Effects of Multi-ingredient Cetylpyridinium Chloride Mouthwash Treatment on the Persistence of Established *Streptococcus mutans* Biofilms

Featured scientist: Abigail Stack, University of Mount Union, Fall 2023

Research Background:

Biofilms are communities of microorganisms, like bacteria, that stick to surfaces and surround themselves with a slimy layer of protective goo called **extracellular polymeric substance (EPS)**. This layer helps them survive harsh conditions, resist antibiotics, and avoid being washed away.

In your mouth, biofilms form on your teeth, gums, tongue, and even braces or retainers. The most familiar biofilm? **Plaque**, that sticky stuff your dentist scrapes off during a cleaning. If plaque isn't removed, it can harden into tartar and lead to **tooth decay**, **gum disease**, and even infections that affect your whole body.

Abigail focused on biofilms formed by *Streptococcus mutans* (Figure 1), a bacterium that loves sugar and is a major cause of cavities.

These biofilms are especially tough because:

- They grow quickly in sugary environments (like your mouth after soda or candy).
- They protect *S. mutans* from mouthwash and antibiotics.
- They can increase resistance to treatment by changing gene expression.

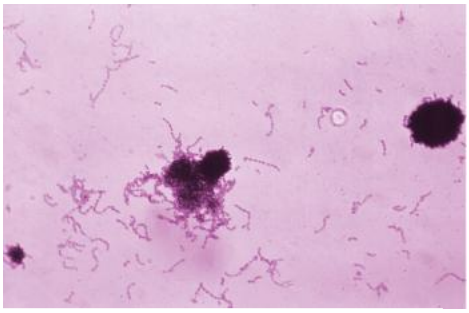

Figure 1

Biofilms are especially tough to treat. They act like a protective shield for bacteria, making it harder for mouthwash or toothpaste to reach and kill the microbes inside. If a natural product can break through that shield, it could be a powerful tool for oral health and a safer alternative for people who want to avoid synthetic chemicals.

In recent years, there's been a surge in consumer interest in natural and organic personal care products, including toothpaste, mouthwash, and skincare. Many people believe these products are safer, healthier, or more environmentally friendly.

Abigail's research tested two natural mouthwashes to see which one could reduce biofilm growth. Her findings help us understand which products are truly effective and which ones might just be riding the wave of consumer trends.

Research Questions:

Abigail wanted to know: Can natural mouthwash break through this biofilm shield and stop *S. mutans* from growing?

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Guiding Questions for Students

1. What is a biofilm, and why is it harder to kill than free-floating bacteria?

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2. What role does *Streptococcus mutans* play in oral health?

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Hypothesis:

3. Write a **null hypothesis** for this experiment?

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4. Write an **alternative hypothesis** for this experiment?

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Experimental Methods:

Abigail wanted to know if natural mouthwash could kill Streptococcus mutans, a bacterium that causes cavities, and stop it from growing in biofilms. To find out, she designed a multi-step experiment using lab-grown bacteria and two different mouthwash products.

Step 1: Growing the Bacteria

She started by growing S. mutans in a nutrient-rich liquid called **TSB-YE** (Tryptic Soy Broth with Yeast Extract). This gave the bacteria everything they needed to multiply and form biofilms, just like they do in your mouth.

Step 2: Preparing the Mouthwash Treatments

Abigail tested two mouthwashes:

- **HPOP** (Hello Peace Out Plaque): marketed as "naturally healthy"
- **TMWF** (Tom's Maine Wicked Fresh): another natural brand

She diluted the mouthwashes in different concentrations to see how strong they needed to be to kill bacteria. These dilutions were used in two types of tests: (1) a test for free-floating bacteria and (2) a test for biofilms

Step 3: Test 1 - Disk Diffusion Test (Planktonic Bacteria)

