Wound Healing Assay (Scratch Test) on Endothelial EaHy.926 Cells Using Caffeine

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Abstract

- Investigated caffeine's effect on endothelial wound healing
- Scratch test performed on EaHy.926 cells
- Treated with caffeine (0, 0.1, 0.5, 1.0, 2.5, 5.0 mM)
- Wound closure monitored over 4 days
- ANOVA analysis conducted to assess significance
- Optimal range (0.1-2.5mM) enhanced healing, while 5.0 mM slowed closure

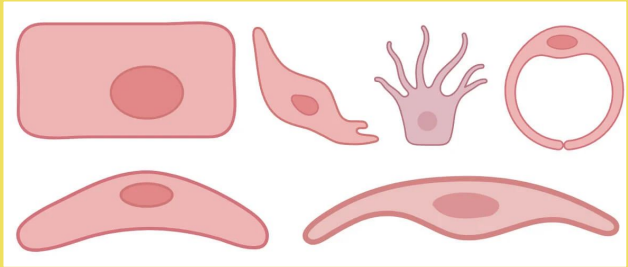
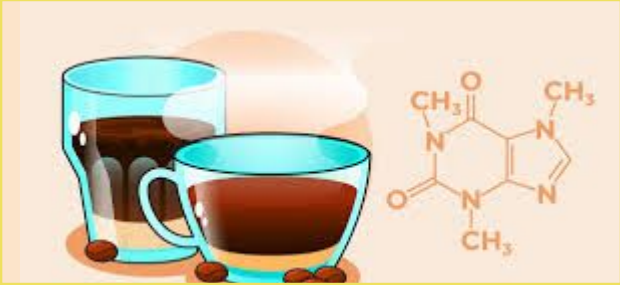


Photo credit: Microbe Notes



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Why did we choose to use caffeine?

Caffeine influences various signaling pathways, including those involved in cell proliferation, migration, and survival. From previous studies, low to moderate doses of caffeine have been associated with improved endothelial cell migration and function, while higher doses may have cytotoxic effects. We are interested is these effects are true or not.

Introduction

- Endothelial cells are crucial for vascular integrity. They line the inner walls of blood vessels and regulating blood flow.
- Background studies on caffeine's impact on wound healing.
    - Wang et al. (2021): Caffeine enhances endothelial motility (50 μM). Potential mechanisms in cell signaling for cAMP/PKA/AMPK pathway involvement.
    - Ojeh et al. (2016): Dose-dependent effects on epidermal cells
    - Jonkman et al. (2014): identified the different ways to statistically graph and analyze the wound healing rate.
    - Chen et al. (2021): Studied the effect of caffeine consumption on skin wound healing and scar formation in mice.
    - Xu et al. (2021): Studies how caffeine induces multiple vascular effects
- Research question: Does caffeine influence endothelial wound closure?

Hypothesis

Caffeine will influence endothelial cell migration as well as wound healing rates based on the concentration of stimulant applied. Therefore, low/moderate doses of caffeine will facilitate the process of wound recovery by enhancing proliferation and migration of endothelial cells. Moreover, we anticipate that elevated caffeine concentration will ultimately decrease the rate of cell migration.

Methods

- Caffeine stock solution prepared (10 mM in sterile water)
- EaHy.926 cells cultured in 18-well plates
- Mechanical scratch created using pipette tip (200ul to keep consistent scratch)
- Caffeine treatment applied per concentration chart
- Wound closure monitored via phase contrast microscopy
- Time points: 9 AM, 1 PM, 5 PM (Day 1); 9 AM (Day 2, 3, and 4)
- Data analyzed using one-way ANOVA

