Journal of Dentistry
Volume 58, March 2017, Pages 34-39

Inhibition of streptococcal biofilm by hydrogen water

Jinkyung Kim, Heon-Jin Lee, Su-Hyung Hong

Show more

https://doi.org/10.1016/j.jdent.2017.01.004
Get rights and content

Abstract

Objectives

The accumulation of oral bacterial biofilm is the main etiological factor of oral diseases. Recently, electrolyzed hydrogen-rich water (H-water) has been shown to act as an effective antioxidant by reducing oxidative stress. In addition to this general health benefit, H-water has antibacterial activity for disease-associated oral bacteria. However, little is known about the effect of H-water on oral bacterial biofilm. The objective of this study was to confirm the effect of H-water on streptococcal biofilm formation.

Methods

In vitro streptococcal biofilm was quantified using crystal violet staining after culture on a polystyrene plate. The effect of H-water on the expression of genes involved in insoluble glucan synthesis and glucan binding, which are critical steps for oral biofilm formation, was evaluated in MS. In addition, we compared the number of salivary streptococci after oral rinse with H-water and that with control tap water. Salivary streptococci were quantified by counting viable colonies on Mitis Salivarius agar-bacitracin.

Results

Our data showed that H-water caused a significant decrease in *in vitro* streptococcal biofilm formation. The expression level of the mRNA of glucosyltransferases (*gtfB, gtfc,* and *gtfI*) and glucan-binding proteins (*gbpC, dblB*) were decreased remarkably in MS after H-water exposure for 60s. Furthermore, oral rinse with H-water for 1 week led to significantly fewer salivary streptococci than did that with control tap water.

Conclusions

Our data suggest that oral rinse with H-water would be helpful in treating dental biofilm-dependent diseases with ease and efficiency.

Introduction

Streptococcus mutans and *Streptococcus sobrinu*s are the major causative agents of human dental caries and are considered to be the principal cariogenic bacteria among the oral streptococci [1]. Therefore, ecologically driven changes in oral biofilms caused by *S. mutans* and *S. sobrinus* are responsible for such diseases. The removal of oral biofilms has been an essential method for the prevention of dental caries [2]. Hence, the importance of effective plaque control has been emphasized over the years. To date, mechanical plaque elimination with assorted devices, like the toothbrush, remains the primary and most widely accepted means of controlling plaque and maintaining good oral hygiene. However, it is difficult for most people to attain adequate plaque removal and control with such mechanical devices. As an adjunct to mechanical methods, oral hygiene products containing chemotherapeutic agents with a variety of antimicrobial mechanisms have been beneficial and desirable.

There are several gargling products that can be used as adjuvants to control dental plaque. The most frequently used agent is chlorhexidine (CHX). CHX mouth rinse is effective in preventing and controlling plaque formation, breaking up existing plaque, and inhibiting and reducing the development of gingivitis [3]. Although the clinical effectiveness of CHX has been well documented, many adverse effects, such as swollen, red, and bleeding gums; desquamation or soreness of oral mucosa; altered taste sensation; staining of the teeth and the tongue; and gastritis, have also been reported [4], [5], [6], [7]. In addition, some oral bacteria develop resistance to the antibacterial activity of CHX [8]. Another antimicrobial mouth rinse product containing triclosan, a chlorophenol, has been suspected to cause resistance in bacterial strains and allergic contact dermatitis [9], [10]. In addition, cetylpyridinium chloride mouth rinse has been found to cause tooth staining and burning sensation [11]. These various limitations led to the development of safer alternative oral hygiene products.

Recently, electrolyzed hydrogen-rich water (H-water) has been shown to be a natural antibiotic material that overcomes such difficulties. Hydrogen (dihydrogen; H_2) acts as an effective antioxidant by reducing oxidative stress [12]. In addition, H-water exhibits antibacterial activity for oral bacteria, such as *Streptococcus mutans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, and *Tannerella forsythia*, which are associated with oral diseases [13]. Furthermore, H-water might be beneficial in suppressing the progression of periodontitis by decreasing gingival oxidative stress [14]. These data suggest that H-water can be used as an adjuvant to prevent dental caries and periodontal disease.