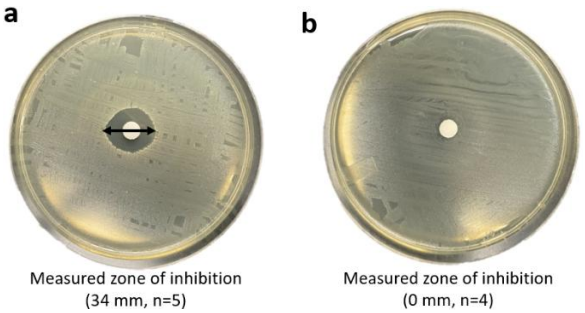
To test how well the mouthwashes killed free-floating bacteria:

1. She soaked sterile paper disks in each mouthwash.
2. She placed the disks on agar plates covered with S. mutans.
3. After incubating overnight at 37°C, she measured the **zone of inhibition**—the clear area around the disk where bacteria couldn't grow.

Results of Test 1 - Disk Diffusion Test:

a. HPOP created a 34 mm zone of inhibition (strong killing power).

b. TMWF had a 0 mm zone (no effect).



Abigail also calculated the **Minimum Inhibitory Concentration (MIC)** and **Minimum Bactericidal Concentration (MBC)** for HPOP:

- MIC: 1:320 dilution (stopped growth)
- MBC: 1:80 dilution (killed bacteria)

TMWF showed no inhibition at any concentration.

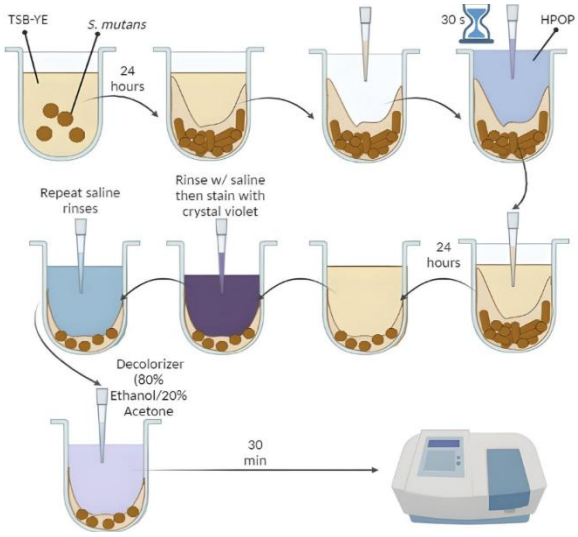
<u>Step 4: Biofilm Treatment</u>

Biofilms are harder to kill than free-floating bacteria, so Abigail tested how well HPOP worked on established biofilms.

She grew biofilms in small wells for 24 or 48 hours, then treated them in two ways:

- **Overnight treatment**: either full-strength or partial-strength HPOP
- **30-second rinse**: mimicking real-world mouthwash use

After treatment, she stained the biofilms with **crystal violet**, a dye that binds to bacterial cells. She then measured **absorbance**—how much dye was retained—to estimate how much biofilm was left.



