

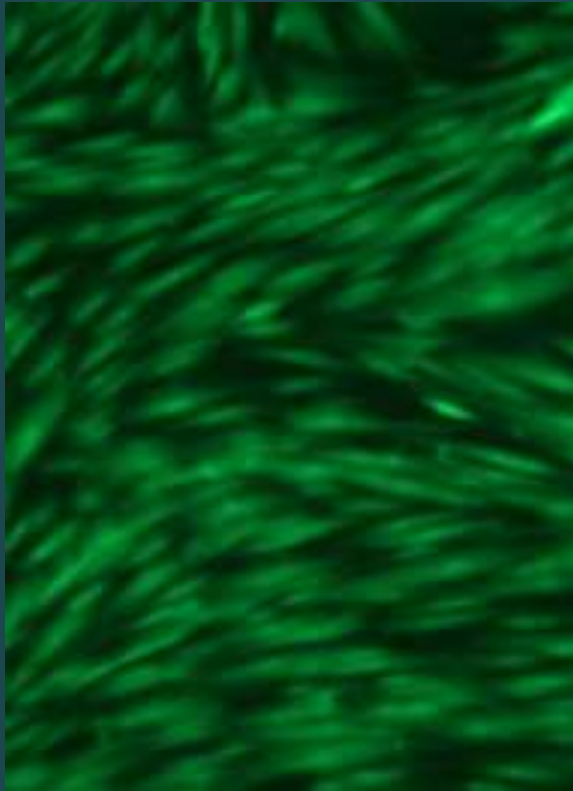
# The Effects of Charcoal Toothpaste on Fibroblasts:

## Gingival Cell Survival, Proliferation, and Migration

Brook Thibodeaux, Dr. Lallier  
LSUSD Periodontics Resident

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# Most of you have seen advertisements like this:



Or more dramatically, this:



# WHAT IS CHARCOAL TOOTHPASTE?



# Addition of Charcoal to Toothpaste

## ■ Activated Charcoal

- *Created by slow burning carbon-rich natural materials at high temperatures*
- *Carbon residue dehydration*
- *Highly porous and excellent for trapping potential stains*

## ■ Why add it to toothpaste?

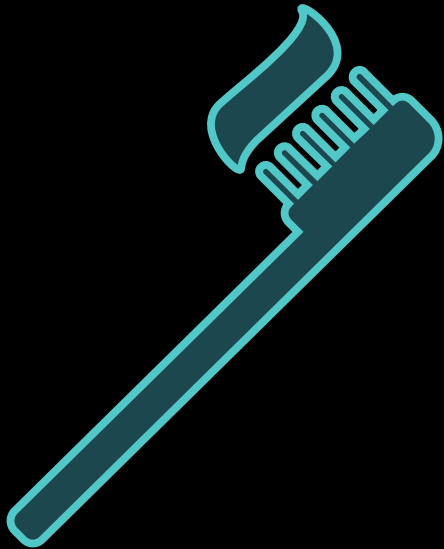
- *For extrinsic stain removal on enamel*
- *It has an abrasive effect within acceptable limits*
- *It is an extracting agent for removal of toxins*

## ■ Potentially carcinogenic



Why do we care as dentists?

# Clinical application



- Patients actively seek treatment for teeth whitening from their dentists
- Is this a safe whitening alternative for patients recovering post periodontal surgery?
- Is this a safe treatment modality for patients healing from periodontal disease?
- Should we be recommending the use of charcoal toothpastes to patient groups with the above concerns?

