Research Article

Eradication of *Porphyromonas Gingivalis* and *Prevotella Intermedia* Biofilms using the Chlorhexidine Gluconate and Matrica Mouthwash (Chamomile Extract)

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Abstract

Introduction: One of the critical pathogenic agents in oral and dental bacteria is biofilm formation. *Porphyromonas gingivalis* (*P. gingivalis*) and *Prevotella intermedia* (*P. intermedia*) biofilms critically contribute to the unsuccessful treatment of oral and dental illnesses, and their permanent presence in dental canals harms the recovery process after treatment. Today, dentists are seeking superior and more beneficial choices with less toxicity as appropriate disinfectants for the mouth and teeth.

Objective: The current research aimed to investigate the effects of Chlorhexidine (CHX) gluconate and matrica mouthwash on one-, three-, and five-day-old *P. gingivalis* and *P. intermedia* biofilms.

Method: The biofilm formation capabilities of *P.gingivalis* and *P.intermedia* were assessed using the microtiter plate method. Subsequently, the Minimum Inhibitory Concentration (MIC) of CHX gluconate and matrica mouthwash at various concentrations against biofilm formation in *P. gingivalis* and *P. intermedia* was specified using the microdilution method.

Results: The biofilm formation capabilities of *P. gingivalis* and *P. intermedia* were approved on a quantitative scale. Both CHX gluconate and matrica mouthwash were effective in eradicating biofilm formation in *P. gingivalis* and *P. intermedia* strains. Notably, according to the results, CHX mouthwash exhibited a greater efficacy in eradicating *P. gingivalis* biofilm. Furthermore, the matrica mouthwash capability to eradicate biofilms in *P. gingivalis* and *P. intermedia* was equivalent, with no significant difference observed.

Conclusion: The CHX mouthwash is recommended as a novel approach for controlling biofilm infections stemming from oral and dental bacteria, including *P. gingivalis* and *P. intermedia*

Keywords: Chlorhexidine gluconate mouthwash; Matrica mouthwash; Biofilm formation; *Porphyromonas gingivalis; Prevotella intermedia*

Introduction

Biofilms are structured communities of non-motile bacteria that can grow on various surfaces, including the oral cavity and teeth. These structured communities are highly resistant to the host immune system and conventional antimicrobial agents, causing critical risks to patients [1]. The capability to form biofilm in bacteria increases resistance to various drugs and antimicrobial substances and diminishes the metabolic activity of cells [2]. Biofilm formation has been extensively investigated in numerous studies due to its effect on over 80% of human bacterial infections [3]. Moreover, the oral cavity that is able to cre-ate an appropriate habitat for about 700 microbial species can create complex and dynamic biofilms known as dental plaque [4,5]. Among these, the *Porphyromonas gingivalis* (*P. gingivalis*) and *Prevotella intermedia* (*P. intermedia*) anaerobic and gram-negative bacteria that are capable of forming sub-gingival biofilms are involved in multiple important periodontal diseases, such as periodontitis and peri-implantitis [6]. The pathogenicity of *P. gingivalis* and *P. intermedia* is connected with various pathogenic agents contributing to the colonization of teeth, tissue destruction and interference with the host defense system

[7,8]. These bacteria also contribute to tooth decay, lip fissures, salivary gland inflammation, etc. [9].

Chlorhexidine (CHX), a commonly used antibacterial mouthwash, is recommended by dentists because of its antimicrobial, antifungal, and antiviral effects. Moreover, CHX gluconate exhibits particularly strong and long-lasting antimicrobial activities, making it a gold standard and the most highly effective anti-plaque agent [10]. However, evidence suggests that CHX gluconate may cause various complications, such as tooth discoloration, burning sensation, and dry mouth [11]. Matrica herbal mouthwash, which contains chamomile extract, is an essential herbal mouthwash produced by Barij Essential Pharmaceutical Company in Iran [12]. Chamomile, the main ingredient of this mouthwash, possesses antibacterial activities against various bacteria. The anti-inflammatory properties of chamomile can be attributed to its main compounds, i.e., flavonoids, including apigenin, luteolin, quercetin, and patuletin, and lipophilic substances such as sesquiterpenes [11,12]. Some studies have also demonstrated the vital role of matrica herbal mouthwash in controlling the growth of dental microbial plaque, disinfecting the gums and oral cavity, reducing inflammatory lesions of the gums and oral cavity, and combating oral-gum inflammations and infections [13]. Therefore, the current study aimed to assess the anti-biofilm activities of CHX gluconate and matrica mouthwash against biofilm formation in *P. gingivalis* and *P. intermedia* isolated strains.

Materials and Method

Bacterial Isolation and Identification

The clinical strains of *P. gingivalis* and *P. intermedia* utilized in previous studies were also employed in the current research. These strains, which were obtained from 38 patients who referred to the Faculty of Dentistry, Hamedan University of Medical Sciences, were cultured on Brucella agar supplemented with 10% defibrinated horse blood, 5 µg/mL hemin, and 0.4 µL/mL vitamin K1, and incubated under anaerobic conditions (5% CO_2, 5% H_2, and 90% N_2) for 7 to 10 days. The identification of the anaerobic bacteria was accomplished using API 20 and rapid ID 32A biochemical tests, as well as gas chromatographic analysis of fatty acids [14]. Following identification, isolated colonies were diluted in fresh Brain Heart Infusion (BHI) broth to achieve a McFarland standard turbidity of 0.5. Additionally, a small quantity of this bacterial suspension was employed for subsequent experiments.

Biofilm Formation by Crystal Violet Assay

Biofilm formation was conducted following the protocol outlined by Kuboniwa et al. [16]. The biofilm formation was determined using the microtiter plate test. In a nutshell, anaerobically grown bacteria from an overnight culture were cultured on BHI broth at a concentration of 0.5 McFarland standards, as determined by absorbance readings (0.08-0.1 at 625 nm), using a spectrophotometer (Shimadzu, model UV-120-01, Japan). The BHI broth medium was supplemented with 1% horse serum, hemin, and vitamin K.

The plate containing the bacterial suspension was then placed in an anaerobic chamber at 37°C for 72 hours to allow for the growth of anaerobic bacteria under anaerobic conditions and the formation of biofilm on the bottom of the wells. Following incubation, the contents of each well were aspirated, and the wells were washed with sterile physiological saline to eliminate non-adherent cells. The attached bacteria were fixed

using absolute methanol for 10 minutes. Crystal violet (1% w/v) was used to stain the plates. The excess stain was removed by washing, and the plates were rinsed using tap water. The bound dye was re-solubilized with 200 µL glacial acetic acid (33% v/v). Each well's Optical density (OD) was measured at 650 nm using an Enzyme-Linked Immunosorbent Assay (ELISA) reader. The un-inoculated wells containing media were used as blanks. A three-grade scale was employed to assess the biofilm formation capability. The first grade represented negative results, with ODs below 0.500. The second grade indicated positive results, with ODs ranging from 0.500 to 1.500. Finally, the third grade denoted strongly positive results, with ODs exceeding 1.500. All experimental steps were repeated three times.

Anti-Biofilm Activities of Chlorhexidine and Matrica Mouthwash

In this research, the impacts of matrica herbal mouthwash and CHX on the formation of biofilms were investigated. The biofilms were established in microtiter plates (Sigma Aldrich, St. Louis, Missouri, USA), as described previously in section 2.2. Following a 72-hour incubation period in an anaerobic atmosphere, the media were thrown away, and the plates were thoroughly washed with Phosphate-Buffered Saline (PBS) (pH=7.4) to eliminate all unattached cells. Subsequently, various dilutions of matrica herbal mouthwash (100%, 50%, and 20%) and CHX (2%, 1%, and 0.5%) were prepared and added separately to the pre-formed biofilms. Control wells were also included, with each medium added separately to a subset of the wells. Additionally, each microtiter plate possessed three wells for sterility controls and three wells for growth controls, each single well for different concentrations of matrica herbal mouthwash and CHX. The plates were then incubated for 24 hours at a temperature of 37°C in an anaerobic atmosphere and stained using crystal violet.

Statistical Analysis

The data analysis was performed using SPSS software version 18 and the analysis of variance (ANOVA) test.

Results

Biofilm Formation in Porphyromonas Gingivalis and Prevotella Intermedia

Microtiter plate techniques were employed in order to assess the biofilm capacity of *P. gingivalis* and *P. intermedia* strains. The findings revealed a robust positive correlation between these strains and biofilm formation. Furthermore, the varying composition of matrix materials is the reason for the intensity of biofilm formation in these bacterial isolates [15].

Anti-Biofilm Activities of Chlorhexidine and Matrica Mouthwash

In the present study, the anti-biofilm activities of matrica herbal mouthwash and CHX against *P. gingivalis* and *P. intermedia* strains were carried out using the standard broth dilution method. Different concentrations of CHX gluconate (2%, 1%, and 0.5%) were tested against these isolates. According to Figure 1, CHX gluconate exhibited significant inhibitory activities against the biofilms of *P. gingivalis*. Moreover, at higher dilutions, CHX could effectively demolish a substantial amount of biofilms in this bacterium (Figure 1B). It was observed that higher concentrations of CHX easily eradicated a significant portion of the biofilm structure in this bacterium. Besides, matrica mouthwash was found to eliminate the biofilm structure

in these bacteria, although the CHX anti-biofilm characteristic exhibited more pronounced impacts (Figure 1A).

The results also indicated that CHX possessed anti-biofilm capabilities against *P. intermedia* biofilm, albeit with weaker impacts compared to *P. gingivalis* (Figure 2A). Additionally, at higher concentrations, CHX exhibited anti-biofilm properties against *P. intermedia* (Figure 2B). Matrica mouthwash also demonstrated the capability to eliminate the biofilm structure in these bacteria (Figure 2B). Overall, based on the findings presented in Figures 1 and 2, it can be concluded that Matrica mouthwash revealed the same effect on biofilm of *P. gingivalis* and *P. intermedia* isolates.

However, the effect of CHX mouth wash on the removal of *P. gingivalis* biofilm was significant.
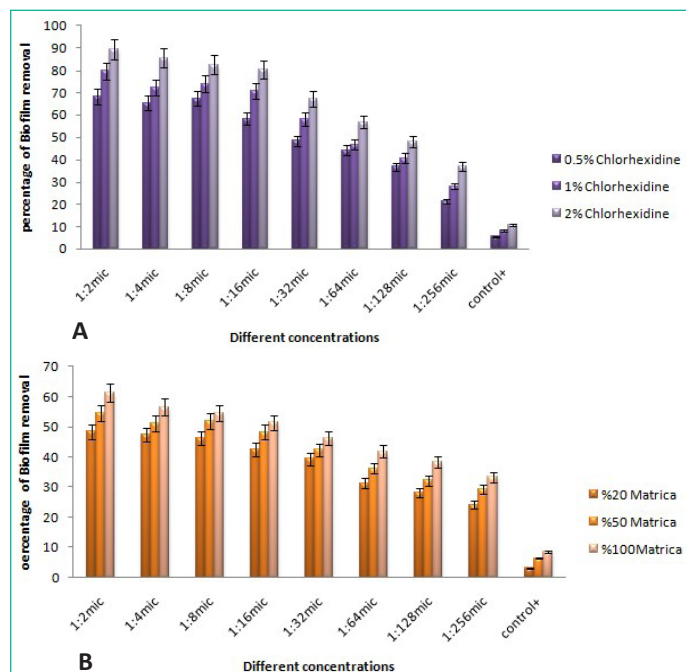

Figure 1: The percentage of biofilm elimination in different concentrations of chlorhexidine gluconate (A), and matrica mouthwash on *Porphyromonas gingivalis* biofilm (B).
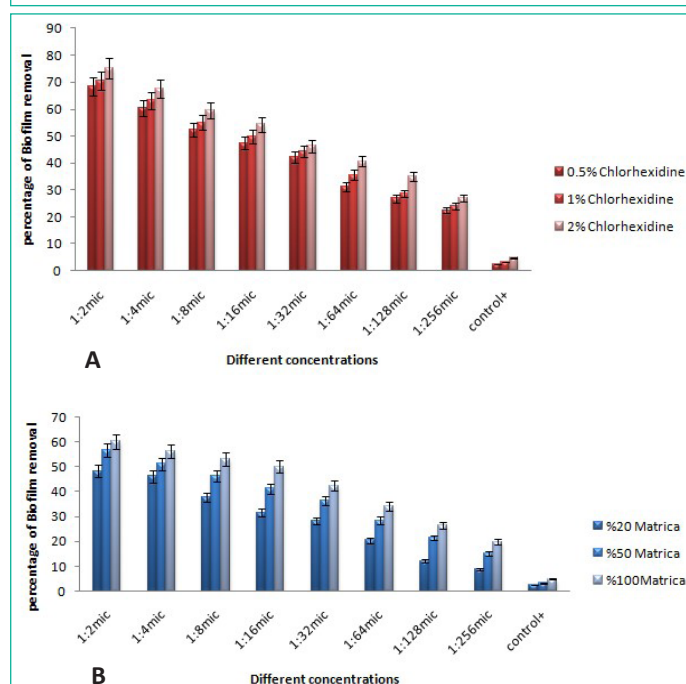

Figure 2: The percentage of biofilm elimination in different concentrations of chlorhexidine gluconate (A), and matrica mouthwash (B) on *Prevotella intermedia* biofilm.

Discussion

Periodontal diseases are now highly prevalent worldwide, affecting up to 90% of the global population [17]. Periodontitis, a chronic inflammatory disease, can cause the progressive destruction of teeth, alveolar bone, and ultimately tooth loss [18,19]. The destruction of soft and bone tissues in periodontal disease primarily stems from chronic infection due to the colonization and invasion of certain bacteria. According to numerous studies, forming bacterial biofilms, particularly *P. gingivalis* and *P. intermedia*, are known as agents of periodontal disease [20]. These biofilms play a crucial role in the development and progression of periodontal disease, accounting for most tissue and tooth damage [19,20]. Moreover, the prevalence rate of opportunistic infections has enhanced due to the high incidence of Acquired Immunodeficiency Syndrome (AIDS) and various types of cancer [21]. Consequently, investigations have been conducted to identify effective solutions to combat *P. gingivalis* and *P. intermedia.* One approach to eliminate this issue is using various antiseptics, disinfectants, and mouthwashes, such as CHX gluconate and matrica mouthwash [22]. In this study, we evaluated the anti-biofilm impacts of two mouthwash types, namely CHX gluconate and matrica mouthwash on biofilm of *P. gingivalis* and *P. intermedia*. Our findings indicate that CHX exhibits greater efficacy than matrica mouthwash against preformed biofilms. Additionally, our study indicates that CHX is particularly effective in eliminating *P. gingivalis* biofims. Therefore, by using CHX mouthwash preformed *P. gingivals* biofilms can be *efficiently removed.* This finding is consistent with the findings of Esfahanizadeh *et al*. study, reporting that 2% CHX mouthwash is more effective than Matrica mouthwash in eradicating biofilm formed by *P. gingivalis* and also in controlling microbial plaque [23]. Furthermore, numerous natural products extracted from traditional and herbal medicines possess antibacterial properties, making them an appropriate option to treat periodontal disease. These herbal mouthwashes can serve as effective antibacterial and anti-biofilm agents. However, there is evidence that researches on herbal mouthwashes is limited, and the antimicrobial spectrum of these mouthwashes remains unclear [24]. Hence, it is recommended that further studies for evaluating antibacterial and antibiofilm properties of herbal mouthwashes should be considered.

Conclusion

The findings of this study indicated that CHX mouthwash exhibited *an even stronger antibiofilm effect than* matrica mouthwash. However, it should be noted that CHX mouthwash has some disadvantages, including an unpleasant taste and the potential to temporarily alter the sense of taste, as well as the ability to cause a brown discoloration on teeth [25]. Consequently, more studies are requied to solve these disadvantages.

Author Statements

Ethical Approval

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