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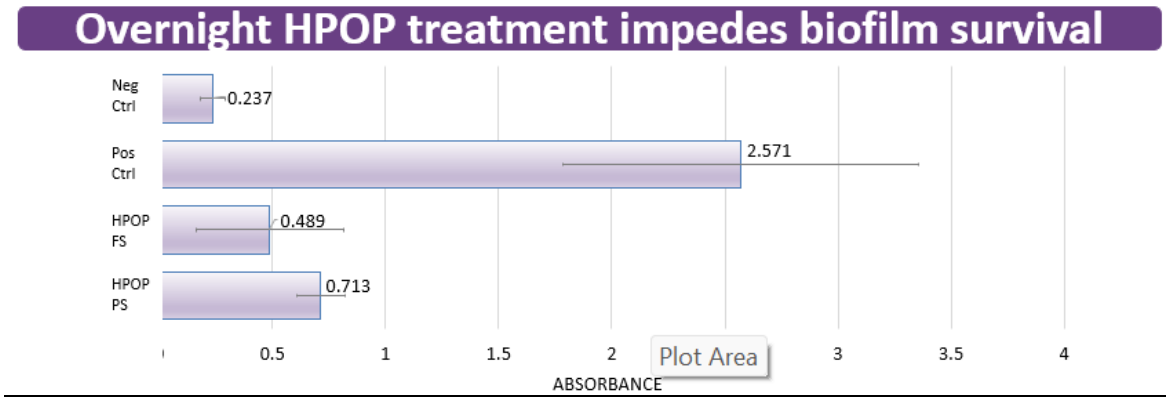
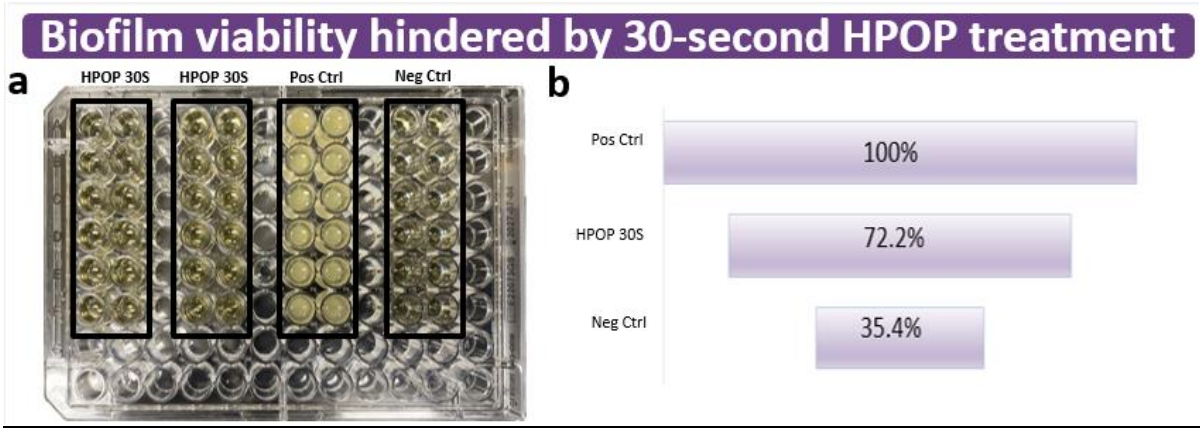
Result of Test 2 - Biofilm Treatment





Guiding Questions for Students – Understanding the Research

1. Why did Abigail test both overnight and 30-second treatments of mouthwash?

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2. What does a "zone of inhibition" tell us about a mouthwash's effectiveness?

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3. Why was TMWF excluded from the biofilm experiments

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Data Interpretation Questions

4. Which treatment had the lowest absorbance reading, and what does that mean?

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5. How did the 30-second HPOP treatment compare to the overnight treatments?

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6. Why is the negative control important in this experiment?

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7. What does the MIC and MBC tell us about HPOP's strength?

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Critical Thinking Questions

8. Do you think natural products should be trusted just because they're labeled "natural"? Why or why not? Provide evidence from Abigail's project.

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9. What are some limitations of Abigail's experiment?

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10. How could this research be applied in real-world settings, like dental care or product development?

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Your Role as a Scientist

11. If you were to design a follow-up experiment, what would you test next?

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12. What other natural ingredients might be worth investigating for oral health?

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13. How could you test mouthwash effectiveness in actual human mouth?

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Citations:

1. **Stack, A. (Fall 2023).** *Effects of Multi-ingredient Cetylpyridinium Chloride Mouthwash Treatment on the Persistence of Established Streptococcus mutans Biofilms*. University of Mount Union, Alliance, H 44601
2. CDC/Dr. Richard Facklam. (1975). *Stain of S. mutans in thioglycolate broth culture* [Photomicrograph]. Wikimedia Commons. https://commons.wikimedia.org/wiki/File:Streptococcus_mutans_in_thioglycolate_broth.jpg

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