## Objective

The purpose of this *in vitro* study is to analyze the effect of various charcoal toothpaste extracts on cell survival, proliferation and migration on human gingival fibroblasts



# Materials

# Materials

## Cells tested

- *Human gingival fibroblasts*

## Toothpastes

- *Charcoal Toothpastes*
  - **Colgate Revitalizing White**
  - **Tom's Activated Charcoal**
  - **Crest Whitening Therapy- Deep Clean Charcoal**
  - **Hello Charcoal, Moon Charcoal**
- *Non-charcoal Toothpastes*
  - **Colgate Optic White**
  - **Tom's Fluoride Free**
  - **Colgate Cavity Protection**

## Control

- *No Toothpaste*

## Test solutions

- *Dissolve 1.3g in 15mL of MEM $\alpha$  media containing 10% FBS and antibiotics*
- *This was defined as 100% solution*

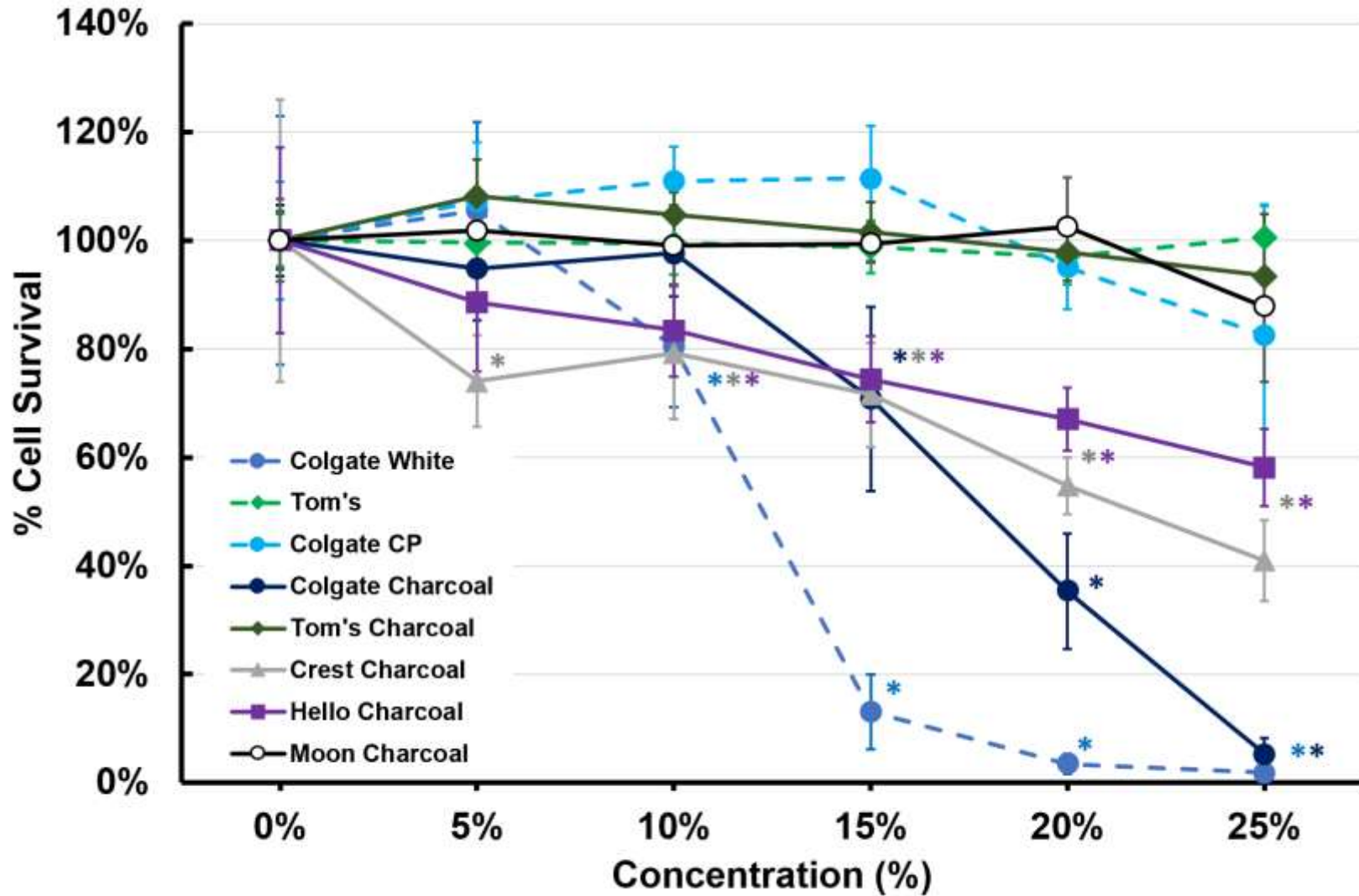
# Experimental design

# Part 1: Survival Assay

# Cytotoxicity Assay- Testing Solution Concentrations

- **Testing:** survival of gingival fibroblasts
- **Exposure time:** 3 minutes
- **Exposure solution concentrations:**
  - *0%, 5%, 10%, 15%, 20%, 25%*
- **Incubation period:** 24 hours post exposure
- **Assessment:**
  - *cells were dyed with calcein and survival was determined using a plate reader*

# Cell Survival



- Gingival fibroblasts were exposed to toothpaste solutions:

- up to 25% concentration
- for 3 minutes
- observed after 24 hours.

- Points represent the mean and standard deviation of eight samples.