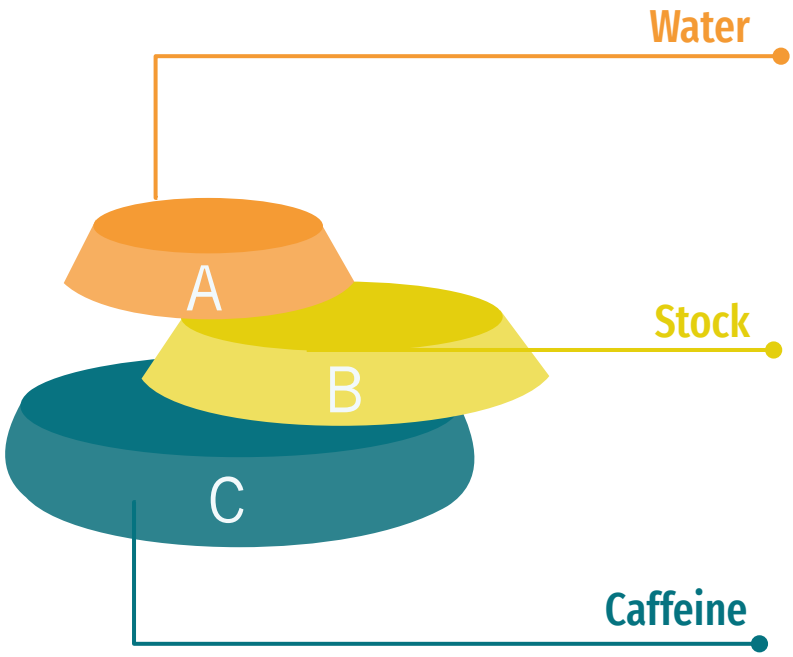
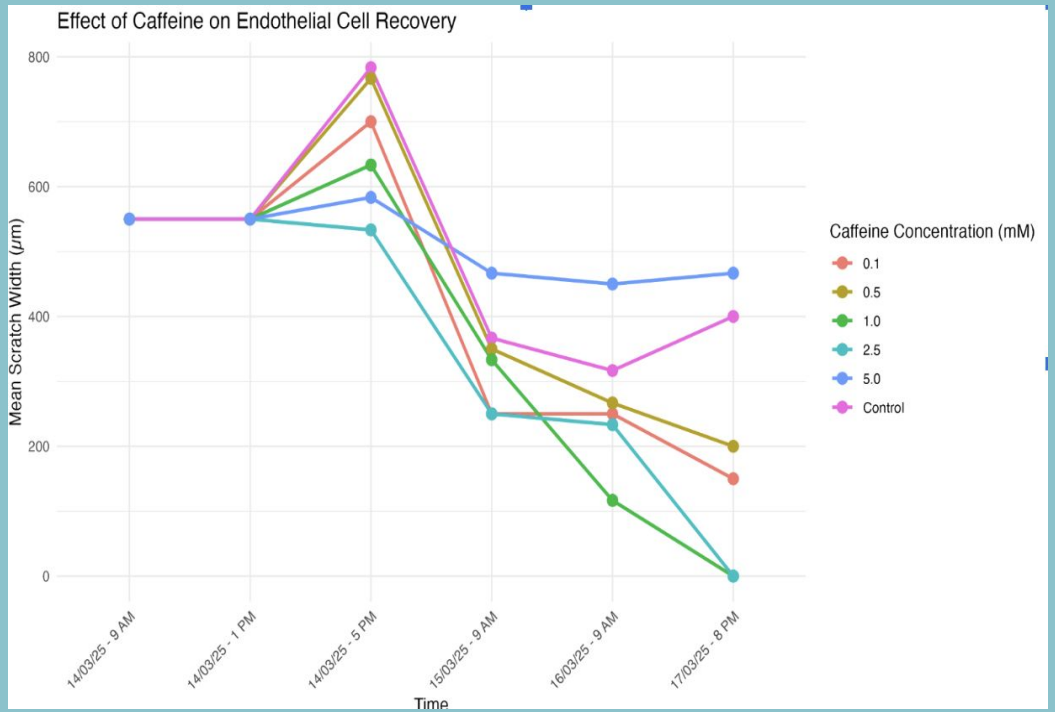


Table 1. Preparation of caffeine concentrations for the scratch test. Different final concentrations (0 – 5.0 mM) were prepared by diluting a 10 mM stock solution in water. The final volume for each well was adjusted accordingly.

Final Concentration (mM)	Volume of 10 mM Stock (µL)	Volume of Water (µL)	Total Volume (µL)
5.0	150	150	300 → take 250uL for each well
2.5	75	225	300 → take 125uL for each well
1.0	30	270	300 → take 50uL for each well
0.5	15	285	300 → take 25uL for each well
0.1	3	297	300 → take 5uL for each well
0 (control)	0	0	0

Results

- General trend: healing accelerates at 0.5–2.5 mM, slows at 5.0 mM
- One-way ANOVA results for caffeine: p = 1.46e-07 (statistically significant)
- One-way ANOVA results for time: p = < 2e-16
- One-way ANOVA results for time x caffeine: p = 4.30e-05
- Confirms our hypothesis



Discussion

- **Statistical analysis (ANOVA)** confirmed time and caffeine affected healing.
- **Unexpected wound expansion** observed initially.
- **Possible causes:** mechanical stress, osmotic pressure, inflammation, or extracellular matrix disruption.
- **Study limitations:** Small sample size, potential variability in cell growth.

Conclusion

- Caffeine influences endothelial wound healing in a dose-dependent manner.
- Low/moderate doses beneficial, high doses detrimental.
- Potential clinical implications for vascular repair strategies.
- **Future directions:** when redoing we have to have better measurements for the scratches in order to get better results.

References

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