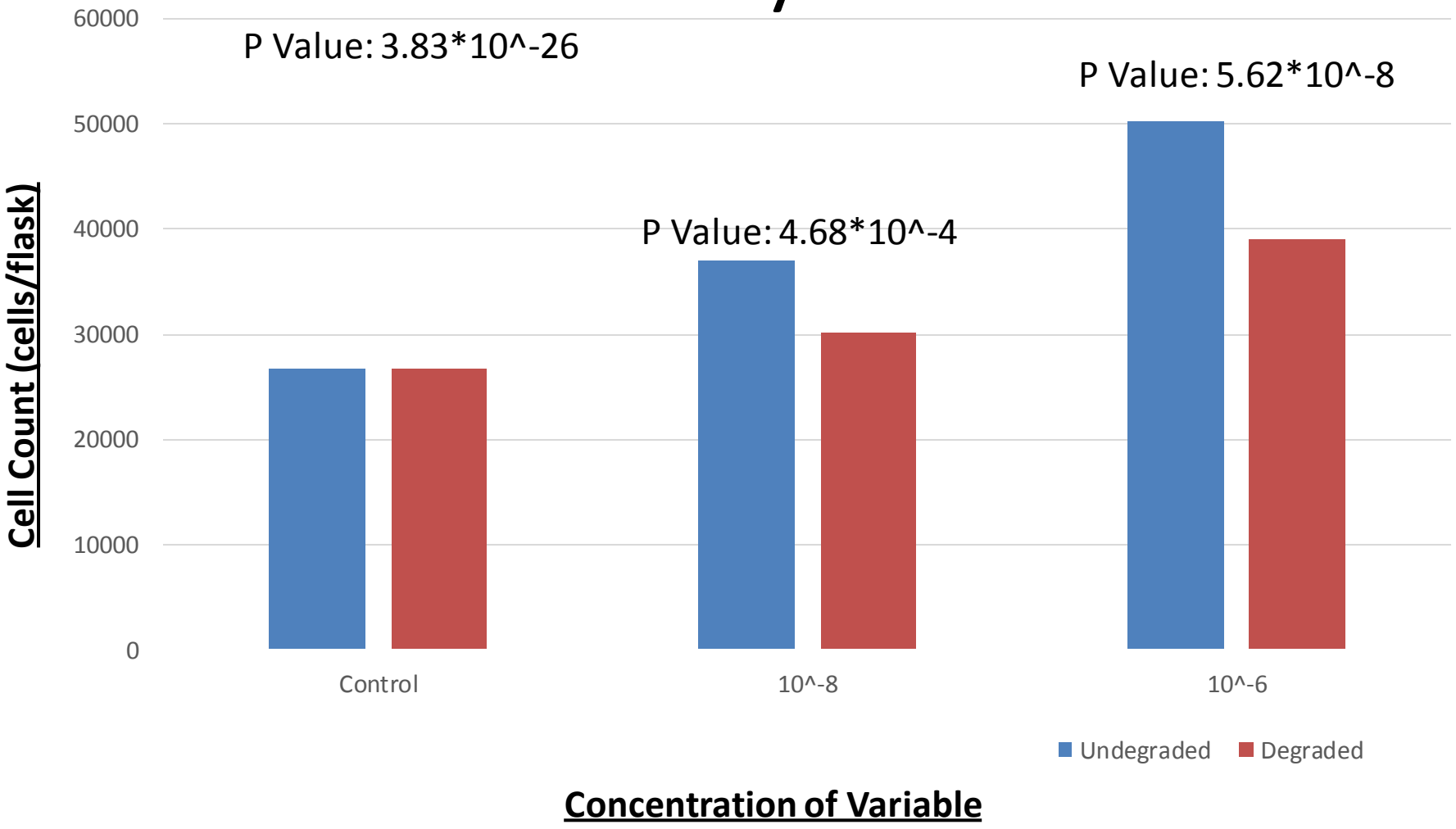
Proliferation Results of Degraded Estradiol