It is generally known that two closely related species of mutans streptococci (MS), namely *S. mutans* and *S. sobrinus*, are the predominantly prevalent caries-associated species in humans. Among the physiological traits of MS that are most relevant to cariogenesis are their syntheses of extracellular polysaccharides from sucrose, which fosters their firm attachment to teeth and promotes tight cell clustering. The enzymes involved in the synthesis of extracellular polysaccharides, such as water-insoluble glucans, are extracellular glucosyltransferases (GTFs) [15], [16]. The GTF genes in *S. mutans* are classified in terms of the solubility of their gene products: insoluble glucan synthesis (*gtfB*), and insoluble/soluble glucan synthesis (*gtfC*) [17]. Mutations of these GTF family genes lead to a reduction in the incidence of dental caries in rats exposed to

MS carrying the mutant genes, indicating that these GTFs in *S. mutans* are responsible for the pathogenesis of dental caries [17]. In *S. sobrinus*, *gtfI* plays an important role in water-insoluble glucan synthesis [18]. Furthermore, binding with insoluble glucan through the glucan-binding protein (GBP) is crucial for the attachment of MS to solid surfaces in biofilm formation [19]. Zhu et al. identified GbpC as the protein that is responsible for sucrose-dependent aggregation in *S. mutans* [20]. Mutants lacking the *gbpC* gene formed biofilms that were structurally different and defective when compared to those of the wild type [21], [22]. On the other hand, the *S. sobrinus dblB* gene, which is one of the four *gbpC* protein gene homologs, is the primary gene responsible for aggregation in this species [23], [24]. Therefore, it is important to reveal the association between *gbpC* or *dblB* gene expression and biofilm formation in MS.

The salivary microbial species reflect the composition of the oral microbial community and could serve as a biomarker of the health and disease status of the oral cavity. Saliva allows dental plaque to flourish and also detaches layers of plaque [25]. The level of certain bacterial species in saliva can reflect their presence in plaque [26]. Previous studies have shown a significant correlation between the salivary concentration of MS and their proportions in plaque [27], [28]. Therefore, saliva would be an appropriate sample from which to estimate the quantity of *S. mutans* biofilm in oral cavity.

The object of this study was to evaluate the effect of H-water on oral streptococci. We first examined whether H-water affects *in vitro* streptococci biofilm formation. We also analyzed the expression of genes involved in glucan binding and insoluble glucan synthesis in *S. mutans* and *S. sobrinus*. Furthermore, we compared salivary streptococcal numbers after oral rinse with H-water and that with control tap water to estimate the effect of H-water on plaque MS levels.

Section snippets

Bacterial culture

The bacteria tested in this study were *Streptococcus mutans* (ATCC 25175) and *Streptococcus sobrinus* (ATCC 27607). Streptococci were maintained on brain heart infusion (BHI) medium and grown under aerobic conditions.…

Effect of H-water on streptococcal biofilm formation

An *in vitro* biofilm formation assay was performed in accordance with the protocol published by Toole [29].

H-water (dissolved hydrogen, 1500 ppb; oxidation-reduction potential, −600 mV to −700 mV) was purchased from Nanotec (nano H ®, South Korea). Streptococcal colonies were inoculated …

Results

Effect of H-water on streptococcal biofilm formation

Streptococcal biofilm formation with exposure to H-water for different durations was compared. A streptococcal biofilm with crystal violet staining is shown in Fig. 1A. Biofilm formation of *S. mutans* decreased significantly when these bacteria were exposed to H-water for 30 s or 60 s (Fig. 1B) (*$P<0.05$,

P<0.01). *S. sobrinus* was more sensitive to H-water; exposure to H-water for only 15 s significantly decreased biofilm formation (Fig. 1C) (P<…

Discussion

Although mechanical plaque control can be an effective strategy for preventing the progression of oral diseases, most individuals do not effectively brush their teeth. Therefore, the daily use of an effective mouth rinse is generally considered a simple strategy to control the formation of oral biofilm [32], which is a common etiological factor for oral diseases. Mouthwashes have been widely used to prevent dental caries. One of the best-selling mouthwashes contains up to 26% alcohol to kill…

Conflict of interest

The authors declare no conflict of interest.…

Authors' contributions

HSH conceived of the study, participated in its design and coordination, bacterial biofilm formation, and drafted the manuscript. KJK carried out the bacterial culture and real-time PCR. LHJ performed statistical analysis, and critically read the manuscript.…

Acknowledgements

This work was supported by the Basic Science Research Program through a NationalResearch Foundation of Korea (NRF) grant funded by the Korean Government (2015R1D1A1A01056880, and MSIP, 2008-0062282).…

References (38)

H.U. Hepso *et al.*
Side-effects and patient acceptance of 0.2% versus 0.1% chlorhexidine used as post-operative prophylactic mouthwash
Int. J. Oral Maxillofac. Surg. (1988)

L.A. Skoglund *et al.*
Desquamative mucosal reactions due to chlorhexidine gluconate. Report of 3 cases
Int. J. Oral Surg. (1982)

W.J. Loesche
Role of Streptococcus mutans in human dental decay
Microbiol. Rev. (1986)

J.J. de Soet *et al.*

Differences in cariogenicity between fresh isolates of Streptococcus sobrinus and Streptococcus mutans
Caries Res. (1991)

J.C. Gunsolley
Clinical efficacy of antimicrobial mouthrinses
J. Dent. (2010)

J. Ainamo *et al.*
Gingival bleeding after chlorhexidine mouthrinses
J. Clin. Periodontol. (1982)

S. Roche *et al.*
Chlorhexidine-induced gastritis
Postgrad. Med. J. (1991)

D. Herrera *et al.*
Differences in antimicrobial activity of four commercial 0.12% chlorhexidine mouthrinse formulations: an in vitro contact test and salivary bacterial counts study
J. Clin. Periodontol. (2003)

T. Bhutani *et al.*
Triclosan: a potential allergen in suture-line allergic contact dermatitis
Dermatol. Surg. (2009)

H. Robertshaw *et al.*
Contact dermatitis to triclosan in toothpaste
Contact Dermat. (2007)

View more references

Cited by (6)

Electrolyzed water for the microbiologic control in the pandemic dental setting: a systematic review
2022, BMC Oral Health

Investigating the anti-streptococcal biofilm effect of ssDNA aptamer-silver nanoparticles complex on a titanium-based substrate
2022, RSC Advances

Electrolyzed water for the microbiologic control in the pandemic dental setting: A systematic review
2022, ResearchSquare

Assessment of antibacterial effect of hydrogen water on plaque from patients with chronic periodontitis
2021, Journal of Indian Society of Periodontology

Identification of non-streptococcus mutans bacteria from predente infant saliva grown on mitis-salivarius-bacitracin agar
2020, Journal of Clinical Pediatric Dentistry

Skin ulcers infected with conditional pathogenic strains treated with local hydrogen water packing in two pemphigus vulgaris patients: Case reports with follow-up for 2 months
2019, Dermatologic Therapy

Recommended articles (6)

Research article

Nanoscale chemical surface characterization of four different types of dental pulp-capping materials
Journal of Dentistry, Volume 58, 2017, pp. 11-18

Show abstract

Research article

Effect of a novel quaternary ammonium silane on dentin protease activities
Journal of Dentistry, Volume 58, 2017, pp. 19-27

Show abstract

Research article

Impact of the CONSORT Statement endorsement in the completeness of reporting of randomized clinical trials in restorative dentistry
Journal of Dentistry, Volume 58, 2017, pp. 54-59

Show abstract

Research article

In vitro dentin barrier cytotoxicity testing of some dental restorative materials
Journal of Dentistry, Volume 58, 2017, pp. 28-33

Show abstract

Research article

The association between loading of restorations and secondary caries lesions is moderated by the restoration material elasticity

Journal of Dentistry, Volume 58, 2017, pp. 74-79

Show abstract

Research article

Mechanical performance of polymer-infiltrated zirconia ceramics

Journal of Dentistry, Volume 58, 2017, pp. 60-66

Show abstract

View full text