Dental Research Journal

Original Article

Antibacterial effect of a disinfectant spray for sports mouthguards on *Streptococcus sobrinus*

Gen Tanabe[1], Hiroshi Churei[1], Yasuo Takeuchi[2], Kairi Hayashi[1], Aki Kanasaki[1], Yuriko Yoshida[1], Jun Toma[3], Yosiaki Araie[4], Toshiaki Ueno[1]

Departments of [1]Sports Medicine/Dentistry and [2]Periodontology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, [3]AGSA JAPAN CO., LTD 1-2-34 Tamatsukuri, Chuo-Ku, [4]LITEC., Ltd 6-33 Minamibori-cho, Tennoji-Ku, Osaka, Japan

Received: 28-May-2020
Revised: 07-Aug-2020
Accepted: 10-Sep-2020
Published: 19-Jul-2021

Address for correspondence:
Dr. Hiroshi Churei,
Department of Sports Medicine/Dentistry,
Graduate School of Medical and Dental Sciences,
Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Japan.
E-mail: chu.spmd@tmd.ac.jp

ABSTRACT

Background: Mouthguard (MG) disinfectant sprays are available for maintaining MG hygiene. The effect of these sprays against *Streptococcus sobrinus* is still unknown. The purpose of this study was to evaluate the antibacterial effect of an MG disinfectant spray against *S. sobrinus* using the modified ISO 22196 standard.

Materials and Methods: In this *in vitro* study, we used the following treatment groups for antibacterial testing: MG spray-1 (left in spray for 30 s), MG spray-2 (60 s), and control (n = 4). All analyses were performed at a statistically significant level (P = 0.05) using JMP® 14.

Results: The log colony-forming units of the MG spray-2 group were significantly lower than those of the other groups. The antibacterial activity of MG spray-2 against *S. sobrinus* was >2.1.

Conclusion: We confirmed the antibacterial effect of the MG spray against *S. sobrinus*, and it was influenced by the treatment duration, with the optimum effect at a longer duration.

Key Words: Antibacterial agents, mouth protectors, *Streptococcus sobrinus*

INTRODUCTION

The World Dental Federation recommends the use of a mouthguard (MG) when playing sports, especially contact sports, to prevent oral injuries.[1] The usefulness of MGs in preventing trauma is widely known, and the number of sports in which players are obliged to wear MGs is increasing. Because MGs are intraoral devices, maintenance is recommended to retain their efficacy over time.[1] The MGs are repeatedly used for several months or years, and their daily storage and cleaning are completely entrusted to each player. Although many players clean their MGs after use, the storage conditions of MGs are often not hygienic enough.[2,3] Sometimes, soil [Figure 1] and intraoral plaque are found attached to MGs [Figure 2]. Unhygienic MGs, with a large number of bacteria, yeasts, and molds, might cause malodor,[4] and even severe inflammatory diseases, gum infection, and tooth decay.[5] Hence, MGs should be maintained appropriately. Glass *et al*. recommend the regular disinfection of MGs with a disinfectant solution, as it significantly decreases the number of microorganisms on the MGs.[6]

Although there are various effective cleaning methods for MG, including mechanical and chemical methods, there is no standard cleaning method.[7] These

Access this article online

Website: www.drj.ir
www.drjjournal.net
www.ncbi.nlm.nih.gov/pmc/journals/1480

How to cite this article: Tanabe G, Churei H, Takeuchi Y, Hayashi K, Kanasaki A, Yoshida Y, *et al*. Antibacterial effect of a disinfectant spray for sports mouthguards on *Streptococcus sobrinus*. Dent Res J 2021;18:59.

methods include simple washing with water after use or cleaning the MG with a soft brush.[2] However, washing ethylene vinyl acetate (EVA) MGs with sterilized water and brushing do not kill the attached microbes.[8] Moreover, brushing significantly damages the surfaces of the EVA sheets.[9] Thus, there is a need for chemical cleaning methods because MGs, similar to dentures, have morphologically difficult parts to clean, and they deteriorate over time.[10]

An MG disinfectant spray containing cetylpyridinium chloride (CPC) was previously developed for chemical cleaning.[7] As CPC is biologically safe and has antimicrobial activity, it is used in quasi drugs such as nasal sprays, troches, and mouthwashes. CPC is a quaternary ammonium salt-type cationic surfactant that adsorbs to the surface of bacteria, destroys the lipid membrane, and disturbs the osmoregulatory function of the lipoprotein membrane. It can also disturb the respiratory activity by forming a membrane on the surface of the bacteria.[11] These are thought to be the mechanisms underlying the antimicrobial effect of CPCs.

