Investigating the Effects of Honey on Gingival Fibroblasts: A Cellular Approach

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Abstract

This study investigates the therapeutic potential of Texas honey to enhance gingival fibroblast activity, aimed at treating periodontal diseases like gingivitis and periodontitis. Gingivitis, primarily due to bacterial plaque, can progress to periodontitis, leading to tissue and bone damage and increasing risks for systemic conditions, such as cardiovascular disease.1 Honey, known for its antioxidant, antibacterial, and growth-stimulating properties, may support gingival fibroblasts, which are vital for tissue repair. A gingival fibroblast cell line will be utilized to assess the impact of honey on cell proliferation, wound healing, and cytotoxicity. Honey solutions will be applied in various assays, and cell viability, wound closure, and cytotoxicity will be measured to determine optimal concentrations for therapeutic use. This study aims to evaluate honey's potential as a natural therapeutic for promoting gingival healing, with implications for periodontal disease management.

Introduction

Periodontal diseases, including **gingivitis and periodontitis**, are among the **most common oral health issues worldwide**. **Gingivitis** is an early, reversible condition caused by plaque accumulation and gum inflammation. If left untreated, it can progress to **periodontitis**, which leads to **irreversible damage** of the periodontal ligament and alveolar bone. Beyond oral health, periodontal disease is associated with systemic conditions such as cardiovascular disease and diabetes, underscoring the need for effective and regenerative treatments.

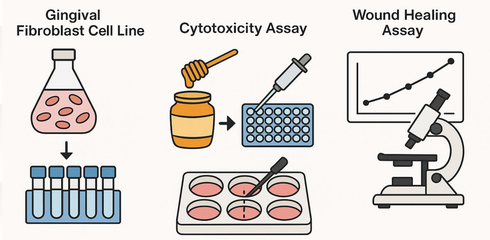
Conventional therapies, including scaling, root planing, and tooth extractions, can be invasive and traumatizing to the gums. While antibiotics are frequently prescribed, they pose risks of side effects and contribute to the rising concern of **antibiotic resistance**. These limitations have increased interest in **natural therapeutic alternatives**.

Honey, particularly **Texas honey**, is known for its **antimicrobial, antioxidant**, and **wound-healing properties**. Its potential to **support gingival fibroblast regeneration** makes it a compelling candidate for future periodontal therapies. Emerging research indicates that honey's phenolic compounds and low concentrations of hydrogen peroxide promote gingival fibroblast proliferation, minimize oxidative stress, and avoid cytotoxic effects.

Objective

We aimed to evaluate the **therapeutic potential of local Texas honey** on **gingival fibroblast activity** by assessing its ability to **promote cell proliferation**, **enhance wound healing**, and **minimize cytotoxic effects**. By testing honey samples with varying levels of bioactivity, we sought to identify **optimal concentrations** that support fibroblast function and contribute to **periodontal tissue repair**. Through this investigation, we provide insight into honey's potential as a **natural supplement treatment for managing periodontal diseases**.

Methods



For cell handling, gingival fibroblasts were rapidly thawed in a 37°C water bath and sterilized with 70% ethanol. Cells were centrifuged at 125 × g for 5–7 minutes, and the resulting pellet was resuspended in prewarmed medium. They were seeded at 2,500–5,000 cells/cm² into T-75 flasks and incubated at 37°C with 5% CO_2, with media changes every 2–3 days. At 75–80% confluence, cells were passaged using a rinse with DPBS, followed by detachment with trypsin-EDTA, neutralization, and either reseeding or cryopreservation.

For the cytotoxicity assay, 5×10³ cells per well were seeded in 96-well plates and treated with honey dilutions (20%, 10%, 5%, 2.5%, 1.25%, and 0.625%) prepared in basal low serum media, in triplicates. After 24-hour incubation, cytotoxicity or live/dead staining assays were performed. Controls included media only (untreated), FGF (positive), and 1% bleach (negative).

In the wound healing assay, cells were grown to ~90% confluence in 24-well plates, scratched with a sterile pipette tip, and treated with honey dilutions in FBM based on cytotoxicity results. Images were taken at 0, 20, 28, and 72 hours using inverted microscopy, and wound closure was quantified using ImageJ.

Results

Honey Samples	24H-14	24H-7	24H-35	Manuka-226
Bioactivity Level	Low	Mod	High	Medical Grade
Antibacterial Activity (ZOI)	8.5	22.5	23.35	18.55
Hydrogen Peroxide Activity	-3.778	175.416	147.55	7.69
Antioxidant Potential (DPPH)	0.2153	0.196	0.029	0.07
pH	3.66	3.69	5.08	4.76
Color	Water White	White	Light Amber	Light Amber

Table 1: *Properties of local Texas honey samples collected in Summer 2024. Samples 24H-7 and 24H-35 showed the strongest antimicrobial and peroxide activity, indicating higher therapeutic potential. All samples were tested under consistent conditions. Honeys were chosen by low, medium, and high bioactivity.*

Results Cont.

