HIGH SCHOOL EDITION
Journal of Student Research
Volume 13 Issue 4 (2024)

OraGel: Delivery of Chlorhexidine Digluconate Through a Hydrogel-Based Powder to Treat Gum Disease

Jamie Cheng[1] and Jiaming Liu[#]

[1]Green Level High School, USA
[#]Advisor

ABSTRACT

Gum disease, affecting over 90% of the global population, leads to serious health complications, including tooth loss and increased risk of other severe diseases. Despite its prevalence, effective and affordable treatments for gum disease are scarce, with current options limited to costly medical procedures and ineffective dental care products. This study introduces OraGel, an in-situ gelling powdered spray formulated with chlorhexidine digluconate. Chlorhexidine digluconate, known for its antiseptic and antibacterial properties in certain mouthwashes, is hypothesized to be more effective in this application, where it is released gradually for sustained drug release and prolonged therapeutic effects. The research assessed OraGel's antibacterial efficacy and stability through bacterial transformation, zone of inhibition testing, live/dead bacteria assays, and stability assays. The results demonstrated that OraGel effectively eliminated over 99.8% of bacteria, exhibiting significant inhibition of bacterial growth compared to control samples. Furthermore, OraGel's novel in-situ gel formation allows for targeted application and enhanced retention at the site of infection, increasing treatment effectiveness. The optimized properties of the gel ensure maximum coverage and adherence to gum tissue, crucial for treating periodontal disease. Additionally, the OraGel hydrogel exhibited strong adhesion to gum tissue even after extensive rinsing, highlighting its stability and sustainability. The sustained antibacterial ability of OraGel confirms its potential as an effective treatment for gum disease. Furthermore, its biodegradable nature, easy application, and affordability characterize OraGel as a promising over-the-counter solution for wider consumer accessibility. Future research directions include in-vivo experimentation and exploration of other antibacterial agents in in-situ gelling powders.

Introduction

Periodontal disease, a significant global health concern, affects a substantial portion of the population, involving various conditions ranging from mild gingivitis to severe periodontitis. Gingivitis, a common form of periodontal disease, affects more than 90% of the population worldwide[1]. It is projected that around 35% of adults in the U.S., aged between 30 and 90, suffer from periodontitis. Out of this, 21.8% experience a mild form of the disease, while 12.6% endure a moderately severe form. Notably, both the prevalence and severity of attachment loss, as well as the overall occurrence of periodontitis, significantly increase with advancing age[2]. Periodontal disease is characterized by gingival recession and gum inflammation caused by oral bacterial overgrowth. In addition to diminishing the appearance of the teeth, periodontal disease involves serious health issues including tooth decay and tooth loss[3]. Furthermore, periodontal disease is directly correlated with other diseases such as heart disease, respiratory disease, diabetes, and stroke[4]. It increases the risk of cardiovascular disease by 19%, a figure that rises to 44% in individuals over 65. In type 2 diabetics with severe periodontal disease, the mortality risk is 3.2 times higher than in those with mild or no periodontitis5. Periodontal therapy has been

found effective in improving glycemic control in these patients[5]. Additionally, periodontitis is linked to maternal health issues like preterm birth and preeclampsia5.

There are four stages of periodontal disease. Stage I, following the onset of gingivitis, is the initial phase where minor attachment loss and less than 15% radiographic bone loss occur6. This stage is critical for early intervention to prevent further progression. Stage II is identified as moderate periodontitis, where there is increased attachment loss of 1-2 mm and radiographic bone loss of 15-33%[6]. This stage still allows for disease management through professional treatment. Stage III represents a more advanced form of the disease, with clinical attachment loss becoming more pronounced, often extending to the mid-third of the root and beyond6. In this stage, the risk of additional tooth loss becomes a significant concern. Stage IV, the most severe form of periodontitis, exhibits similar attachment loss to Stage III but is compounded by the need for extensive dental rehabilitation[6]. In this stage, the patient often faces disabled masticatory function and severe occlusal trauma, necessitating a comprehensive treatment approach. Periodontal disease can be caused by a variety of risk factors including smoking, poor oral hygiene, diabetes, certain medications, age, genetics, and stress[7]. Smokers experience more severe periodontal diseases, characterized by greater bone attachment loss, tooth loss, gingival recession, and pocket formation compared to non-smokers, with the severity increasing with the number of cigarettes smoked daily[8]. Poor oral hygiene, often resulting from improper tooth brushing techniques, lack of interdental cleaning, and infrequent dental visits, leads to the accumulation of dental plaque and calculus, causing gingival inflammation and potentially progressing to periodontitis[9].

Current treatment for periodontal disease includes costly medical procedures and ineffective dental self-care products. Not only are medical procedures expensive, but they also require follow-up appointments which may be inconvenient and time-consuming. The most common treatment, scaling and root planing, costs $200-$300, excluding additional visits and procedures[10]. Other treatment methods include local drug delivery systems (LDDS) such as fibers, gels, strips and films[11] Fibers, though effective in delivering antimicrobials, require skilled placement and often a follow-up visit for removal if non-biodegradable, which can be inconvenient and uncomfortable for patients[11]. Gels, while easy to apply, may not sustain drug concentrations effectively in the periodontal pocket due to dilution by gingival crevicular fluid[11]. Strips and films, although easily adaptable to pocket dimensions, face challenges in maintaining effective drug levels over extended periods and can sometimes provoke an inflammatory response if remnants are left in the pocket[11]. These limitations highlight the need for a more efficient delivery system, leading to the development of OraGel, an in-situ gelling system. OraGel overcomes these drawbacks by providing a sustained, targeted drug release directly at the site of infection, minimizing side effects and enhancing treatment efficacy.

In-situ gelling drug delivery systems represent a revolutionary approach in the administration of therapeutic agents. These systems exist in a sol state before being introduced into the body but transform into a gel once inside, due to various stimuli such as temperature change, pH alteration, ion activation, or ultraviolet irradiation[12]. Polymers that induce in-situ gelation include carbopol 934P, chitosan, sodium carboxymethyl cellulose, hydroxypropyl methylcellulose (HPMC), hydroxypropyl cellulose, and methyl cellulose[13]. We used tannic acid which is a polyphenol and a natural tannin characterized by a core glucose unit with ten attached gallic acid molecules. This compound can be derived from various plant sources, including both herbaceous and woody varieties[14]. Tannic acid can form hydrogen bonds and hydrophobic interactions with a variety of polymers, thus inducing in-situ gelation[15]. Not only does tannic acid serve as an in-situ gelling system, but it also has antiviral and antibacterial properties[14]. OraGel involves a powder containing tannic acid that forms a protective film-like hydrogel once sprayed onto the gum pocket surface and fused with saliva. This hydrogel slowly releases the drug chlorhexidine digluconate throughout the gum surface, achieving sustained antibacterial ability.

The formation of such gels, which can be composed of natural or synthetic polymers like gellan gum, alginic acid, and poly(DL-lactic acid), ensures a controlled and sustained release of the encapsulated drug, making them highly effective for targeted treatment[16]. The process of gelation in these systems is influenced by

a range of factors including the presence of specific ions or molecules, with natural polymers responding to environmental conditions like body temperature or calcium ion presence to initiate gelation16. This transformation from a liquid to a gel state is key to the efficacy of in-situ gelling systems, as it allows for a prolonged and localized drug release at the site of application. Moreover, these systems offer several advantages over traditional formulations. Their ability to provide sustained drug release reduces the frequency of administration, enhances the convenience of application, and improves patient compliance. In addition, in-situ gels protect the drug from environmental changes, ensuring stability and effectiveness[17].

In-situ gelling systems can be administered via various routes, including oral, ocular, and intraperitoneal, making them versatile for different therapeutic needs[18]. In-situ gelling systems have emerged as a significant advancement in targeting periodontal disease. These systems, utilizing hydrogels in various forms such as films, micro-/nanoparticles, and implants, can effectively deliver drugs directly to the affected areas. By incorporating biopolymers, particularly polysaccharides and their derivatives, these systems ensure the targeted and controlled release of biologically active compounds, offering a promising alternative to traditional periodontal disease treatments[19]. Considering these advantages, in-situ gelling systems like OraGel present a more effective alternative for drug delivery, especially in treating localized conditions such as periodontal disease. Their ability to form gels in situ ensures that the drug remains concentrated at the desired site, providing effective treatment while minimizing systemic exposure and side effects.

Chlorhexidine digluconate is a key agent in periodontal disease management due to its extensive antimicrobial action. Structurally, it consists of four chlorophenyl rings and two biguanide groups linked by a hexamethylene bridge, which impart strong basic properties with pH levels above 3.5 and positive charges at both ends of the bridge[20]. This unique structure enables chlorhexidine digluconate to disrupt bacterial cell membranes, increasing permeability and resulting in cell lysis. Its wide spectrum of activity encompasses gram-positive and gram-negative bacteria, yeasts, dermatophytes, and some lipophilic viruses, making it highly effective against the microbial species involved in periodontal disease, such as Staphylococcus aureus[21]. Chlorhexidine digluconate is used in several dental applications, including mouthwashes, gels, and slow-release forms like chips. It is effective in reducing plaque and gingival inflammation, crucial in periodontal disease management[22]. However, its use is not without adverse effects, such as dry mouth, altered taste sensations, and tooth staining, which can occur with extended use. Tooth staining is particularly common and is attributed to non-enzymatic browning and pigmented metal sulfide formation on the teeth[23].

Despite these side effects, chlorhexidine digluconate remains a valuable tool in periodontal therapy. Its anti-plaque properties have been shown to significantly reduce the microbial flora associated with periodontal diseases, effectively managing oral hygiene and controlling disease progression[24]. Concentrations between 0.12% and 0.2% are typically used for maximal effectiveness with minimal side effects[25]. Additionally, chlorhexidine digluconate is known to have substantive properties, maintaining its presence and activity in the oral cavity for extended periods, which is particularly beneficial in managing chronic periodontal conditions[26]. In treating periodontal disease, chlorhexidine digluconate can be used as an adjunct to mechanical therapies, offering significant benefits in managing periodontal pathology[22]. Its effectiveness in delivering high concentrations of antimicrobial agents directly to the site of infection allows for a considerable reduction in plaque and inflammation while minimizing systemic uptake of the medication[27]. OraGel leverages the antimicrobial properties of chlorhexidine digluconate through an advanced in situ gelling system. This novel delivery method enables the powdered spray to transform into a hydrogel upon contact with saliva in the oral cavity. This reaction ensures a targeted, sustained release of chlorhexidine digluconate directly at the site of periodontal infection, offering effective treatment while minimizing systemic exposure and side effects[25]. This approach represents a significant advancement in the management of periodontal disease, making OraGel a promising option for localized treatment.

In this study, we investigated the effectiveness of OraGel, an innovative in-situ gelling system utilizing chlorhexidine digluconate, as a novel approach to managing periodontal disease. We hypothesized that OraGel

would demonstrate sustained and stable antibacterial ability by inhibiting oral bacterial growth and forming stable bonds with gum tissue. We tested OraGel's efficacy against Staphylococcus aureus, a common bacteria in the oral environment, through bacterial transformation tests, zone of inhibition assays, and live/dead bacteria assays. The results substantiated our hypothesis, demonstrating that OraGel effectively inhibited bacterial growth, as indicated by its potent bacterial transformation, significant zone of inhibition, and the results of live/dead bacteria assays. Notably, OraGel was shown to kill over 99.8% of bacteria, confirming its robust antibacterial properties. Furthermore, the study revealed OraGel's high stability and sustainability, maintaining its hydrogel consistency and adherence to gum tissue even after rinsing. Our study highlighted OraGel's exceptional efficacy in providing sustained antibacterial action and its potential as a convenient, affordable, and accessible product for effective periodontal disease treatment.

Methods

Preparation of Microparticles

To prepare microparticles, a combination of 250mL corn oil and 0.25mL Tween-80 was first placed in a flask and heated in a 55℃ water bath. Next, a solution containing 10 mL water, 1.5 mL acrylic acid, and 0.5g gelatin was prepared in a separate flask and also heated to 55℃. To this second solution, 50mg of acrylic acid NHS and 50mg chlorhexidine digluconate were added, with the aid of an ultrasonic cleaner for thorough mixing. This solution was then combined with the first solution, maintained at 55℃ for 20 minutes, and subsequently transferred to an ice bath for a two-hour cooling and stirring process. The next step involved filtration using a funnel, 0.45 μm nylon filter paper, a flask with a side tube, and cold acetone. 250mL of acetone was added to the solution, stirred, and allowed to separate. The upper layer was then vacuum filtered and washed with acetone, and the resulting product was transferred using tweezers to a Petri dish for drying and grinding. 5mg of these chlorhexidine digluconate microspheres were then combined with 10mg of tannic acid, establishing a 2:1 ratio, and filled into a precision needle-tip spray bottle, finalizing the preparation of the hydrogel microparticles.

Preparation of Bacteria Cultures

In a sterile laboratory hood, a vial of 106 Staphylococcus aureus was opened, and 1 mL of LB broth was added using a serological pipette controller to create a bacteria suspension. This suspension was then carefully transferred into a clean tube. To promote consistent growth of the bacteria, the tube containing the suspension was placed in a shaker set at 200 rpm and incubated at 37°C overnight. To prepare the solutions for the bacterial transformation experiment, 5mg of chlorhexidine digluconate powder was combined with 10mg of tannic acid. This mixture (CD + TA) was then transferred into a microcentrifuge tube. To this, 1 mL of phosphate-buffered saline (PBS) was added, ensuring thorough shaking using a vortex to prevent hydrogel formation. Subsequently, 99μl of a 109 Staphylococcus aureus bacteria solution was added to the tube, followed by vigorous shaking for uniform mixing. A control solution was also prepared, consisting of 1mL of PBS and 99 μl of the 109 bacteria solution, in a separate tube. Both tubes were then incubated for 30 minutes as part of the preparation for the bacterial transformation experiment.

Bacteria Transformation

For the bacterial transformation experiment, an alcohol burner was used to sterilize the bacterial cell spreader, which was then allowed to cool for three minutes. Once cooled, 100 μl of the prepared chlorhexidine digluconate and tannic acid (CD+TA) solution was added to each of the three designated culture dishes. Similarly,

the control solution was applied to another set of three separate culture dishes. The bacterial cell spreader was employed to evenly distribute the solutions across each dish, ensuring sterilization of the spreader between each application to prevent cross-contamination. The dishes were then flipped upside down and incubated at 37°C for varying time periods of 0.5, 1, and 2 hours. After each specified incubation period, the dishes were examined and bacterial quantifications were counted using the Promega Colony Counter App to assess the bacterial growth and the effectiveness of the OraGel treatment. Pictures of the dishes were taken and charts were constructed for data purposes.

Zone of Inhibition Test

In the zone of inhibition experiment, the control solution containing 1 mL of phosphate-buffered saline (PBS) and 99 µl of a 109 Staphylococcus aureus bacterial solution was evenly spread across a culture dish using a bacterial cell spreader. The dish was then divided into two distinct sections using a marker. For the OraGel test, a microcentrifuge tube was filled with 1 mL of PBS, and a small amount of the CD+TA powder was gently sprinkled in the tube to form a thin coat to just cover the surface. After waiting for 30 seconds, a hydrogel film formed, which was carefully transferred using tweezers to one section of the culture dish. Similarly, for the control, another microcentrifuge tube was filled with 1 mL of PBS, and tannic acid was added to the surface. After 30 seconds, the hydrogel film formed and was placed on the other section of the dish. Each section of the plate was labeled, and the culture dish was then incubated for 0.5 hour. Subsequently, at intervals of 0.5 hour, 1 hour, and 2 hours, the dish was removed for photography to aid in later examination. Following the completion of all incubation times, the photographs were analyzed to determine the size of the zone of inhibition. This analysis compared the section treated with OraGel hydrogel versus the control hydrogel, providing insights into the effectiveness of the OraGel treatment in inhibiting bacterial growth.

Live/Dead Cell Assay

A tube of the CD+TA solution, consisting of 15mg of the CD+TA, 1mL of PBS, and 99µl of 109 Staphylococcus aureus bacteria, and a tube of the control solution, consisting of 1mL of PBS and 99µl of the 109 Staphylococcus aureus bacteria, at 37℃ were incubated for varying incubation times of 0.5, 1, and 2 hours. After each incubation period, 1mL of the CD+TA was added into one well of a 6-well culture plate and 1mL of the control was added into another well. This resulted in wells designated as CD+TA 0.5h, control 0.5h, CD+TA 1h, control 1h, CD+TA 2h, and control 2h. Following the final incubation period, 10 µL of propidium iodide and 10 µL of SYTO 9 stains were added to each well. These specific amounts were chosen to ensure optimal staining while avoiding excess fluorescence that could obscure the results. The plate was then incubated at room temperature in the dark for 15 minutes, allowing the stains to interact with the bacterial cells. After the staining period, the solutions in each well were gently mixed to ensure an even distribution of the stains. Samples from each well were then transferred onto glass slides using a pipette, with each sample carefully spread out and covered with a coverslip. The prepared slides were subsequently examined under an ECHO microscope, using appropriate filters to detect the green fluorescence of live bacteria (stained by SYTO 9) and the red fluorescence of dead bacteria (stained by propidium iodide). This method allowed for a clear differentiation between live and dead cells, providing a visual representation of OraGel's antibacterial efficacy.

Stability Assay

For the stability assay of OraGel, different ratios of tannic acid (TA) to chlorhexidine digluconate (CD) microspheres were tested to determine their stability in a hydrogel form. The ratios used were 1:8, 1:4, 1:2, 1:1, 2:1, and 4:1, corresponding to mixtures of 0.625mg TA with 5mg CD, 1.25mg TA with 5mg CD, 2.5mg TA with

5mg CD, 5mg TA with 5mg CD, 10mg TA with 5mg CD, and 20mg TA with 5mg CD, respectively. Each mixture was placed into a separate well of a 6-well plate containing 1mL of PBS. The powders were gently poured over the surface of the PBS to form a light coat, triggering the formation of a hydrogel in each well. Photographs were taken at intervals of 0h, 1h, 3h, 6h, and 24h to assess the physical appearance and stability of the hydrogels. These photographs provided visual evidence to evaluate the stability and consistency of the hydrogels over time, crucial for determining the most effective ratio for OraGel's sustained application in periodontal treatment.

Tooth Model Experiment

For the tooth model experiment, a needle-tip spray bottle was filled with the CD+TA powder. This powder was then sprayed onto a gum pocket of a teeth model, simulating the application of OraGel in a real-world scenario. To mimic the interaction with saliva, a mixture of 10μL of Rhodamine B fluorescent dye and 50mL of water was prepared and sprayed onto the gum pocket, covering the previously applied powder. The interaction between the powder and the dye mixture resulted in the formation of a hydrogel. To assess the stability of the hydrogel, the gum pocket was rinsed several times with water using a pipet, washing out all of the fluorescent dye. The persistence of the hydrogel on the gum pocket was then examined under a UV flashlight. If the hydrogel remained intact, it would exhibit a bright orange fluorescence under UV light. This fluorescence would indicate that the hydrogel retained the fluorescent dye water due to hydrogen bonding during the in-situ gelation process. The visibility of the hydrogel after rinsing determined the stability of the hydrogel and its adherence to the gum tissue. A visual representation of the methodology can be found in Figure 1.

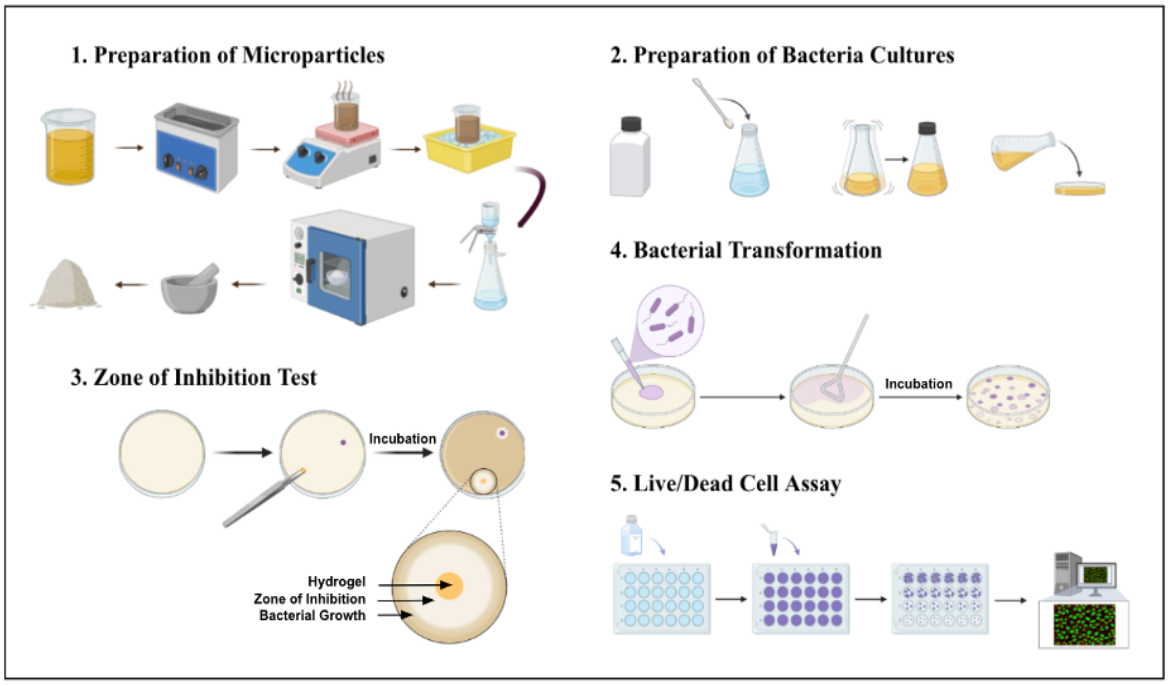

Figure 1. Methodology of OraGel. 1) Preparation of Microparticles. 2) Preparation of Bacteria Cultures. 3) Zone of Inhibition Test. D) Bacterial Transformation. E) Live/Dead Cell Assay.

Results

Bacterial Transformation Assay

In our study, the bacterial transformation experiment was designed to test OraGel's antibacterial effectiveness against Staphylococcus aureus. This bacterium was chosen due to its prevalence as a common oral pathogen. The experimental setup involved two distinctive solutions - one comprising a mixed powder of chlorhexidine digluconate microspheres and tannic acid, while the other served as a control. After incubation, these solutions were applied to culture dishes with meticulous spreading to ensure even distribution. The plates were then incubated for various durations which were 0.5 hour, 1 hour, and 2 hours. Upon examination of the culture plates after each duration, a markedcontrast was observed between the control and OraGel-treated plates. The control plates exhibited rapid bacterial growth, whereas the OraGel-treated plates exhibited a significant reduction in bacterial colonies. We observed a stark difference between the control plates and those treated with OraGel (Figure 2A). The control plates had vast clusters of dots which represent bacteria colonies, while the OraGel-treated plates were almost completely clear with the exception of 1 dot, indicating OraGel's strong antibacterial capability.The quantifications of bacterial colonies for various incubation times with and without OraGel treatment revealed a consistent trend where OraGel effectively reduced bacterial populations almost completely compared to the control (Table 1). The percentages of bacteria killed by OraGel for different treatment durations confirmed its potent bactericidal property (Figure 2B). On average, OraGel was found to kill more than 99.8% of bacteria, highlighting its potential as an effective antimicrobial agent.

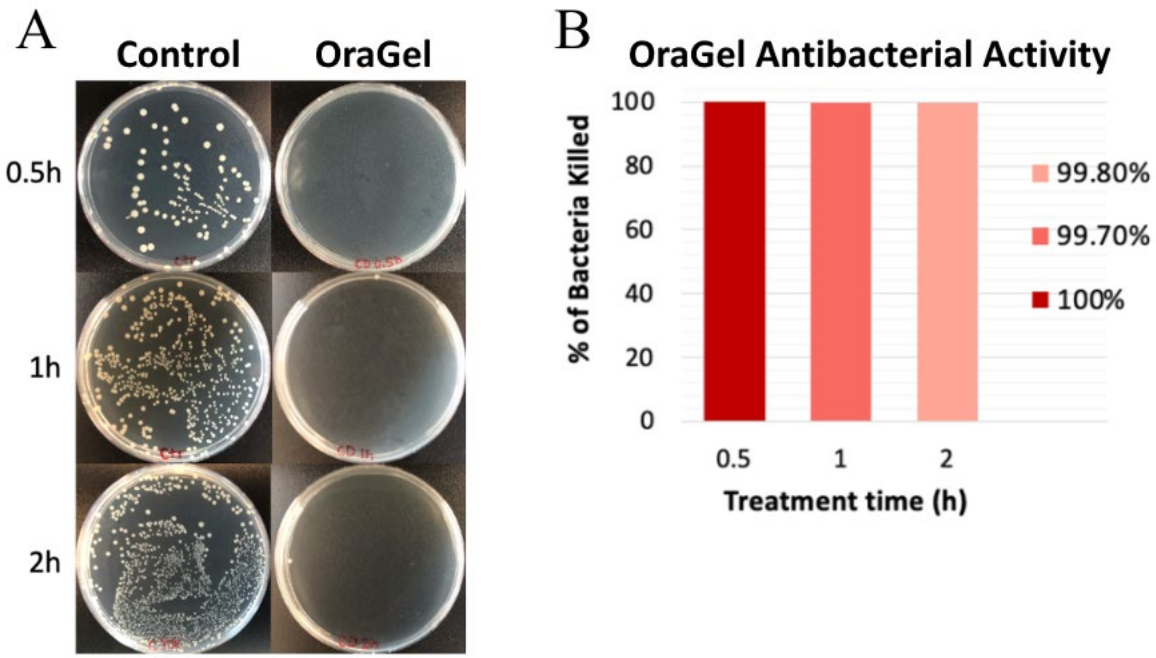

Figure 2. Bacterial transformation results. (A) Visible bacterial colonies after OraGel treatment/control. (B) Percentages of bacteria killed by OraGel.

Table 1. Quantifications of bacterial colonies after OraGel treatment/control.

# of Bacteria	Control	OraGel
0.5h	94	0
1h	352	1
2h	588	1

Zone of Inhibition Test

Further delving into OraGel's antibacterial ability, we conducted a zone of inhibition test. This experiment was crucial to visualize the extent of OraGel's impact on bacterial spread. The OraGel hydrogel, consisting of chlorhexidine digluconate microspheres and tannic acid, was applied to bacterial culture plates. We also applied a control hydrogel, which contained raw microspheres and tannic acid. The test involved applying the two hydrogels on bacterial culture plates and observing the area around the application site after various incubation times which were 0.5 hour, 1, hour, and 2 hours. The clear, circular area around the application site represents the zone of inhibition. OraGel-treated areas displayed a significantly larger zone of inhibition compared to the control (Figure 3). Although the control hydrogel exhibited weak bacterial inhibition due to tannic acid's minimal antibacterial ability, it significantly contrasted with the expansive antibacterial reach of OraGel. This phenomenon illustrates OraGel's not just bactericidal but also bacteriostatic capabilities, effectively containing the spread of harmful oral bacteria.

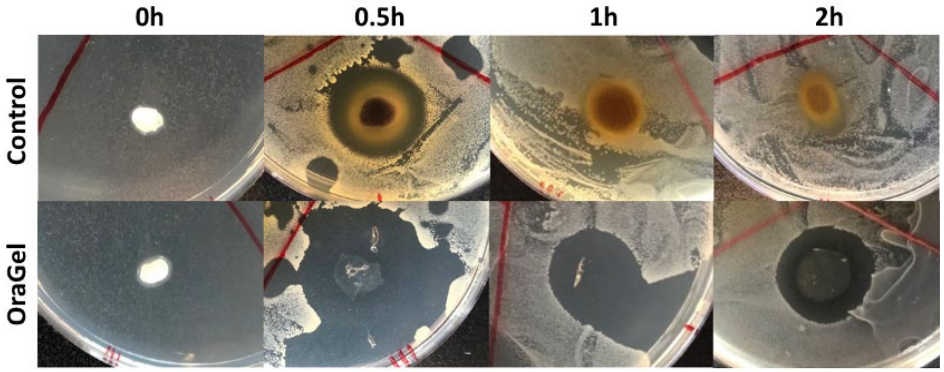

Figure 3. Zones of bacterial inhibition after different incubation times of OraGel treatment or control.

Live/Dead Bacteria Assay

The live/dead bacteria assay provided a deeper understanding of OraGel's bactericidal capability. This assay employed a dual staining technique using SYTO 9 and propidium iodide, allowing for the differentiation of live (green fluorescence) and dead (red fluorescence) bacterial cells. This color-coded system provided a clear visual representation of OraGel's effectiveness. Samples treated with OraGel primarily showed red fluorescence, indicating a predominance of dead bacterial cells. In contrast, the control samples, which were not treated with OraGel, predominantly exhibited green fluorescence, signifying a larger population of live bacterial cells. These observations were quantitatively supported by the data collected, which highlighted OraGel's ability not just to

inhibit but to actively kill bacterial cells, further emphasizing its potential as an effective antibacterial agent. Overlay images of bacterial culture wells after different incubation times of OraGel treatment were compared with the control (Figure 4). The control samples predominantly displayed live bacteria cells, whereas the OraGel-treated samples displayed predominantly dead bacteria cells, demonstrating OraGel's ability to effectively kill bacteria.

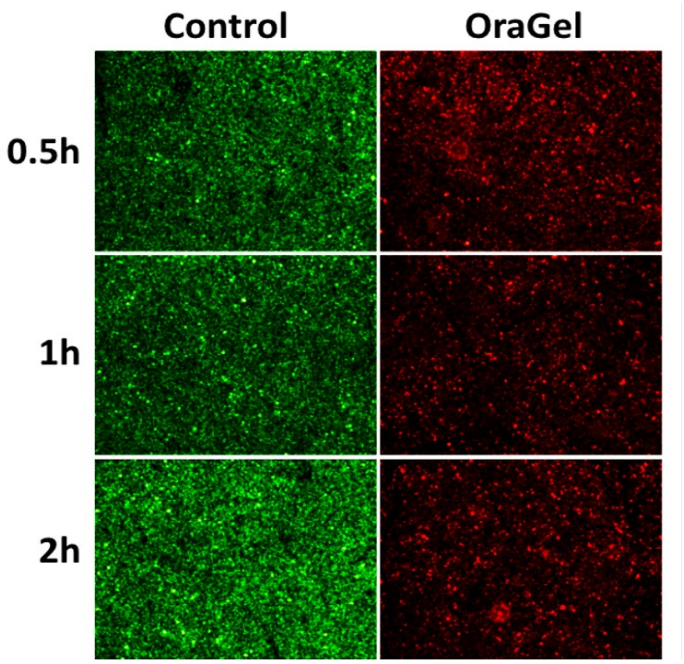

Figure 4. Overlay images of bacterial culture wells from live/dead bacteria assay.

Stability Assay of OraGel

The stability of OraGel was a critical aspect of its effectiveness as a periodontal treatment. To assess this, various ratios of tannic acid to chlorhexidine digluconate microspheres were tested to determine the optimal composition for the hydrogel. The ratio that stood out was 2:1, which consistently produced a thick, cohesive hydrogel without any observable disintegration or spaces (Figure 5). This ratio was key in maintaining the hydrogel's structural integrity and ensuring its sustained presence on the gum tissue. The resilience and stability of OraGel's hydrogel, as demonstrated in the assay, are indicative of its potential as a reliable and effective treatment for periodontal disease, capable of withstanding the dynamic conditions of the oral cavity.

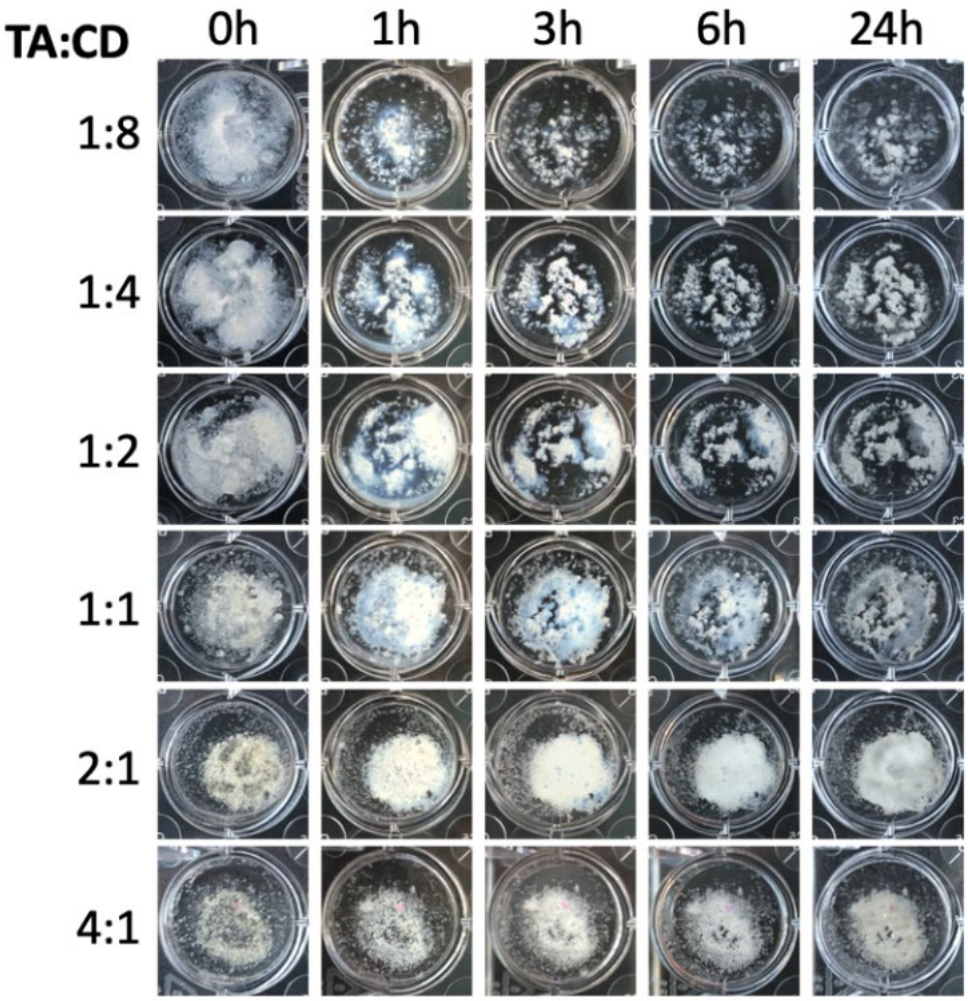

Figure 5. Stability levels of hydrogels formed by in-situ gelation, with different ratios of tannic acid to chlorhexidine digluconate microspheres.

OraGel Delivery in a Tooth Model

To further assess the stability of the OraGel hydrogel, we conducted an experiment using a teeth model that mimicked a gum pocket. This simulation was crucial to replicate the oral environment where OraGel would be used. The hydrogel's stability was remarkable even after undergoing rigorous rinsing and washing processes, which are common in oral hygiene practices. This was indicative of OraGel's ability to remain effective and intact within the oral environment. OraGel powder was sprayed onto a gum pocket in a tooth model, followed by the addition of a mixture of fluorescent dye and water that simulated saliva, resulting in the formation of an antibacterial hydrogel film (Figure 6A-B). Post-rinsing, the hydrogel remained attached to the gum pocket under UV light, demonstrating OraGel's resilience and sustainability, critical for its effectiveness in oral healthcare (Figure 6C-D).

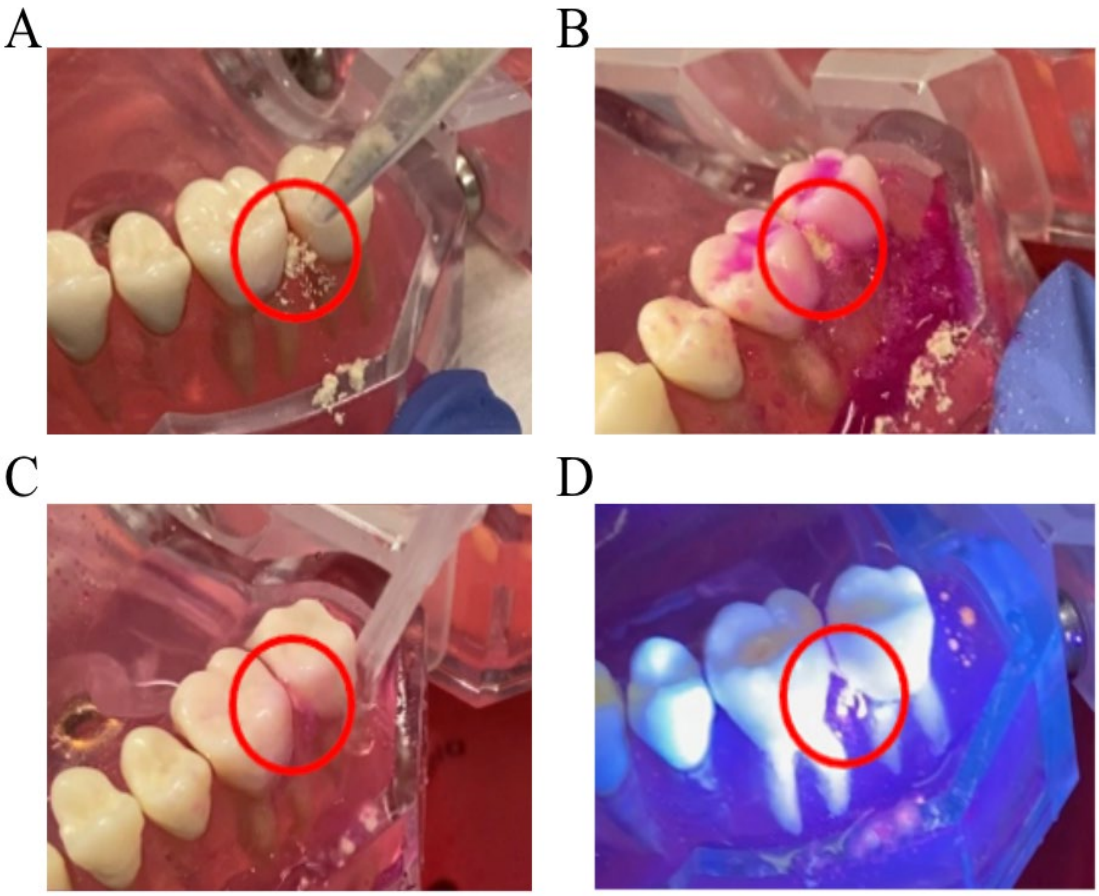

Figure 6. OraGel delivery in a tooth model. (A) Powder spraying. (B) Fluorescent dye water spraying. (C) Rinsing with clean water. (D) UV lighting.

Discussion

Our study delved into evaluating the efficacy of OraGel, a novel in-situ gelling system, as a targeted treatment for periodontal disease. The experimental results demonstrated OraGel's potent antibacterial properties. Specifically, the bacterial transformation results revealed a significant reduction in bacterial growth when treated with OraGel, as shown by the rapid bacterial inhibition compared to the control group. This was further quantified, showing an average bacterial kill rate of over 99.8%. Additionally, the zone of inhibition results highlighted OraGel's strong antibacterial ability, where the OraGel hydrogel exhibited a significantly larger area of inhibition compared to the control hydrogel. The live/dead bacteria assay corroborated these findings, with a marked increase in dead bacterial cells in the OraGel-treated samples. The stability assay illustrated OraGel's resilience and effectiveness in the oral environment, maintaining its structural integrity and efficacy even after exposure to conditions simulating oral hygiene practices.

The significance and impact of the results are considerable, particularly in advancing the understanding and treatment of periodontal disease. OraGel, an in-situ gelling hydrogel incorporated with chlorhexidine digluconate, demonstrated remarkable efficacy in inhibiting bacterial growth and eradicating bacterial cells, specifically targeting common oral pathogens like Staphylococcus aureus. The hypothesis was supported by the

results which revealed OraGel's capabilities to effectively inhibit bacterial growth while maintaining high stability by staying attached to the gum tissue of the tooth model. The incorporation of chlorhexidine digluconate and tannic acid into a hydrogel that forms upon contact with a saliva-like solution is a significant advancement in localized drug delivery for oral health. The ability of OraGel to deliver chlorhexidine digluconate directly to the site of infection, coupled with its sustained release mechanism, represents a significant advancement in local drug delivery technologies for periodontal therapy. This study's findings, therefore, represent a significant stride in periodontal disease treatment, offering insights that could lead to more efficient and accessible treatments in the future of oral healthcare.

Conclusion

In conclusion, our research on OraGel presents promising implications for the management of periodontal disease. We reached the result that OraGel's strong antibacterial ability and hydrogel stability verify its sustained antibacterial ability to treat periodontal disease. While our results are compelling, they represent a preliminary step in understanding the full potential of in-situ gelling systems in periodontal therapy. The continued exploration of this technology could ultimately lead to more effective, less invasive treatments for periodontal disease, contributing significantly to the field of oral healthcare. By demonstrating the efficacy of an in-situ gelling system in targeting and eliminating bacterial growth, our findings suggested that OraGel could offer a more effective and user-friendly alternative to traditional periodontal treatments, empowering individuals to take control of their oral health. Moreover, OraGel's biodegradable nature, ease of application, and affordability as an over-the-counter product greatly enhance its practicality and accessibility. This positions OraGel not just as a clinical innovation but also as a user-centric solution that addresses the affordability and usability concerns often associated with periodontal care, emphasizing the profound impact of our study in the broader context of oral health management.

For future research, the progression from in vitro to in vivo experiments will be critical in establishing the real-world efficacy and safety of OraGel. This transition to animal models and eventually human trials will provide valuable insights into the systemic effects, long-term implications, and overall patient response to the treatment. Additionally, experimenting with other drugs through in-situ gelling powders could broaden the scope of applications, potentially catering to a wider range of oral health issues. A refinement in the microsphere technology, particularly focusing on minimizing the size of the OraGel microspheres, would improve its ability to permeate deeper into gum pockets, thereby increasing its therapeutic reach. Finally, conducting clinical trials with human participants would be a pivotal step in validating OraGel's effectiveness and safety in a clinical setting, setting the stage for its introduction as a mainstream periodontal treatment option.

Limitations

Several factors could have influenced our results. The choice of Staphylococcus aureus as the bacterial model, while relevant due to its prevalence in oral environments, might limit the generalizability of our findings to other oral pathogens. Future studies could expand on this by including a wider range of bacteria commonly associated with periodontal disease. Additionally, the in vitro nature of our experiments, though insightful, cannot fully replicate the complex dynamics of the oral microbiome and its interaction with periodontal tissues in vivo. Therefore, caution must be exercised in extrapolating these findings to clinical scenarios. Lastly, a notable limitation encountered in our study during the bacterial transformation experiments, where there was a possible uneven distribution of the OraGel solution on the culture plates. There were instances where the solution may not have been spread thoroughly, as shown by the presence of an isolated bacterial colony at the edge of the plate which was observed in the OraGel-treated plates for the 1-hour and 2-hour incubation periods.

These isolated occurrences of bacterial growth suggest that the application method could be further refined to ensure a more uniform distribution of the OraGel solution across the entire surface of the culture plates.

Acknowledgments

I would like to thank my advisor for providing mentorship, guidance, and encouragement as well as North Carolina State University for providing the research environment and resources. I would also like to thank my teacher for her guidance and for providing a platform for student research. Lastly, I would like to thank my mom, who has gum disease, for inspiring me to conduct this research.

References

1. Eke, Paul I et al. “Recent epidemiologic trends in periodontitis in the USA.” Periodontology 2000 vol. 82,1 (2020): 257-267. doi:10.1111/prd.12323
2. Albandar, J M et al. “Destructive periodontal disease in adults 30 years of age and older in the United States, 1988-1994.” Journal of periodontology vol. 70,1 (1999): 13-29. doi:10.1902/jop.1999.70.1.13
3. Takedachi, Masahide et al. “Evaluation of periodontitis-related tooth loss according to the new 2018 classification of periodontitis.” Scientific reports vol. 12,1 11893. 13 Jul. 2022, doi:10.1038/s41598-022-15462-6
4. Arigbede, Abiodun O et al. “Periodontitis and systemic diseases: A literature review.” Journal of Indian Society of Periodontology vol. 16,4 (2012): 487-91. doi:10.4103/0972-124X.106878
5. Nazir, Muhammad Ashraf. “Prevalence of periodontal disease, its association with systemic diseases and prevention.” International journal of health sciences vol. 11,2 (2017): 72-80.
6. Ossowska, Agata et al. “Evaluation of the Progression of Periodontitis with the Use of Neural Networks.” Journal of clinical medicine vol. 11,16 4667. 10 Aug. 2022, doi:10.3390/jcm11164667
7. Nazir, Muhammad Ashraf. “Prevalence of periodontal disease, its association with systemic diseases and prevention.” International journal of health sciences vol. 11,2 (2017): 72-80.
8. Zhang, Yixin et al. “Effect of tobacco on periodontal disease and oral cancer.” Tobacco induced diseases vol. 17 40. 9 May. 2019, doi:10.18332/tid/106187
9. Lertpimonchai, Attawood et al. “The association between oral hygiene and periodontitis: a systematic review and meta-analysis.” International dental journal vol. 67,6 (2017): 332-343. doi:10.1111/idj.12317
10. Harchandani, Namrita, et al. “How Much Does a Scaling and Root Planing Cost in 2023?” Authority Dental, 15 Dec. 2023, www.authoritydental.org/scaling-and-root-planing-cost.
11. H R, Rajeshwari et al. “Local drug delivery systems in the management of periodontitis: A scientific review.” Journal of controlled release : official journal of the Controlled Release Society vol. 307 (2019): 393-409. doi:10.1016/j.jconrel.2019.06.038
12. Madan, M et al. “In situ forming polymeric drug delivery systems.” Indian journal of pharmaceutical sciences vol. 71,3 (2009): 242-51. doi:10.4103/0250-474X.56015
13. Kolawole, Oluwadamilola M, and Michael T Cook. “In situ gelling drug delivery systems for topical drug delivery.” European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V vol. 184 (2023): 36-49. doi:10.1016/j.ejpb.2023.01.007
14. Kaczmarek, Beata. “Tannic Acid with Antiviral and Antibacterial Activity as A Promising Component of Biomaterials-A Minireview.” Materials (Basel, Switzerland) vol. 13,14 3224. 20 Jul. 2020, doi:10.3390/ma13143224

15. Zhang, Xiumei et al. “Abundant tannic acid modified gelatin/sodium alginate biocomposite hydrogels with high toughness, antifreezing, antioxidant and antibacterial properties.” Carbohydrate polymers vol. 309 (2023): 120702. doi:10.1016/j.carbpol.2023.120702
16. Kouchak, Maryam. “In situ gelling systems for drug delivery.” Jundishapur journal of natural pharmaceutical products vol. 9,3 e20126. 1 Jun. 2014, doi:10.17795/jjnpp-20126
17. Okur, Neslihan Üstündağ et al. “Promising Polymeric Drug Carriers for Local Delivery: The Case of in situ Gels.” Current drug delivery vol. 17,8 (2020): 675-693. doi:10.2174/1567201817666200608145748
18. Vigani, Barbara et al. “Recent Advances in the Development of In Situ Gelling Drug Delivery Systems for Non-Parenteral Administration Routes.” Pharmaceutics vol. 12,9 859. 10 Sep. 2020, doi:10.3390/pharmaceutics12090859
19. Baranov, Nicolae et al. “Polysaccharide-Based Drug Delivery Systems for the Treatment of Periodontitis.” Molecules (Basel, Switzerland) vol. 26,9 2735. 6 May. 2021, doi:10.3390/molecules26092735
20. Thangavelu, Arthiie et al. “Chlorhexidine: An Elixir for Periodontics.” Journal of pharmacy & bioallied sciences vol. 12,Suppl 1 (2020): S57-S59. doi:10.4103/jpbs.JPBS_162_20
21. Kim, Ga-Yeon, and Chong Heon Lee. “Antimicrobial susceptibility and pathogenic genes of Staphylococcus aureus isolated from the oral cavity of patients with periodontitis.” Journal of periodontal & implant science vol. 45,6 (2015): 223-8. doi:10.5051/jpis.2015.45.6.223
22. Sholapurkar, Amar et al. “Professionally Delivered Local Antimicrobials in the Treatment of Patients with Periodontitis-A Narrative Review.” Dentistry journal vol. 9,1 2. 22 Dec. 2020, doi:10.3390/dj9010002
23. Brookes, Zoë L S et al. “Effects of chlorhexidine mouthwash on the oral microbiome.” Journal of dentistry vol. 113 (2021): 103768. doi:10.1016/j.jdent.2021.103768
24. Brookes, Zoë L S et al. “Current uses of chlorhexidine for management of oral disease: a narrative review.” Journal of dentistry vol. 103 (2020): 103497. doi:10.1016/j.jdent.2020.103497
25. Poppolo Deus, Frank, and Aviv Ouanounou. “Chlorhexidine in Dentistry: Pharmacology, Uses, and Adverse Effects.” International dental journal vol. 72,3 (2022): 269-277. doi:10.1016/j.identj.2022.01.005
26. McDonnell, G, and A D Russell. “Antiseptics and disinfectants: activity, action, and resistance.” Clinical microbiology reviews vol. 12,1 (1999): 147-79. doi:10.1128/CMR.12.1.147
27. Killoy, W J. “Chemical treatment of periodontitis: local delivery of antimicrobials.” International dental journal vol. 48,3 Suppl 1 (1998): 305-15. doi:10.1111/j.1875-595x.1998.tb00721.x