An MG disinfectant spray must exert its effect immediately. Moreover, it should be safe, palatable, and convenient to use. It should also reach parts that cannot be accessed by standard brushes. Previous studies on the antimicrobial effects of MG disinfectant sprays have proved their effectiveness.[7,8] However, further validation of the effectiveness of these sprays is necessary to promote their application. The disinfectant effect of sprays is affected by their concentration, MG treatment duration, and temperature. If the treatment duration is insufficient, the optimal effect cannot be achieved even if an appropriate concentration of the spray is used.[12] Thus, MGs should be treated for a certain period for optimal disinfection, and the disinfection duration depends on the disinfectant and target microorganisms. Suzuki *et al*. tested the effectiveness of an MG disinfectant spray against *Streptococcus mutans* and reported that it was effective in 30 s.[7] *Streptococcus sobrinus* is a common bacterium in the oral cavity and is an important cariogenic agent. Recently, the importance of *S. sobrinus* in the progression of tooth caries has been reported.[13] However, the antibacterial effect of MG disinfectant sprays against *S. sobrinus* is not known. The purpose of this study was to evaluate the antibacterial effect of an MG disinfectant spray against *S. sobrinus* using the modified ISO 22196 standard.

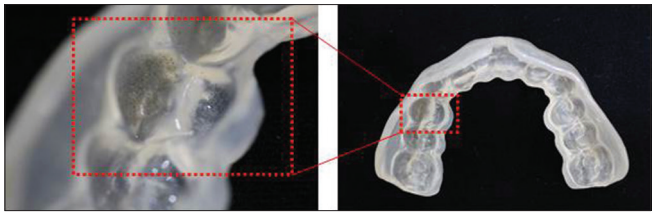

Figure 1: Example of an unhygienic mouthguard contaminated with soil.

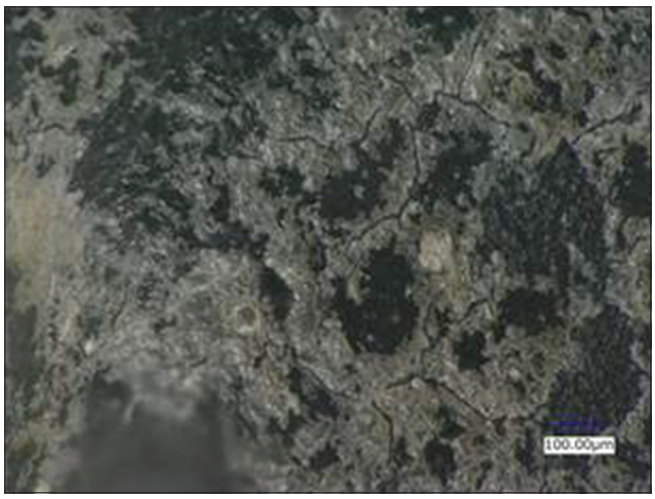

Figure 2: Intraoral plaque attached inside a mouthguard. Mouthguards have many morphologically difficult parts to clean.

MATERIALS AND METHODS

Mouthguard disinfectant spray

In this *in vitro* study, the MG disinfectant spray Kirei-chan® (Agsa Japan Co., Ltd., Osaka, Japan) was used [Figure 3]. It is based on an existing MG spray and contains CPC 500 ppm as a bactericidal component, glycerin as a sweetening component, and 1-menthol to improve the taste and create a refreshing feeling. Table 1 shows the components of the MG spray.

Determination of the antibacterial effect *in vitro*

The experimental procedure was based on the ISO 22196 standard,[14] which is an internationally recognized test to evaluate the ability of treated

Table 1: Components of the mouthguard disinfectant spray

Kirei-chan®	Composition
Bactericidal component	CPC 500 ppm
Other components	Polysorbate 80
	Ethanol
	Glycerin
	1-Menthol

CPC: Cetylpyridinium chloride

plastic materials to inhibit the growth of or kill test microorganisms. In the ISO 22196, *Staphylococcus aureus* is used as the test organism; however, in this study, we used *S. sobrinus* OMZ176 to investigate the effect of the spray on oral bacteria.