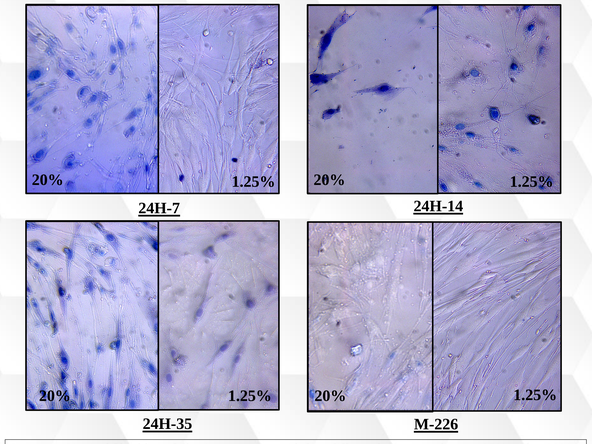
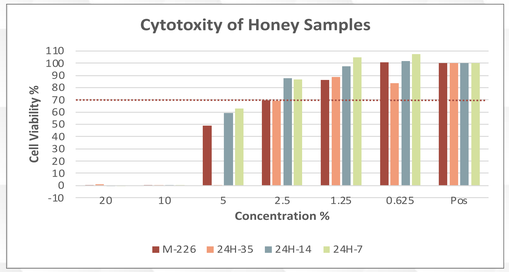


Image 1: *Gingival fibroblasts following treatment with four different honey samples at 1.25% and at 20% concentration during the cytotoxicity assay on day two. Comparing the differences between a high cytotoxic honey and a low cytotoxic honey.*



Graph 2: *Cytotoxicity Assay of Honey samples 24H-7, 24H-14, 24H.35, and M-226. Concentration and Cell Viability percentage are compared.*

Ongoing Experiment

This study is currently in progress. We will perform the **wound scratch assay** using selected **Texas honey dilutions** alongside a **media-only positive control**. The goal is to evaluate **wound closure**, **fibroblast migration**, and **cytotoxicity**, which will help determine the **regenerative potential of honey in periodontal therapy**.

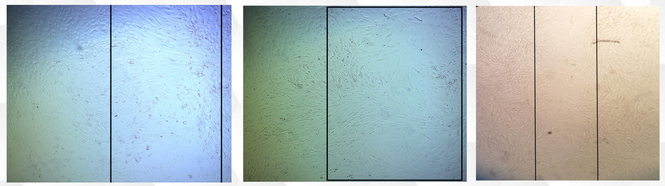


Image 3: *Practice Wound Scratch Assay After 24 Hours. A practice wound scratch assay performed at a cell density of 5.00 × 10³ and a honey concentration of 1.25% after 24 hours, illustrating the experimental setup and typical wound closure patterns. The parallel vertical lines indicate the initial location of the scratch, where cells were mechanically displaced to simulate a wound. These markings serve as a reference point for assessing fibroblast migration during subsequent time points in future investigations.*

Conclusions

- **Texas honey promotes gingival fibroblast proliferation and wound healing**.
- **Medium to low concentrations** of honey increased cell viability and enhanced wound closure **without inducing cytotoxic effects**.
- Findings **support the hypothesis** that **bioactive compounds in honey aid tissue regeneration**.
- This study helps **fill a gap in research** by identifying honey concentrations that optimize oral cell health.
- Results **support the integration of sustainable, natural therapies** in periodontal care using **complementary medicine products**.

Future Directions and Applications

- A promising application of this research is the development of **honey-infused oral care products**, such as **therapeutic gum strips**, **mouth rinses**, or **toothpaste additives** designed to **promote tissue regeneration** and **reduce bacterial load** in patients with periodontal disease.
- Moving forward, we plan to investigate **honey's antimicrobial effects** against **common plaque-forming oral bacteria**, focusing on its ability to **inhibit bacterial growth and biofilm formation**.
- In the long term, we aim to expand this work into **in vivo models** to evaluate the **clinical effectiveness**, **biocompatibility**, and **formulation stability** of **honey-based dental therapeutics**.

References

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Acknowledgements

- We would like to give a huge thank you to Alejandro Morales Betancourt, Amber Arooj, and Shahabullah Mandozai.
- Dr. Kelly Nash for supporting us throughout out our research and Dr. Ferhat Ozturk for being an inspiring and encouraging mentor.
- Thank you to the CURE Program of Biology, Health, Environment Dept!
- Visual concept and design support provided using ChatGPT by OpenAI (2025).