- n=8

- \* =  $P < 0.05$

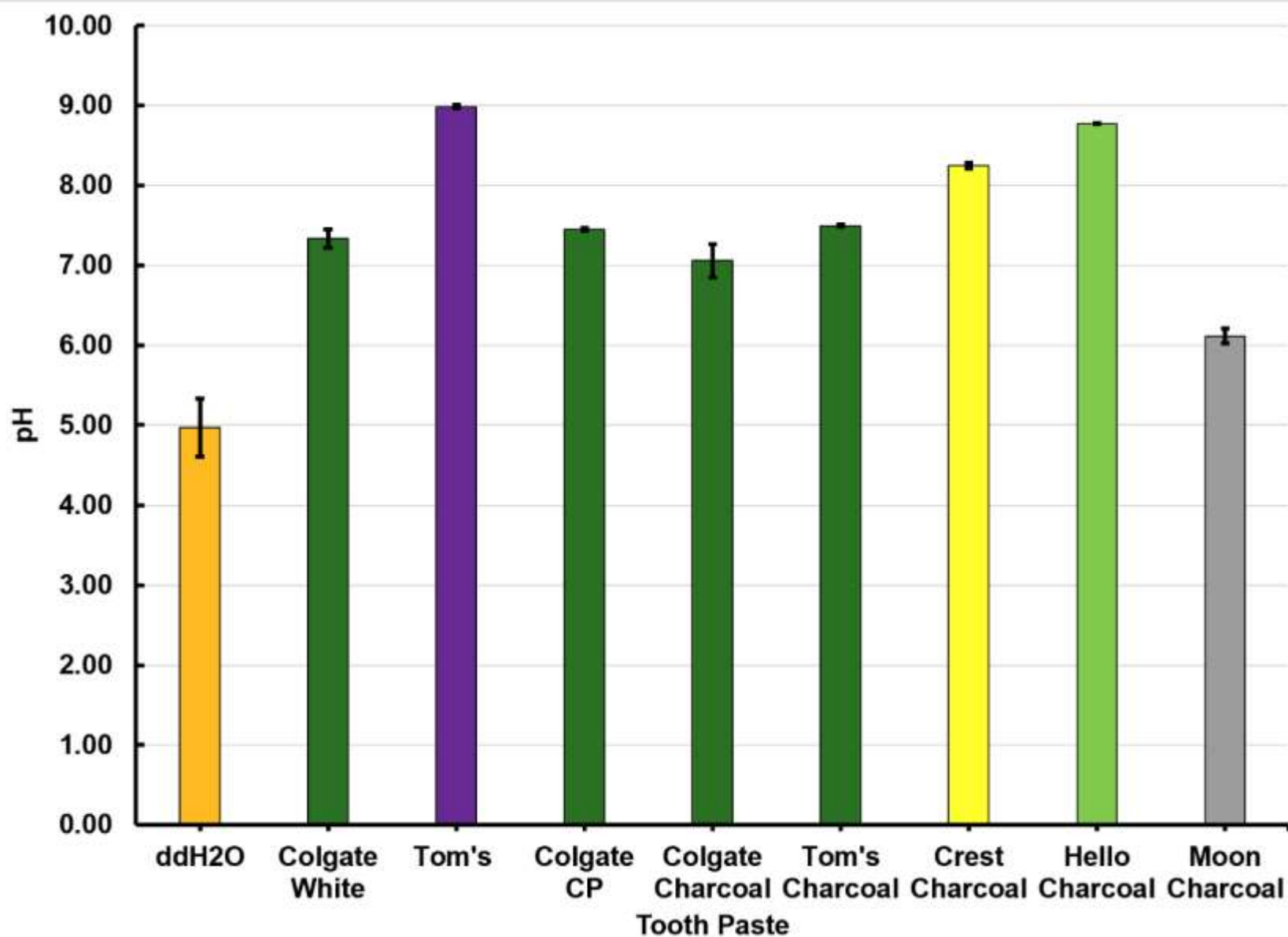
# Part 1a: pH

# pH of Toothpaste Solutions

- **Testing:** pH of Toothpaste Solutions
- **Control:** tap water
- **Calibrated digital pH tester**
- **Measurements:** 3 readings per solution
- **Assessment:**
  - *Comparison to Cytotoxicity Assay*

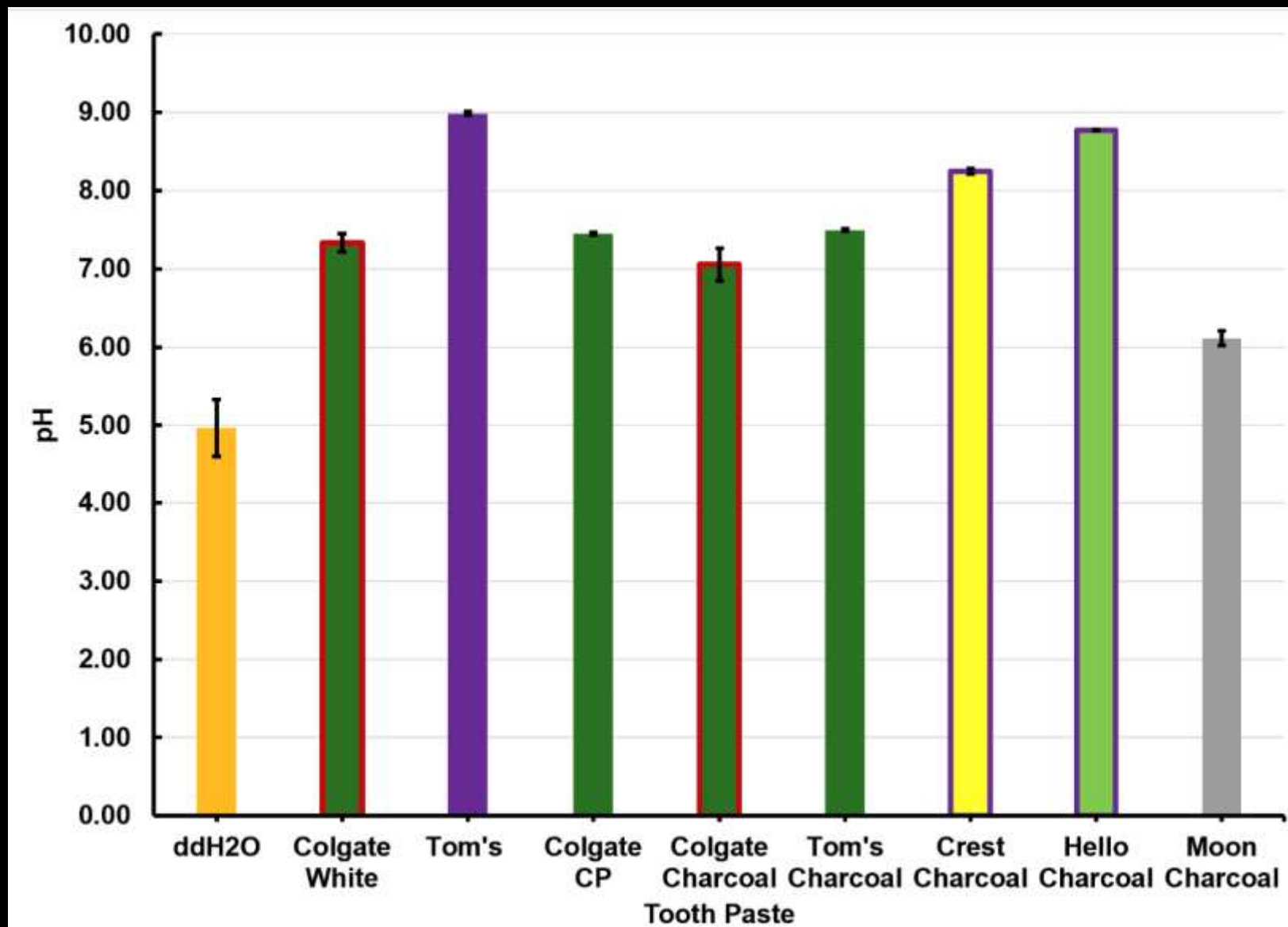


# pH



- Average of 3 measurements of pH of each toothpaste extract.
- Points represent the mean and standard deviation of 3 measurements.
- Bars of same color are statistically similar.
- Bars of different colors are statistically different.

# pH with Toxicity Indicators



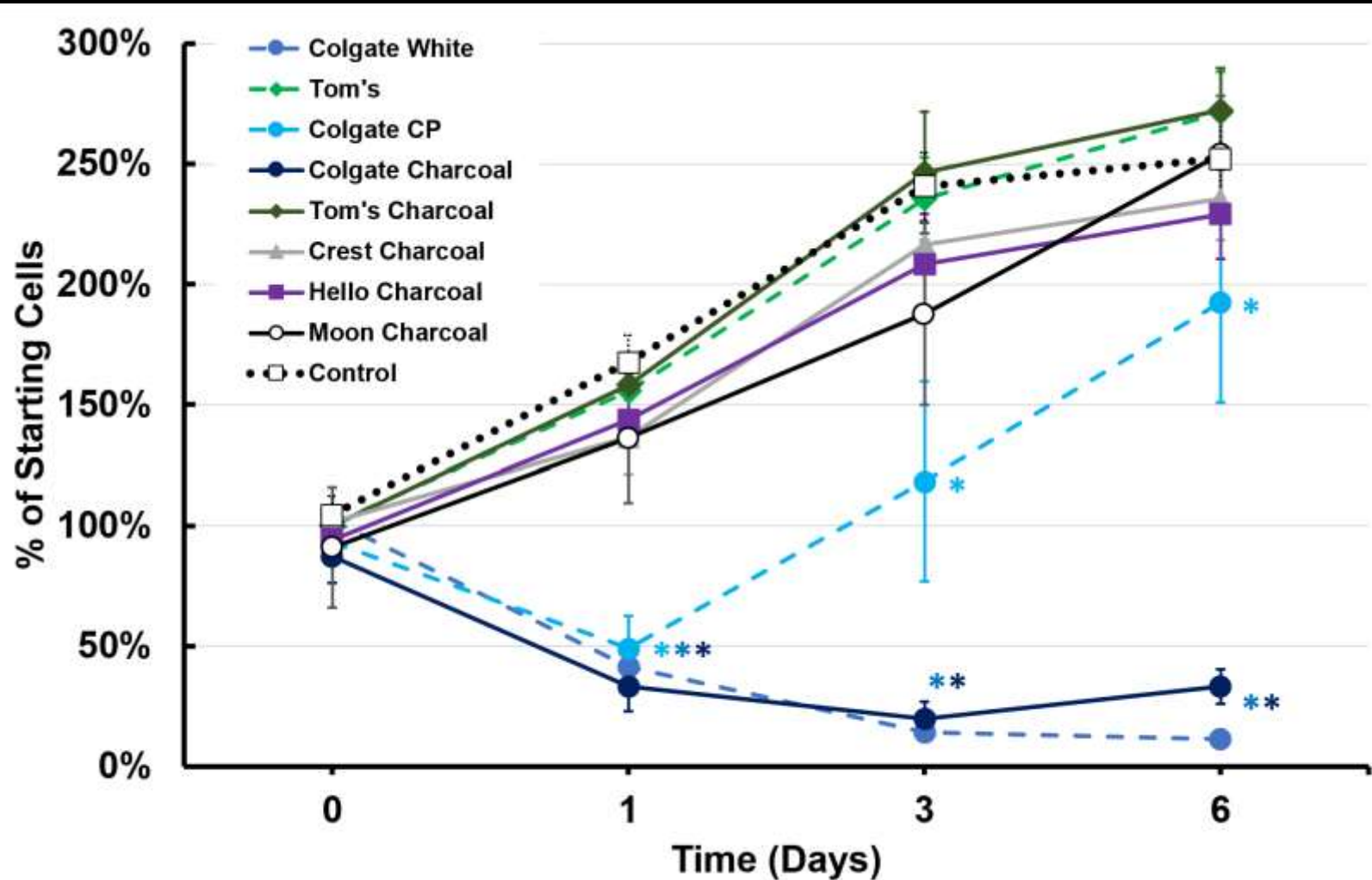
- Average of 3 measurements of pH of each toothpaste extract.
- Points represent the mean and standard deviation of 3 measurements.
- Red outline was highly cytotoxic
- Purple were moderate cytotoxic

# Part 2: Cell Proliferation

# Cell Proliferation

- **Testing:** proliferation of gingival fibroblasts
- **Exposure time:** 3 minutes
- **Exposure solution concentrations:** 25%
- **Incubation period:** 0 days, 1 day, 3 days, 6 days
- **Assessment:**
  - *After incubating for specific time intervals, cells were frozen for lysis, DNA exposed and stained by binding with CyQuant*

# Cell Proliferation



- Gingival fibroblasts were exposed to toothpaste solution
  - 25%
  - 3 minutes exposure
  - observed after 1, 3 and 6 days.
- Points represent the mean and standard deviation of eight samples.
- n=8
- \* = P<0.05

# Part 3: Cell Motility Assay

# Cell Motility Assay- To Assess Cell Motility

- Testing:

- *Motility of Gingival Fibroblasts Post Scrape of 1.5mm*

- Exposure time: 3 minutes

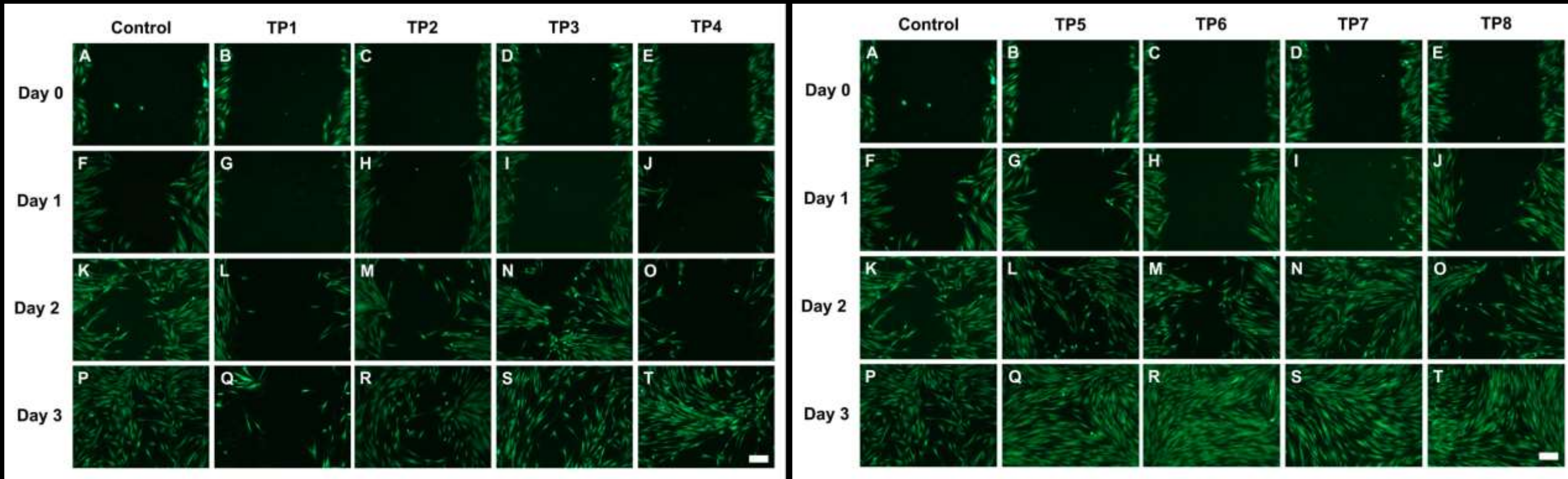
- Exposure solution concentration: 25%

- Incubation period: Day 0, Day 1, Day 2, Day 3

- Assessment:

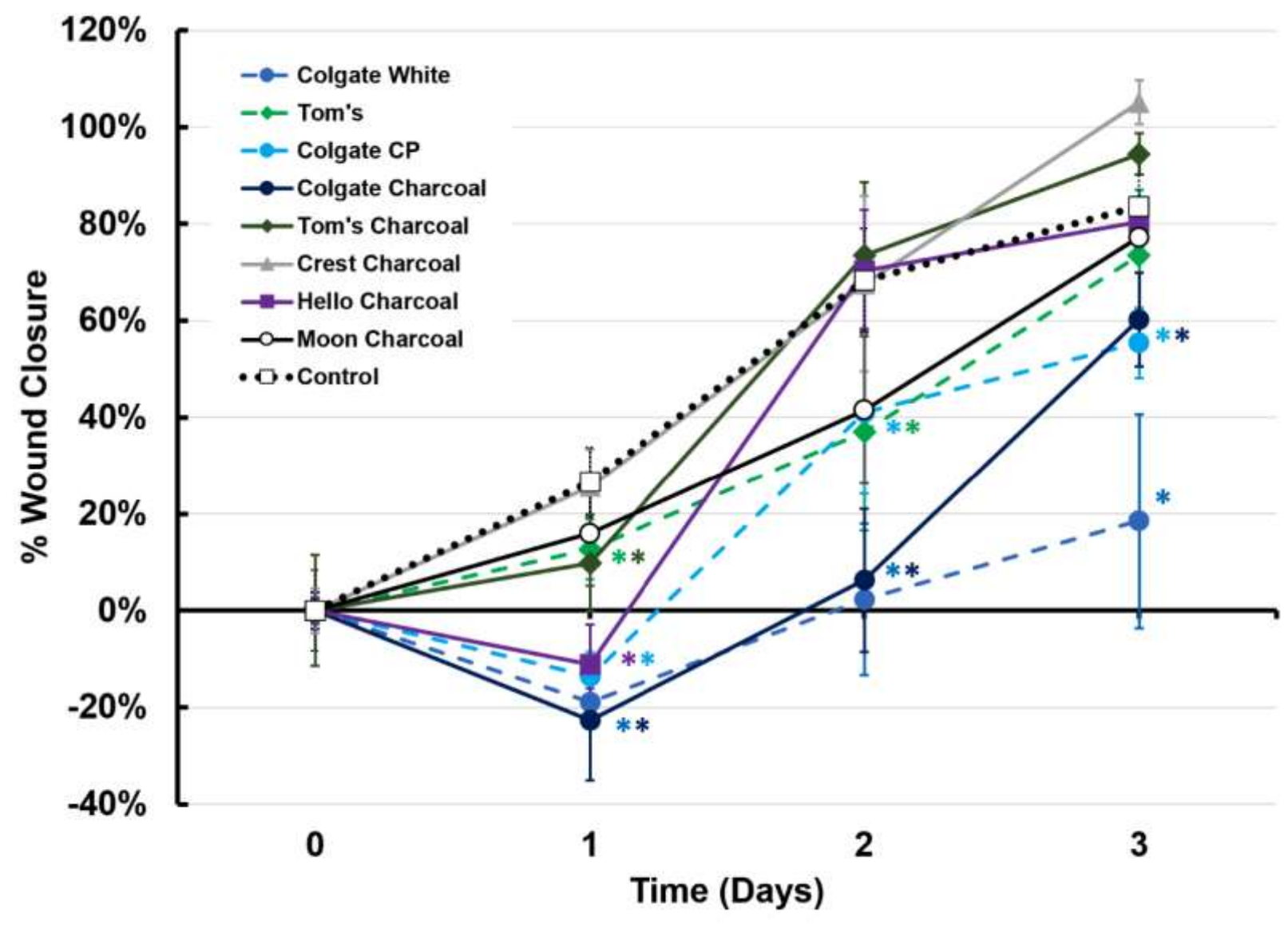
- *Cells were dyed with calcein and motility was determined by measurement of the scrape to determine wound closure*

# Cell Motility Assay





# Cell Motility Assay



- Gingival fibroblasts were exposed to toothpaste solutions
  - 25% concentration
  - 3 minute exposure
  - observed after 1, 2, and 3 days.
- Points represent the mean and standard deviation of eight measurements.
- n=8
- \*= P<0.05

# Findings

## Cytotoxicity:

- *Charcoal toothpastes were less toxic than Colgate whitening toothpaste*
- *Colgate Charcoal, Crest Charcoal and Hello Charcoal were moderately cytotoxic*

## pH:

- *pH did not correlate with gingival fibroblast survival*

## Cell Proliferation:

- *Charcoal toothpastes did not alter cell proliferation*
- *Unlike Colgate toothpastes*

## Cell Motility:

- *After 1 day wound closure was reduced for*
  - *All Colgate toothpastes and Hello Charcoal*
- *By 3 Days wound closure remained delayed for*
  - *All Colgate toothpastes*

# Conclusion

Charcoal toothpastes may be a good alternative to whitening toothpastes for periodontal disease patients and for those recovering from periodontal surgery.

- **Following 3-minute exposure:**
- Charcoal toothpastes
  - Tom's and Moon Charcoal had no effect on cell survival
    - Unlike Colgate Charcoal, Crest Charcoal and Hello Charcoal
  - No effect on Cell Proliferation
    - Except Colgate Charcoal
  - Moon Charcoal had no effect on Cell Motility
    - Tom's, Hello, and Crest charcoal delayed wound closure
    - Colgate Charcoal inhibited wound closure
- All Colgate Toothpastes
  - Reduced Cell survival
    - Except Colgate Cavity Protect
  - Reduced Cell Proliferation
  - Reduced Cell Motility

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THANK YOU!