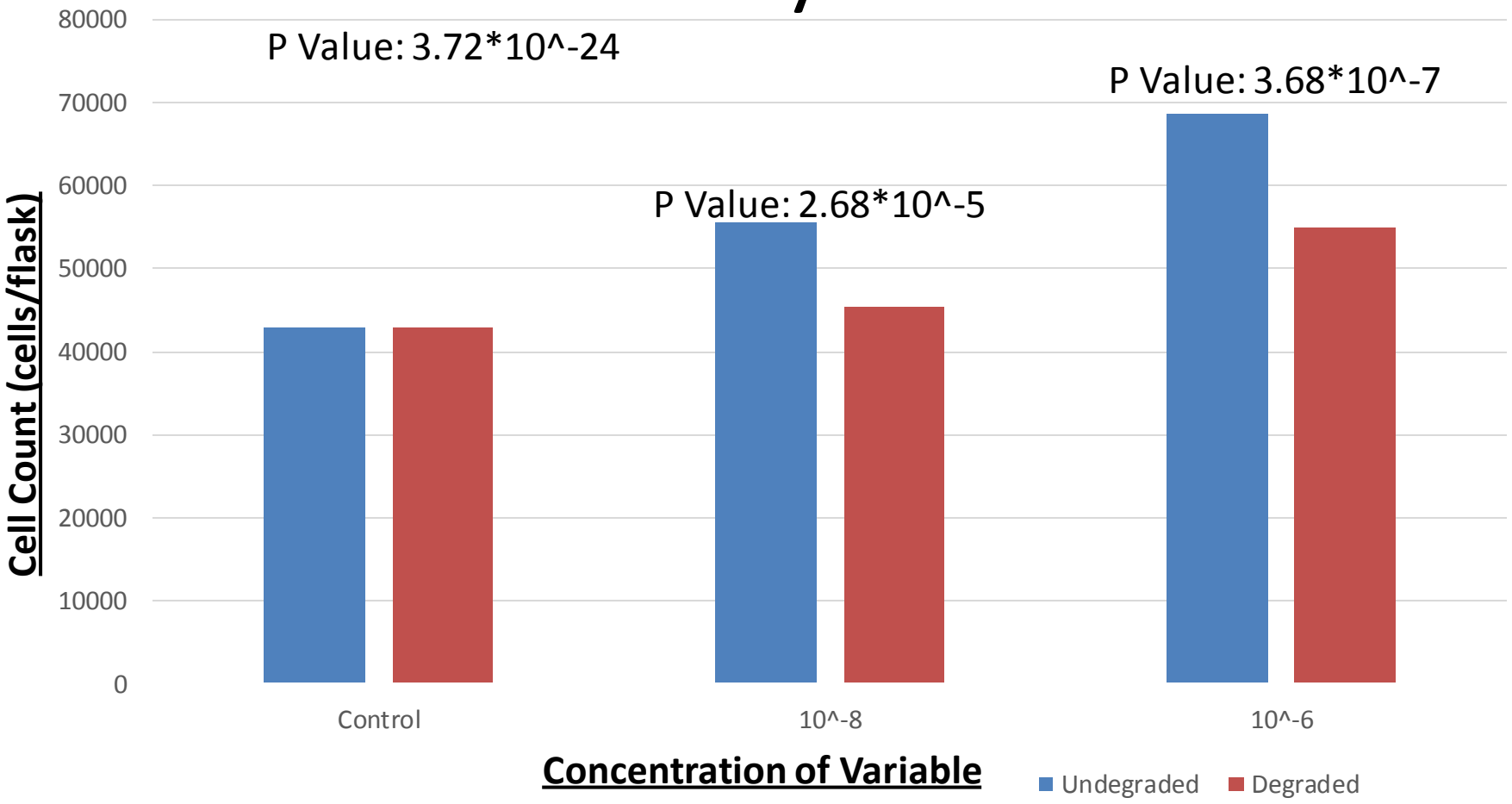
Dunnett's Test Degraded

Concentration	t-value	t-critical (.05)	t-critical (.01)	Variation
10^{-6} Day 1	11.549	2.110	2.567	Significant
10^{-8} Day 1	3.716	2.110	2.567	Significant
10^{-6} Day 3	13.503	2.110	2.567	Significant
10^{-8} Day 3	2.574	2.110	2.567	Significant