MG sheet (MG21; CGK Corp., Hiroshima, Japan) was used in the antibacterial test. It was cut into 15 mm × 15 mm pieces. All sheets were 2-mm thick and transparent. Before the test, the test pieces were immersed in ethanol for 15 min, wiped with sterile gauze, and dried for 30 min for disinfection.

The test bacteria were cultured in blood agar plates for 48 h at 35°C. The cells were then incubated in a brain–heart infusion medium, which was supplemented with 5 mg/L hemin and 50 μg/L Vitamin K1, for 16 h at 35°C. Subsequently, the medium was replaced with fresh brain–heart infusion medium, and the cells were incubated again for 16 h at 35°C. The bacterial solution was diluted, and the bacterial density was adjusted to approximately 1.0×10^4 cells/mL. A 10-fold-diluted bacterial solution (10 mL) was added to the wells in a six-well plate, and MG test pieces were immersed in the wells; the plate was aerobically incubated for 2 h at 35.6°C.

After removing the test pieces immersed in the bacterial solution, they were divided into two MG spray and one control groups (n = 4). In the MG spray groups, the front and back of the test piece were sprayed with MG spray twice, left for 30 s (MG spray-1) or 60 s (MG spray-2), and washed in physiological saline. In the control group, the test piece was washed with only physiological saline. Each test piece was placed in a 50-mL Falcon tube with 5 mL of physiological saline and stirred for 1 min to collect the bacteria surviving on the surfaces. The agitated physiological saline was aerobically cultured for 24 h at 35°C on blood agar medium, and then the colonies were counted.

After incubation, colony-forming units (CFUs) were visually counted, and then transformed to log CFUs to evaluate the antimicrobial activity of the test substance using the following formula:

$$R = (\log [B/A] - \log [C/A]) = (\log [B/C]).$$

Where R represents the antibacterial activity, A is the average number of viable bacteria immediately after inoculation on the control specimen, B is the average number of viable bacteria on the control specimen after 24 h, and C is the average number of viable bacteria on the antibacterial specimen after 24 h.[14] The logarithm of difference between the two values was used to calculate the antibacterial activity. Samples with $R \geq 2$ were considered to have antibacterial effects and be associated with a death rate of >99% (ISO22196).[14]

Statistical analysis

All recorded values were tabulated using JMP® 14 (SAS Institute Inc., Cary, NC, USA) for statistical analyses. Data were presented as mean ± standard deviation. Differences in log CFUs among the groups were compared using the one-way analysis variance and Tukey's honestly significant difference test. Statistical significance was considered at $P < 0.05$.

RESULTS

Visual inspection revealed that the number of CFUs in the MG spray-2 group was less than that in the other groups. The log CFUs are shown in Figure 4. The log CFUs of the MG spray-2 group were significantly

Figure 3: Mouthguard disinfectant spray.

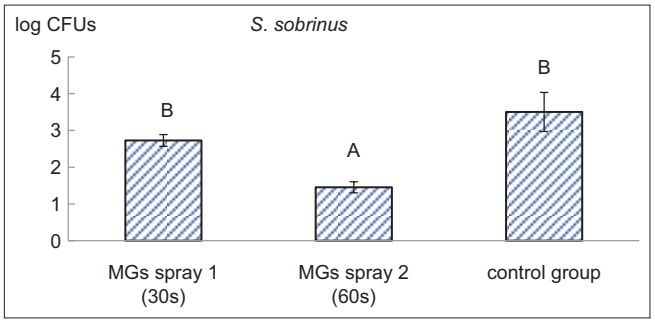

Figure 4: Results of the antibacterial test with the mouthguard disinfectant spray. All values were expressed as mean ± standard deviation (n = 4). Values with the same letter were not statistically significantly different ($P < 0.05$). Bacterial colonies in mouthguard spray Group 2 (application for 60 s) were significantly inhibited.

lower than those of the other groups; this suggested that colony forming in the MG spray-2 group was significantly inhibited. The antibacterial activity of MG spray-2 treatment against *S. sobrinus* was 2.1, whereas that of MG spray-1 treatment was 0.8.

DISCUSSION

As mentioned earlier, the storage conditions of MGs are often not hygienic enough.[2,3] Moreover, only a few players brush their teeth before and after using MGs;[15] thus, the oral conditions when using MGs are not sufficiently hygienic. The use of MGs also affects the oral cavity environment, such as the pH buffering capacity of saliva and bleeding indicators.[16] Currently, there are no established standards and guidelines for the hygienic use of MGs. Consequently, the methods of MG storage differ among individuals and the quality of hygienic management varies widely.

A strength of our study is we used an immersion time of 2 h, which is the estimated average daily wear duration of MG by an athlete. In addition, we used a modified ISO 22196 test with a common bacterium in the oral cavity, *S. sobrinus*, instead of *S. aureus*. *S. sobrinus* is a Gram-positive, facultatively anaerobic bacterium.[13] Although *S. sobrinus* is less frequently detected than *S. mutans* in the oral cavity, it is more virulent due to its high acidogenicity and acid tolerance.[17,18] It might be used as a predictive marker for caries at the supragingival biofilm.[19] It has been reported that the co-existence of both species is associated with a higher incidence of dental caries in children.[20] The antibacterial effect of MG sprays containing CPC has been studied against *S. mutans*,[7] but not against *S. sobrinus*.

In this study, we confirmed the antibacterial effect of an MG disinfectant spray against *S. sobrinus*. The duration for which the test pieces were left after chemical spraying was found to be an important factor influencing the antibacterial effect. Our results showed that the adhered *S. sobrinus* on MGs was not sufficiently removed or killed by simply washing with only physiological saline. The MG disinfectant spray was effective in the removal of the adhered *S. sobrinus* on MGs. These results confirmed the effectiveness of the chemical cleaning method, and they were consistent with the findings of previous studies.[7,8]

The antibacterial activity of the MG spray-1 (30 s) treatment was 0.8, indicating an insufficient inhibitory effect. However, the antibacterial activity of the MG spray-2 (60 s) treatment against *S. sobrinus* was 2.1; furthermore, the number of bacterial cells on the surface after MG disinfectant spraying decreased and the death rate was >99%. The antibacterial effect was optimal when the spray was left for 60 s. The optimal antibacterial effect of the MG disinfectant spray might not be achieved in a short time. Thus, the duration of treatment is an important factor influencing the antibacterial effect of the MG disinfectant spray. Therefore, MG users should be encouraged to leave the MGs for approximately 1 min after spraying.

As not only bacteria in the oral cavity but also those in the soil can attach to the surface of MGs, further investigation with more bacterial species is necessary. The duration of treatment for each bacterium should also be investigated. Several disinfectant MG sprays are commercially available, and their antimicrobial properties should be compared in MG users. A comparison of the antimicrobial characteristics of different products will help determine appropriate products for different situations, such as indoor and outdoor activities. Furthermore, it is necessary to perform physical property analyses, such as tensile, elongation, wear resistance, and water absorption tests, to evaluate the physical influence of the spray on MG sheet and the effect of different spray times. The MG sheet used in this study was a novel sheet, and its surface is smooth. Micro-cracks and innumerable scars might develop on the MG surface due to daily use. In future, studies on the effectiveness of MG disinfectant spray should focus on how these factors affect the antibacterial effect and elucidate the time of effect onset.

CONCLUSION

In this study, we demonstrated the antibacterial effect of an MG disinfectant spray against *S. sobrinus*. The optimal antibacterial activity of this MG disinfectant spray was achieved when the test piece was left for 60 s. The treatment duration is an important factor in influencing the antibacterial effect of MG disinfectant sprays.

Acknowledgment

The authors thank Editage (www.editage.jp) for English language editing.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial, in this article.

REFERENCES

1. FDI World Dental Federation. FDI policy statement on sports dentistry: Adopted by the FDI general assembly, September 2016, Poznan, Poland. Int Dent J 2017;67:18-9.
2. Himeno K. Importance of oral hygiene education for mouthguard using athletes. Int J Dent Hygine 2004;33:29-33.
3. Namba EL, Strapasson A, Jarzynski J, Baratto SP, Tomazinho PH. