Antimicrobial Efficacy of Chlorhexidine and Hyaluronic Acid Mouthwashes on Streptococcus Viridans: An In-Vitro Study

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Abstract

Streptococcus Viridans can cause diseases of the heart valves known as Infective Endocarditis, in addition to causing periodontal disease. As an adjunct to mechanical plaque control, mouthwashes aid in the control and prevention of dental plaque biofilm. The aim of this study was to find out whether there is any difference in the antimicrobial efficacy between chlorhexidine and hyaluronic acid on the CFUs of Streptococcus Viridans.

Commercially available Streptococcus Viridans isolates obtained from the American Type Culture Collection (ATCC 12392, USA) were used in this study. 0.1 mL volume of the prepared bacterial suspension was added to 3 serial dilutions (100%, 50% and 25% concentration) of Chlorhexidine mouthwash (CHX) and Hyaluronic acid mouthwash (HA) including NaCl (0.9%) which was the control. Following the incubation (for 0.5, 1, 1.5 and 5 min, at 37 °C).

Chlorhexidine at 0.2% concentration showed significant reductions of CFUs/ml; (1,859 at 30 seconds, 1,105 at 1 minute, 936 at 1.5 minutes and 234 at 5 minutes) as compared to the control (CFU >106). Hyaluronic acid did not have an effect on the number of colonies with >106 CFUs/ml in all concentrations.

Hyaluronic acid did not exhibit any antimicrobial effect on Streptococcus Viridans.

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Introduction

Periodontitis is a condition where there is a loss of attachment of the periodontal tissues from the tooth structure.[1] Periodontitis is the sixth most common human disease, and it is estimated to affect 11.2% of the global population. Periodontal disease is the leading cause of tooth loss with a global prevalence of about 90%. In the United States of America (USA) alone, 80% of its adults have experienced some form of periodontal disease which is more prevalent in individuals with low-income and non-educated levels. The consequences of untreated periodontal disease can have a devastating effect on the remaining dentition that could lead to halitosis, gingival bleeding, pathological migration of teeth, periodontal disease, tooth loss and aesthetic disharmony. Periodontal disease can also contribute to systemic diseases and a lot of evidence supports this association between periodontal and systemic diseases.[2-4]

The possible direct and indirect mechanisms related to the link between periodontal disease and systemic conditions is through bacteremia and inflammatory response to periodontal bacteria or their byproducts respectively. Bacteremia is the process of bacterial transmission into the systemic circulation. Bacteria can enter the bloodstream during normal daily activities like tooth brushing and chewing, in addition invasive dental procedures like scaling, root planing and open flap surgeries can reinforce the entrance of

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bacteria into the bloodstream. Bacteremia and low-grade periodontal infection carry a high risk for systemic diseases, particularly in the cardiovascular system which increases the risk of developing cardiovascular diseases to 19% and this relative risk is pushed up to 44% in individuals whose age is above 65.[5] As a result of periodontal bacteremia, certain manifestations can arise such as atherosclerosis, coronary heart disease, and valvular stenosis and infective endocarditis.[6] *Streptococcus Viridans* is a heterogenous group of mostly gram-positive α-hemolytic bacteria that produce a green coloration on blood agar plates. These organisms are facultative anaerobic species which can colonize in the oral cavity, gastrointestinal tract, and heart valves. The development of ulcerated epithelial surfaces in periodontal pockets allows periodontal bacteria to penetrate easily into the systemic circulation.[7] Translocation of this bacteria from the periodontal pocket into the bloodstream to the heart muscles can lead to diseases of the heart valves known as Infective Endocarditis.

Non-surgical therapy includes mechanical debridement where scaling and root planning is done to remove plaque biofilm and calculus, diseased cementum tissue and granulation tissue. As an adjunctive to mechanical plaque control, chemical plaque control agents such as mouthwashes aid in the control and prevention of dental plaque biofilm. Mouthwashes can help to reduce the proportion of pathogenic bacteria by altering the ecosystem of periodontal pockets which in turn makes it unsuitable for bacteria to survive in it. CHX raises the salivary pH, exerts a buffering action and increases the oxygen saturation. Chlorhexidine and hyaluronic acid mouthwash are prescribed after non-surgical and surgical therapies to prevent bacterial reinfection and facilitate of healing of tissues respectively.[8]
Chlorhexidine is an antimicrobial chemical agent with a broad spectrum of action and used in dentistry for root canal disinfection, denture cleaning, irrigation of dry sockets and aphthous ulcers, irrigation of periodontal pockets. Chlorhexidine exhibits “substantivity” which is the capacity to remain retentive on root surfaces for twenty-four hours.[9]

Hyaluronic Acid possesses anti-inflammatory effects and is known for its healing properties. It facilitates re-epithelization, increases microvascular density and collagen production in the connective tissue. Due to these reasons, hyaluronic acid is recommended to be prescribed pre-surgically and post-surgically to improve the treatment prognosis.[10] There is also evidence of improved bone healing when Hyaluronic acid is used with bone grafts post extraction.[11]

Hyaluronic acid as a bactericidal agent is still a controversy. Few studies have demonstrated antibacterial effects but not very conclusive. If hyaluronic Acid possess antimicrobial properties like chlorhexidine, it will prove to be a suitable option for treating periodontal disease by suppressing bacterial growth and providing the convenient environment for tissue healing to take place. Recently, there have been reports of allergic responses and cytotoxic effects of Chlorhexidine mouthwashes.[12,13] Hence, it is imperative that we look for newer agents with comparable bactericidal properties. Therefore, in our study we aimed to evaluate and compare the antibacterial efficacy of chlorhexidine and hyaluronic acid mouthwash on *Streptococcus Viridans.*

Materials and methods

Commercially available *Streptococcus Viridans* (ATCC 12392, USA) was used in this study. *Streptococcus Viridans* was grown aerobically on a blood agar plate for 24 hours at 37 °C.

- **Sample preparation**

Bacterial suspension containing approximately 1×10^7 CFUs/ mL (0.5 McFarland standard) was prepared. Three serial dilutions of 100%, 50% and 25% concentration was prepared for the Chlorhexidine mouthwash (CHX) and Hyaluronic acid mouthwash (HA) each. NaCl (0.9%) was used as the control.
0.1 mL volume of the prepared bacterial suspension was then added to each 10 mL serial dilution of CHX, HA and 0.9% NaCl (control). Following the incubation (for 0.5, 1, 1.5 and 5 min, at 37 °C), 0.1 mL aliquots of each dilution at each time point were aseptically inoculated on a plate of agar medium and incubated at 37 °C for 24hrs. The 0.9% NaCl control bacterial suspensions were incubated and treated in the same manner as test ones.[12]

- **Enumeration of bacterial colonies**

After incubation of the inoculated agar plates for 24 hours at 37 °C, the colonies were then counted using a digital colony counter machine and the number of CFUs/mL were plotted against time for each dilution.

Results

The results of this study showed that there was a significant change in the number of CFU/ml when exposed to different concentrations of Chlorhexidine in different time intervals. The number of CFU/ml in the control group was $>10^6$. (Figure 1)

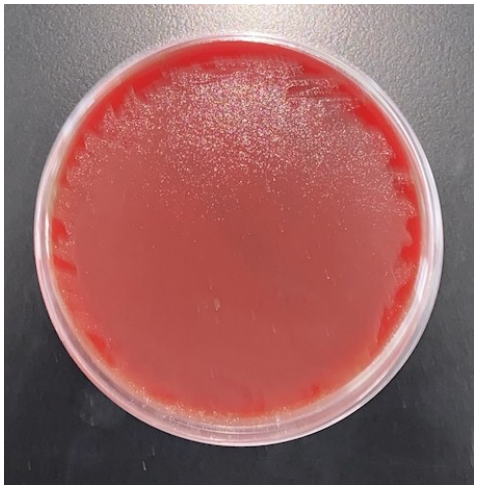


Figure 1. Bacterial Control Blood Agar Plate.

The number of CFU/ml after exposure to Chlorhexidine at 0.2% concentration was 1,859 at 30 seconds, 1,105 at 1 minute, 936 at 1.5 minutes and 234 at 5 minutes (Figure 2). Chlorhexidine at 0.1% and 0.05% concentration showed no significant reduction in the bacterial count in different time intervals. (Table 1)

	NaCl Bacterial Control	Chlorhexidine Digluconate				Hyaluronic Acid			
Time / %	-	30 seconds	1 minute	1.5 minutes	5 minutes	30 seconds	1 minute	1.5 minute	5 minute
At 100% Conc.	>100,000	1,859	1,105	936	234	> 100,000	> 100,000	>100,000	>100,000
At 50% Conc.	>100,000	>100,000	>100,000	>100,000	>100,000	> 100,000	> 100,000	>100,000	>100,000
At 25% Conc.	>100,000	>100,000	>100,000	>100,000	> 100,000	> 100,000	> 100,000	>100,000	>100,000

Table 1. Bacterial CFUs/ml after exposure to three different concentrations of chlorhexidine and hyaluronic acid under four-time frames.

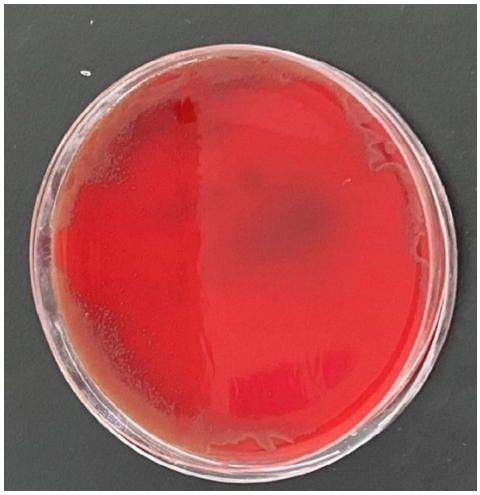


Figure 2. 0.2% CHX under 5 minutes caused the greatest reduction of CFUs.

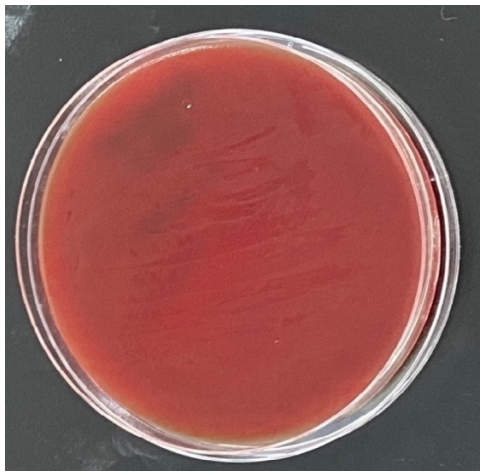
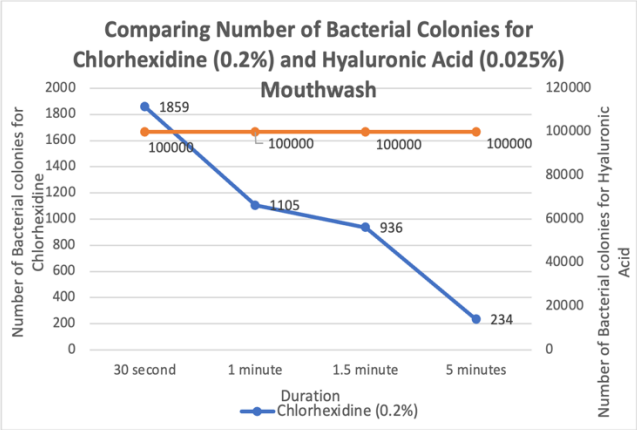


Figure 3. 0.025% HA under 5 minutes didn't exert much of reduction of CFUs.

Hyaluronic Acid at 0.025%, 0.0125% and 0.00625% showed no reduction in the CFU/ml at different time intervals (30 seconds, 1 minute, 1.5 minutes and 5 minutes). The number of CFUs/ml were $>10^6$ in all concentration and time intervals (Figure 3). This means that none of the three concentrations showed a bacterial reduction compared to the concentrations used with chlorhexidine. (Table 1)

When comparing the number of bacterial colonies in the highest two concentrations, Median number of bacterial colonies in the Chlorhexidine and the hyaluronic group was 1020.5 and 100,000 respectively. There was a statistically significant reduction in CFU/ml with the chlorhexidine mouthwash as compared to the Hyaluronic mouthwash with a p-value 0.014 (Graph 1).



Graph 1. Comparison between the effects of 0.2% chlorhexidine with 0.025% hyaluronic acid on the CFUs/ml of *Streptococcus Viridans*.

Discussion

Mouthwashes are recommended after scaling and root planing to reduce pathogenic organisms in the surrounding periodontal structures. Chemical plaque control with the use of mouthwashes is mainly used to control the progression of periodontal disease by preventing further break-down of tooth-supporting tissues.[14-17]

In our study we evaluated the efficacy of CHX and HA mouthwash in different concentrations and time duration on the *Streptococcus Viridins* group of microorganisms. These bacteria belong to the initial colonizers and can be translocated into the bloodstream during active periodontal disease. *Streptococcus Viridins* is the primary etiologic agent behind infective endocarditis.[7] Bacteremia can be controlled by treating periodontal conditions to prevent further bacterial transmission, rinsing with mouthwashes and by administering antibiotics.[18,19]

CHX is currently the gold standard in mouthwashes and due to its anti-bacterial properties,[20] it is widely used as an adjunct to traditional periodontal therapies including implants and as a pre-procedural rinse to reduce the microbial load and limit bacteremia. HA has good wound healing properties, and few reports show a bacteriostatic effect and is gaining popularity these days.

Ali et al in their clinical study concluded that CHX was the most effective antiplaque mouthwash. HA and antioxidants were as effective as CHX in reducing gingival bleeding. Patient acceptance was better with HA and hence they concluded that it could be a potentially good alternative to CHX.[21]

Results from our current study illustrated that chlorhexidine was very effective in reducing CFUs of *Streptococcus Viridans* when compared to hyaluronic acid. 0.2% Chlorhexidine with achieved the most reduction of CFUs in contrast to other concentrations of 0.05% and 0.1%. Maximum reduction of CFUs was witnessed when 0.2% chlorhexidine was applied for 5 minutes. This reduction achieved by chlorhexidine in *Streptococcus Viridans* CFUs was due to its broad-spectrum antibacterial effect. On the other hand, hyaluronic Acid in all its concentrations did not show any reduction in CFUs of the *Streptococcus Viridans*. This was contrary to the findings of a study by BinShabaib et al.,[22] where they concluded that 0.8% HA was more effective in reducing the *Porphyromonas gingivalis* CFUs/ml compared with 0.2% CHX. The concentration of HA mouthwash used in our study is lower (0.025%) and hence could be a reason to not exhibit an antibacterial effect. Also, *Porphyromonas gingivalis* is a gram-negative bacteria whereas *Streptococcus viridans* are gram positive bacteria.

Bansal et al.[23] showed that high concentration of medium and lower molecular weight HA exhibits bacteriostatic properties against a variety of pathogenic microbes including *Prevotella oris, Aggregatibacter actinomycetemcomitans and Streptococcus species.* They also stated that hyaluronate modulated wound healing and that its administration in sites with periodontal defects would be beneficial. Eliezer et al. showed that cross-linked HA augments osseous wound healing by slowing down collagen membrane degradation.[24]

In a clinical study the authors evaluated the efficacy of 0.025% hyaluronan-containing mouthwash in comparison with 0.2% chlorhexidine and a water-based mouthwash to evaluate its antibacterial efficacy on isolated strains of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia*. They found that Hyaluronan (0.025%) containing mouthwash was comparable to chlorhexidine (0.2%) in inhibiting plaque growth *in vivo*, and it significantly reduced the growth of *Aggregatibacter actinomycetemcomitans* and *Prevotella*

intermedia in vitro.[25]

The differences in the outcome could be attributed to the use of different concentrations of HA, time durations and different modes of delivery like gels instead of mouthwashes. The differences in research methodologies and experimental designs could lead to contrasting results as well. *The in-vitro nature of this study* makes it difficult to directly apply these laboratory-based results into a clinical setting. Further research both *invitro* followed by clinical studies must be done with higher concentrations of HA mouthwash at different time intervals to evaluate if it possesses any antibacterial properties. Also, a comparison of the effect of this mouthwash on different types of bacteria will give us clear picture if this mouthwash is effective against specific groups of bacteria. As per our study, HA mouthwash did not have any effect on the CFU/ml of *Streptococcus viridans* group as compared to the CHX mouthwash which showed a significant reduction.

Conclusions

Hyaluronic acid did not show any anti-bacterial effect on the *Streptococcus viridans* group of bacteria within the scope of this in vitro study.

Recommendation: To further compare and evaluate the effectiveness of chlorhexidine with other mouthwashes than hyaluronic acid like Povidone Iodine and Hydrogen Peroxide or any other new products in different formulation.

Declaration of Interest

The authors report no conflict of interest.

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