Comparison of Degraded and Undegraded Day 1



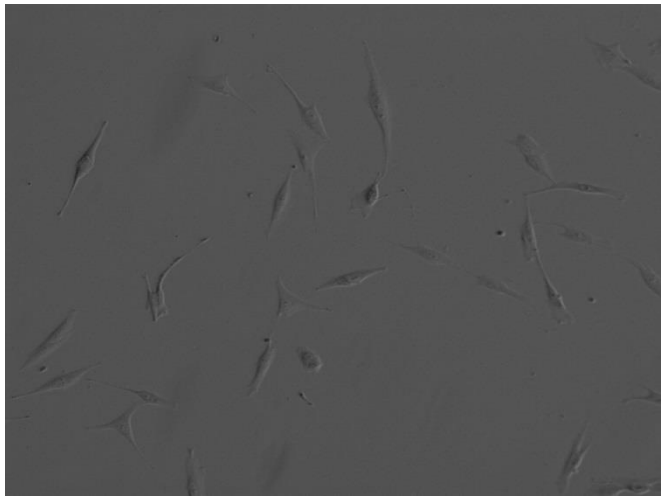
Comparison of Degraded and Un-Degraded Day 3



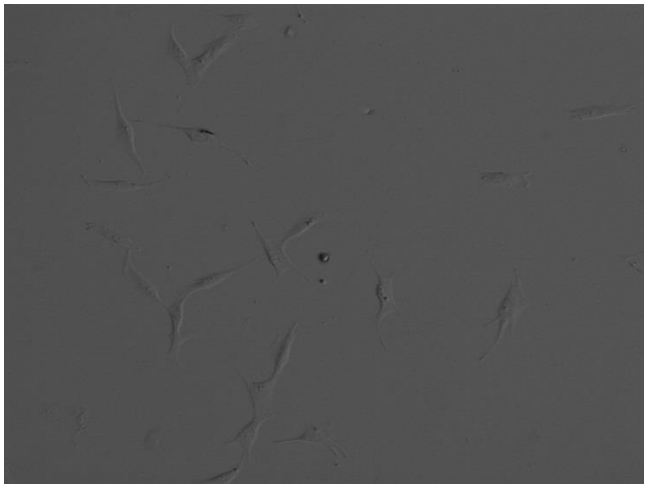
Dunnett's Test Degraded vs Undegraded

Concentration	t-value	t-critical (.05)	t-critical (.01)	Variation
10^{-6} Day 1	9.697	2.110	2.567	Significant
10^{-8} Day 1	7.416	2.110	2.567	Significant
10^{-6} Day 3	14.108	2.110	2.567	Significant
10^{-8} Day 3	11.62	2.110	2.567	Significant

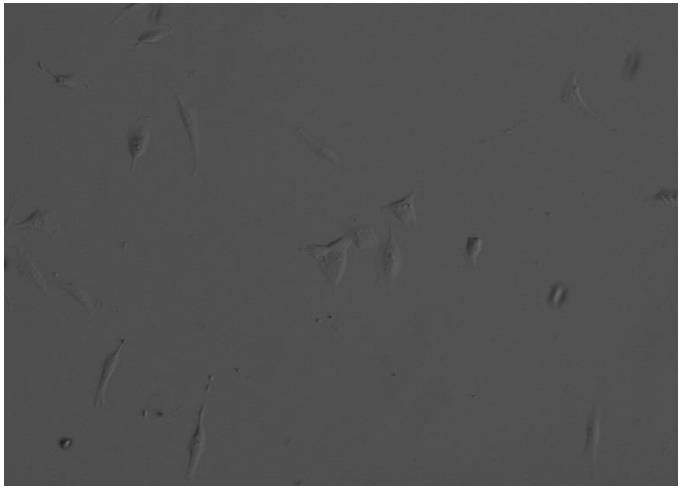
Proliferation Results Day 1



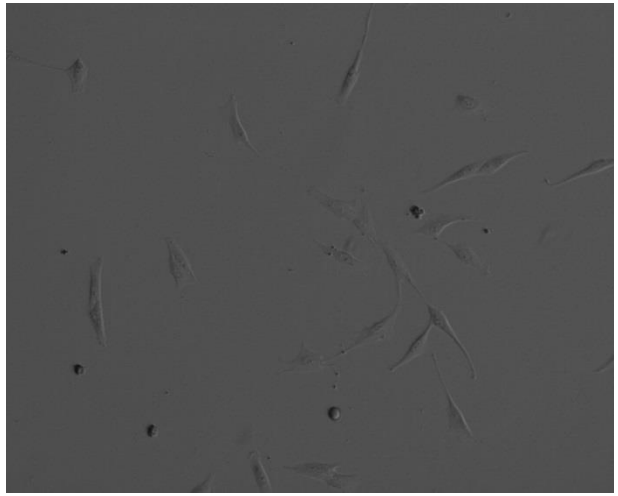
10^-6



10^-6Degraded

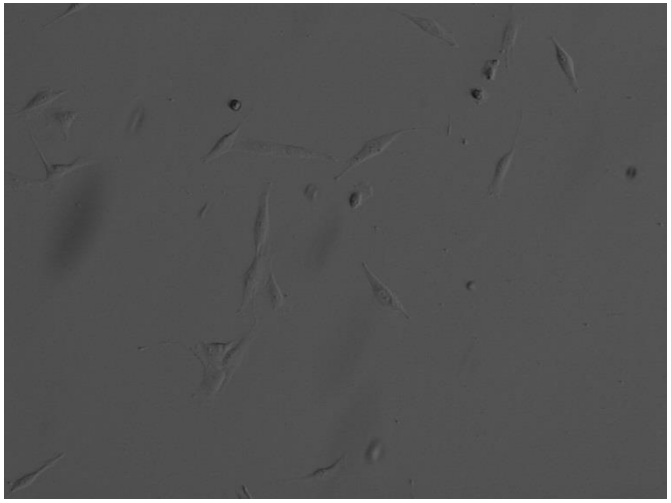


10^-8

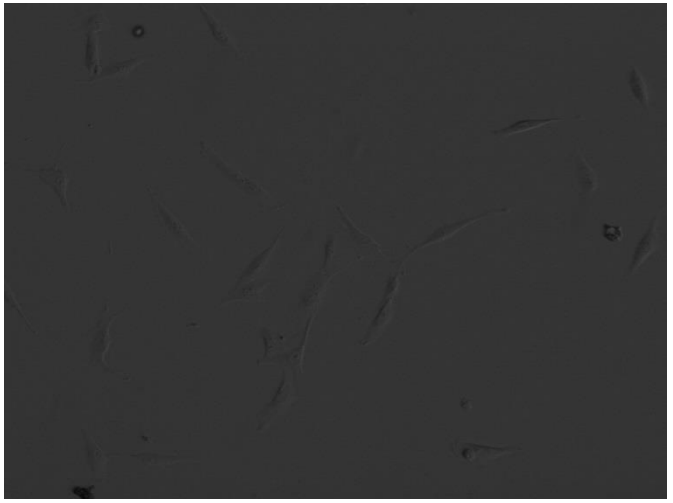


10^-8 Degraded

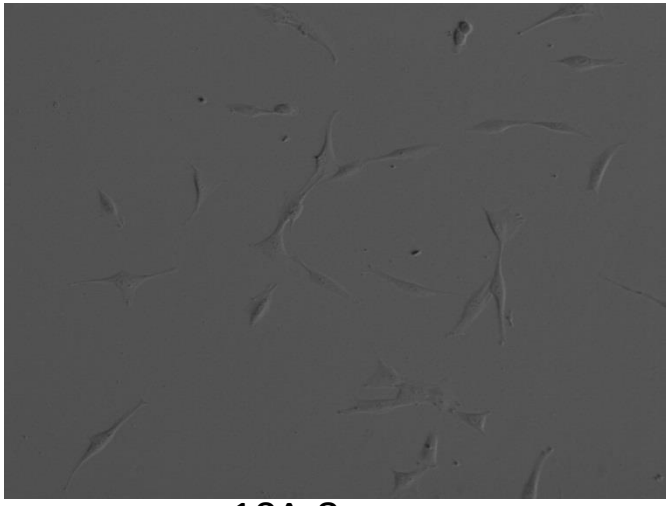
Proliferation Results Day 3



10^-6



10^-6 Degraded



10^-8

10^-8 Degraded

Conclusions

- Null Hypothesis was rejected
  - Addition of estradiol significantly affected the proliferation of MG-63 cells.
  - TAMLs caused a significant degradation in estradiol's effects on MG-63 cells.

Limitations

- Possible sources of error:
  - Cells may have been dead/dying on hemocytometer
  - Hemocytometer counts can vary
  - Hydrogen Peroxide may have been remaining after the TAML reaction took place
  - Catalase may have become partially inactive before addition to TAML reaction mix

Extensions

- Use a wider range of concentrations
- Test TAML degradation on other hormones
- Determine a LD 50% concentration of the hormone

Works Cited

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