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ANTIBACTERIAL EFFECT OF CITROFORTUNELLA MICROCARPA EXTRACT ON STREPTOCOCCUS BACTERIAL GROWTH ON ORTHODONTIC BRACKETS

MASTER OF SCIENCE

In

DENTISTRY MAJOR IN ORTHODONTICS

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Abstract

The antibacterial effect of calamansi (Citrofortunella microcarpa) was explored in this study. Focusing on its ability to inhibit Streptococcus mutans growth on orthodontic brackets; as S. mutans is a key contributor to plaque accumulation during fixed orthodontic treatment. The literature noted that biofilm formation on metal and ceramic brackets increases caries risk, highlighting the need for natural microbial alternatives such as citrus extracts. This in vitro experiment evaluated whether calamansi (Citrofortunella macrocarpa) at varying concentrations, could reduce S. mutans bacterial growth on metal and ceramic orthodontic brackets. The study also aimed to identify the optimal concentration capable of producing significant bacterial inhibition and examined changes in antibacterial activity across different exposure periods (1, 3, and 7 days). Calamansi extract was freshly prepared and applied in concentration of 100%, 50%, and 25%, and bacterial growth was analyzed through colony- forming unit (CFU) counts on BHI agar. Across all conditions, results demonstrated that a dose-dependent reduction in S. mutans growth, with 100% concentration showing the strongest inhibitory effect, and metal brackets consistently exhibiting slightly lower adhesion than ceramic brackets. Overall, the finding supports that calamansi extract as a promising, natural and cost-effective antibacterial agent for orthodontic care, offering a sustainable alternative to chemical-based products reducing plaque during treatment.

CHAPTER 1
Introduction

The antibacterial effect of calamansi on S. mutans in relation to plaque accumulation on orthodontic brackets is a promising area of research. Oral hygiene neglect during orthodontic treatment may lead to remarkable dental problems, specifically an increased risk in the incidence of dental caries.

According to research, it has been shown that patients who underwent fixed orthodontic treatment have encountered an increased risk of dental caries, and this is due to the accumulation of plaque around the orthodontic brackets. As a consequence, this could lead to dental caries and other oral infections such as periodontal diseases. (Contaldo, 2021)

Additionally, (Contaldo, 2021) has indicated the crucial alterations and changes in dental caries and oral hygiene amongst patients undergoing fixed orthodontic treatment. The study observed that sustaining and maintaining a proper oral hygiene becomes a challenging obstacle because of the fixed orthodontic appliance, as a result, more and increased plaque accumulation is noted. The orthodontic appliances are designed in a way that traps food and bacteria, therefore aggravating hygiene issues. Hence, patients with fixed orthodontic appliances are more prone and liable to dental caries, and this is due to the change in the oral microbiota that contributes to increasing their risk.

Specifically, orthodontic fixed appliances create an additional cariogenic environment when compared to removable appliances, and this contributes to the growth of unfavorable bacteria. Also, as early as one week into orthodontic treatment, plaque changes may be observed, and this

becomes more consistent after three months, which in turn increases the risk of caries. (Contaldo, 2021)

This highlights the essential requirement for refined and improved oral hygiene practices among patients and those regular dental check-ups to diminish the risk of dental caries and other factors to support a healthy oral health during the orthodontic treatment. (Contaldo, 2021)

Orthodontic brackets are an ideal niche for the accumulation of plaque, and this can cause dental caries if not properly maintained. (Contaldo, 2021). Patients with fixed orthodontic appliances exhibit a notable change in their oral microbiota, and this results in increased risk of harmful bacteria that are linked to periodontal diseases and dental caries. (Contaldo, 2021). This signifies that fixed orthodontic appliances notably alter the quantitative and qualitative contents of oral plaque, increasing the risk of oral infections. (Contaldo, 2021)

A longitudinal study by (Pinto, 2020), observed the relationship between the occurrence of active caries lesions and fixed orthodontic appliances over a one-year timeframe. Results revealed that patients with fixed orthodontic treatment had a significantly increased risk of developing caries when compared to patients without. This indicated that patients with fixed orthodontic appliances encounter a significant increase in active caries lesions that are active, and this highlights the importance of monitoring caries activity throughout the treatment. (Pinto, 2020)

According to the World Health Organization (WHO), an estimated 80% of the global population depends on herbal medicines for treating numerous

Different illnesses.This liking is attributed to the cost effectiveness, availabilit y, and decreased incidence of side effects.

The usage of remedies that are herbal has been a foundation of medical pharmacology and has had a significant effect on conventional medicine practices. The increasing popularity of herbal remedies can be linked to their wider acceptance by patients (Ahmad H, 2022)

Background of the Study: -

Calamansi or Citrofortunella microcarpa is considered to have originated from China. It is thought to be a result of a cross-link between two other types of citrus species and a kumquat. The calamansi pertains to the Rutaceae family, and this includes other citrus fruits such as the pomelo, tangerine, and lime. Following its original emergence in China, the calamansi has expanded its presence to various areas and regions, and this includes Southeast Asia, India, Hawaii, Central America, North America, and the West Indies.

The process of extracting the calamansi usually includes the extraction of its juice as well as its byproducts. These may include the seeds, the pulp, and the peels of the fruit. It can also be prepared into several types or forms, like forming it into syrup or powder, or can also be made into a jam or juice.

As for the peel of the calamansi, it is recognized for the extraction of essential oils from it. They are abundant in D-Limonene and other bioactive compounds, which are the main reason behind their several health benefits. (Venkatachalam, 2023)

The calamansi in the Philippines is harvested and produced greatly and highly, and is usually utilized alternately and in preference more than limes

due to its resistance to diseases and pests. It is involved and constitutes a huge amount in the country's economy, since it is harvested and cultivated locally for local utilization and for international trade. (Venkatachalam, 2023) The small citrus fruit known as calamansi is highly prized not just for its taste but also for its possible health advantages, such as its antibacterial properties. Abundant in Vitamin C, which is recognized for its immune- boosting characteristics, this vitamin is essential in bolstering the body's ability to fight off infections, which may help contribute to its antibacterial properties. (Philippines, 2024)

In different cultures, calamansi has long been utilized as a natural treatment for illnesses. Its acidic properties could potentially hinder the proliferation of specific bacteria, which is why it is favored for home treatments. (Philippines, 2024)

The use of herbal products is gaining traction due to their antibacterial and anti-inflammatory properties, which can help maintain periodontal health during orthodontic treatment (Marya, 2023). Patients prefer these natural alternatives to avoid adverse effects associated with conventional medications (Marya, 2023)

Herbal medicine could serve as a beneficial alternative to modern pharmaceuticals, while the traditional use of herbs is supported by current literature, further empirical evidence is required to establish their effectiveness. (Pasupuleti, 2023)

While specific studies on calamansi's antibacterial properties are limited in the provided contexts, its relatives in the citrus family, such as lemons and limes, have been studied for similar effects. (Philippines, 2024)

In conclusion, calamansi is regarded for its antibacterial qualities mainly because of its high Vitamin C content, traditional applications, and its ability to create conditions that are hostile to bacteria. Additional studies could yield clearer information about its antibacterial effectiveness. Overall, calamansi is a multifaceted fruit with a rich history, important extraction methods, broad popularity in the Philippines, and cultivation practices that enhance its economic significance.

Statement of the problem

The study will focus on examining the antibacterial effect of calamansi extract on the inhibition or destruction of S. mutans bacteria on orthodontic brackets.

Specifically, it seeks to address the following questions:

1. What is the effect of the different concentrations of calamansi juice extract on the growth of S. mutans on the metal brackets:

1.1 100% calamansi extract

1.2 50% calamansi extract

1.3 25% calamansi extract

1.4 control group (distilled water)

2. What is the effect of the different concentrations of calamansi juice extract on the growth of S. mutans on the ceramic brackets:

2.1 100% calamansi extract

2.2 50% calamansi extract

2.3 25% calamansi extract

2.4 control group (distilled water)

3. How do the effects of different concentrations of calamansi extract on different days compare between ceramic and metal orthodontic brackets?

Objectives of the Study: -

The main objective of the study is to assess the antibacterial effect of calamansi extract on the growth of S. mutans on orthodontic brackets. It will contribute to the development of natural solutions to prevent bacterial growth and improve oral health during orthodontic treatment.

Specifically, this study aims to address the following:

1. Assess the effect of the different concentrations of calamansi juice extract on the growth of S. mutans on the metal and ceramic brackets.
2. Determine the optimal concentration of calamansi juice extract that inhibits the growth of S. mutans on the metal and ceramic brackets, and;
3. To compare how the effects of the different calamansi extract concentrations change over time (on different days) between metal and ceramic orthodontic brackets.

Theoretical Framework: -

The study's theoretical framework is based on various interconnected theories concerning microbiology, dental health, and the characteristics of natural antimicrobial agents. It incorporates and includes these primary constituents:

1. The biofilm formation theory: This theory demonstrates how biofilms are bacterial communities that adhere superficially to surfaces and envelop themselves in an autogenous extracellular matrix. This protective layer facilitates bacteria such as S. mutans to multiply and proliferate in the oral cavity, especially on orthodontic appliances in patients undergoing orthodontic treatment, whether fixed or removable. It is critical to understand the mechanism by which biofilms are formed in order to evaluate how the calamansi extract can interrupt the process of adherence and inhibit the adhesion

of the bacteria on orthodontic brackets. (Park, 2022)

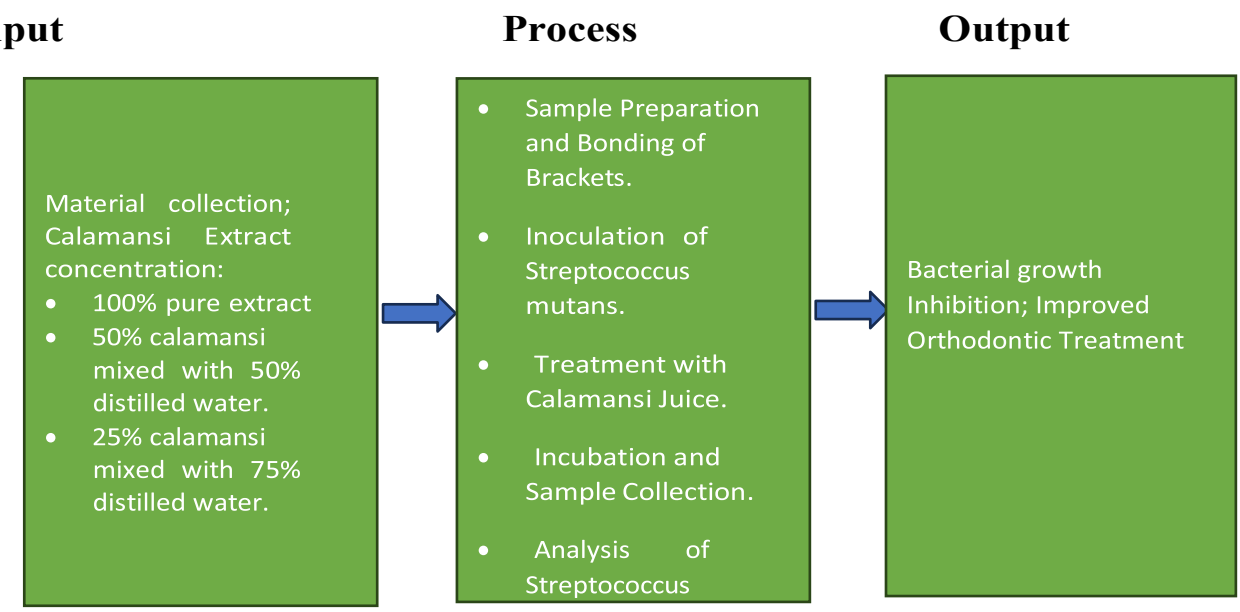
2. Antimicrobial agents that are natural theory: this research will investigate the efficacy and success of using calamansi extract as an all-natural antibacterial agent. This may be due to its high citric acid content and presence of essential oils that can reduce the S. mutans and the formation of biofilm on orthodontic appliances.

Conceptual Framework: -

The main fundamentals of the study are combined in the conceptual framework, indicating how biofilm formation on orthodontic brackets may be inhibited by different calamansi extract concentrations, and this corresponds to the antibacterial effects on S. mutans, while also displaying the variables related to orthodontic treatment.

Figure 1.

Research Paradigm.



Hypothesis: -

Null Hypothesis-Calamansi extract does not affect the growth of S. mutans on orthodontic brackets. Also, there is no difference in the effect of different calamansi concentrations on metal or ceramic brackets. This advocates that there is no influence on bacterial accumulation or related problems in orthodontic treatment.

Scope, Limitations, and Delimitations of the Study: -

The primary aim of this research is to assess the antibacterial impact of calamansi extract on inhibiting S. mutans bacteria, particularly on metal and ceramic orthodontic brackets.

The main bacteria studied was S. mutans exclusively, which is recognized as a dominant contributor to plaque development and oral infections in orthodontic patients.

The study will assess how different concentration levels of calamansi extract impact bacterial attachment. The research was carried out in vitro, indicating that

the experimental conditions was regulated in a laboratory setting, involving the application of various calamansi concentrations on extracted premolar teeth with two different types of orthodontic brackets.

The results might not entirely reflect and replicate real-life oral environments, like saliva production, eating habits, or the variations in natural oral bacteria, because the research is carried out in a laboratory setting.

The study is focused on S. mutans bacteria, but there are several other types and kinds of microorganisms that may perhaps cause and induce oral health problems during orthodontic treatments, possibly affecting the wide- ranging pertinence of the results.

As well, the time span for bacterial growth and the period of calamansi extract contact exposure may not reflect the long-term effects.

The examination in this study concentrates exclusively on metal and ceramic orthodontic brackets, and abstains from including further orthodontic materials like the wires, bands, and elastics, that possibly may also be prone to bacterial adhesion.

Significance: -

The possible benefits of calamansi extract on orthodontic treatment are being investigated in this research, with an exact emphasis on its capability to decrease S.mutans bacteria, and this in turn will improve oral health outcomes throughout orthodontic treatment. The results may perhaps have substantial implications for patients, orthodontists, and manufacturers.

This research explores the efficacy of calamansi extract in reducing the development of S. mutans bacteria on metal and ceramic orthodontic brackets. If results show that it is indeed useful and effective, this may offer an efficient,

cost-effective, all-natural substitute for orthodontic patients in avoiding oral infections and accumulation of plaque, and this eventually will support and assist the orthodontist in enhancing the oral health results during orthodontic treatment.

Also, the conclusions of this research might likewise contribute to manufacturers and future researchers in creating an original, pioneering, biologically all-natural dental product by taking advantage of the antibacterial constituents of the calamansi extract. This may as well emerge and evolve into a justifiable progression in orthodontic care and direct the rising necessity for natural alternatives in the specialty.

Definition of Terms: -

In order to achieve a thorough and clear grasp of the research, the following terms are defined:

Antibacterial: refers to substances that inhibit or prevent bacterial growth; it is often utilized in medical and hygiene products.

BHI Agar/Broth: a highly nutrient rich culture medium containing infusion of brain and heart tissue plus peptones and glucose; it is used for the cultivation of a wide variety of fastidious and non-fastidious microorganisms (including aerobic and anaerobic bacteria, yeasts and molds). (Aryal, 2022)

Calamansi, scientifically identified as Citrofortunella microcarpa, is a small citrus fruit native to the Philippines, renowned for its tangy flavor and high vitamin C content. It is commonly used in beverages and as a condiment in Filipino cuisine. Beyond its culinary uses, calamansi has garnered attention for its potential health benefits and applications in various industries. (Philippines, 2024)

Ceramic orthodontic brackets: They are made of alumina; in polycrystalline or monocrystalline forms and are used to provide an aesthetic alternative to stainless-steel brackets in fixed orthodontic appliance therapy. (Mundhada VV, 2023)

Colony-forming unit (CFU count): is a measure used in microbiology to estimate the number of viable bacteria in a sample, representing a single bacterial cell or a group of cells that can form a colony (Meyer, 2023) Culture Media. It is a nutrient material prepared for the growth of microorganisms in a laboratory. (Chauhan, 2020)

Dose-dependent relationship: a connection between the amount of something and the effect it produces; as the amount changes (whether increasing or decreasing) the effect also changes. (Feye, 2020)

Metal Orthodontic Bracket. The bracket is made from stainless steel, and it is the conventional and widely used bracket in orthodontics.

Microbial Contamination. It is the inclusion or growth of harmful microorganisms in an item used. (WHO)

Sterility. It is freedom from the presence of viable microorganisms. (WHO)

S. Mutans: Streptococcus mutans bacteria, is a Gram-positive bacterium primarily found in the human oral cavity, recognized as a significant contributor to dental caries. Its ability to adapt to the oral environment and its virulence factors, such as acid production and adhesion, facilitate its role in tooth decay and systemic health issues (Kannan et al., 2024; Bedoya- Correa et al., 2019). It develops in diverse conditions, leading to the formation of biofilm on teeth, where it comprises a considerable percentage of the oral microbiome.

Chapter 2 Methods

Study Design: -

This in vitro experimental study evaluated the antibacterial effect of calamansi (Citrofortunella microcarpa) juice extract on Streptococcus mutans colonization of orthodontic brackets. Two bracket types (metal and ceramic) were tested across three extract concentrations (100%, 50%, 25%) and a distilled-water control. S. mutans accumulation was quantified as colony-forming units (CFU) at 1-, 3-, and 7-day intervals.

Sample Collection and Preparation

Seventy-two (72) extracted human premolars were randomly selected from private dental clinics in Metro Manila. Teeth were stored in 1% thymol solution and rinsed with distilled water before use. Teeth with fractures, caries, restorations, or visible defects were excluded. Metal and ceramic brackets (American Ortho AO, identical prescription) were bonded using standardized orthodontic bonding procedures and light-cured. Samples were stored in sterile artificial saliva to simulate oral conditions.

Calamansi Extract Preparation

Fresh calamansi fruits were washed, manually squeezed, and filtered to remove solids. Extracts were prepared at:

- 100% calamansi juice (pH ~5),
- 50% calamansi juice (1:1 with distilled water, pH ~5),
- 25% calamansi juice (1:3 with distilled water, pH ~6).

Bacterial Inoculation: -

A pure S. mutans culture was obtained from the Philippine National Collection of Microorganisms, transported on ice, and cultured in Brain Heart Infusion Broth (BHIB) at 37°C under anaerobic conditions for 24 hours. The inoculum was standardized to McFarland Standard 5 (~3×10^8 CFU/mL). Bracket

surfaces were inoculated using sterile swabs, then incubated in artificial saliva at 37°C for 24 hours to allow bacterial colonization.

Treatment Protocol: -

After 24 hours of incubation, samples were treated with their respective calamansi concentrations. Each specimen was immersed twice daily (5 min each), rinsed with distilled water, and returned to artificial saliva. Controls were immersed in distilled water (pH 6.5) only. Treatments continued until assessment at Days 1, 3, and 7.

Bacterial Enumeration: -

At each time point, two samples per group were processed for CFU analysis. Bracket surfaces were vortexed with sterile diluent, then serially diluted. Aliquots were spread plated on Brain Heart Infusion Agar and incubated at 37°C for 24 hours. Plates with 30–300 colonies were counted, and CFU/mL was calculated:

$$\text{CFU/mL} = \frac{\text{colonies} \times \text{dilution factor}}{\text{volume plated (mL)}}$$

Counts from the third dilution (T3) were averaged for reliability.

Statistical Analysis: -

Descriptive statistics (mean ± SD) were calculated for all CFU counts. Between-group comparisons across concentrations, bracket types, and time points were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc tests when appropriate. A significance threshold of $\alpha = 0.05$ was adopted.

Chapter 3 Results and Discussion

Antibacterial Effects of Calamansi Extract on Streptococcus mutans

Across all treatment groups, calamansi (Citrofortunella microcarpa) juice extract demonstrated a dose-dependent inhibitory effect on Streptococcus mutans growth on both metal and ceramic orthodontic brackets.

At Day 1, the 100% calamansi extract produced the lowest bacterial counts on both ceramic (1.25 ± 0.07) and metal brackets (1.15 ± 0.07). As days progressed (Day 3 and Day 7), CFU counts increased in all groups, indicating reduced antibacterial activity over time, but the 100% concentration consistently remained most inhibitory, followed by 50% and then 25%. Control groups (distilled water) exhibited the highest bacterial counts at all time points.

These results suggest a clear dose-dependent and time-dependent antibacterial trend: higher extract concentration yields stronger inhibition, and antibacterial effectiveness declines with sustained bacterial exposure.

Bracket Material Effects

Although independent t-tests revealed no statistically significant differences ($p > 0.05$) between ceramic and metal brackets at each extract concentration and time point, mean values consistently showed lower bacterial adhesion on metal brackets. Metal surfaces appeared less conducive to bacterial retention than ceramic, aligning with prior evidence that smoother, less porous materials reduce microbial attachment.

This finding supports existing literature indicating that surface characteristics influence microbial adhesion, with ceramic brackets offering more micro-irregularities that may trap bacteria and enhance biofilm formation.

Statistical Findings: -

One-way ANOVA demonstrated:

- Only the 100% calamansi extract yielded significant differences across time points for both metal and ceramic brackets ($p < 0.05$).
- 50% and 25% concentrations did not demonstrate significant changes across days, reflecting weaker antibacterial action.

These results confirm that the strongest inhibitory effect occurs early with full-strength extract, while lower concentrations lack sufficient antimicrobial potency to drive significant temporal changes.

Mechanistic Interpretation and Comparison with Literature

Calamansi contains bioactive compounds (e.g., terpenoids such as d- limonene and flavonoids) that disrupt bacterial cell membranes, leading to reduced viability. Previous studies have shown moderate to strong antibacterial effects of calamansi essential oils against oral pathogens, including S. mutans. Other citrus extracts also exhibit inhibitory effects on Gram-positive oral bacteria, attributed to similar phytochemical profiles.

Although the material type did not yield statistically significant differences, the consistent directional trend of lower bacterial counts on metal brackets aligns with established findings that surface free energy and roughness influence biofilm formation.

Summary: -

- 100% calamansi extract showed the strongest antibacterial activity, especially early (Day 1).
- Lower concentrations (50%, 25%) showed reduced and inconsistent inhibition.
- Metal brackets tended to have lower bacterial counts than ceramic, though not statistically significant.
- The antibacterial effect of calamansi extract is dose-dependent and

decreases over time

Overall, the findings support the potential of calamansi extract as a natural antimicrobial agent with application in reducing bacterial accumulation during orthodontic treatment.

Chapter 4

Summary, Conclusions, and Recommendations Summary of Findings
This study evaluated the effect of calamansi juice extract on S. mutans growth on metal and ceramic orthodontic brackets at concentrations of 100%, 50%, and 25%, compared against distilled-water controls. Key results include:

Metal Brackets: -

- 100% extract demonstrated the strongest antibacterial action with the lowest CFU counts.
- 50% extract showed moderate inhibition.
- 25% extract had minimal effect, approaching control levels.
- Control groups showed robust bacterial growth.
- Metal brackets generally exhibited lower bacterial adhesion than ceramic.
-

Ceramic Brackets: -

- 100% extract showed effective inhibition, but less than on metal.
- 50% extract produced moderate inhibition, less consistent than on metal.
- 25% extract showed negligible inhibition.
- Controls allowed substantial bacterial colonization.
- Ceramic brackets consistently showed higher bacterial retention.

Across Time and Concentrations: -

- Only 100% extract demonstrated statistically significant changes across days, with strong early inhibition that weakened over time.
- 50% and 25% did not show significant changes across observation points.
- Numerical trends suggested lower adhesion on metal brackets at all concentrations and time points.

Conclusions: -

1. 100% calamansi extract was the most effective concentration in inhibiting S. mutans growth on both metal and ceramic brackets.
2. Metal brackets tended to show lower bacterial adhesion than ceramic brackets, likely due to smoother surface characteristics.
3. The antibacterial activity of calamansi extract declined over time, with the strongest effect observed at Day 1.
4. The optimal inhibitory concentration was 100% calamansi extract, with reduced effectiveness seen at 50% and minimal at 25%.
5. Calamansi extract presents potential as a natural, cost-effective antimicrobial agent in orthodontic hygiene, though its sustained efficacy may require enhanced formulation or delivery.

Recommendations: -

Based on these findings, the following are recommended:

1. Develop calamansi-based oral hygiene products (e.g., rinses, gels, varnishes) for use as adjuncts in orthodontic care to reduce S. mutans levels and prevent white spot lesions.
2. Compare calamansi extract with standard antimicrobial agents such as chlorhexidine to assess relative clinical effectiveness.
3. Expand research to other cariogenic bacteria (e.g., Lactobacillus spp.) to explore broader caries-prevention applications.
4. Conduct in vivo/clinical trials to evaluate long-term efficacy, safety, and patient acceptability.
5. Investigate interactions with additional orthodontic materials (e.g., elastomeric modules, bonding adhesives) to understand broader applicability in clinical settings.

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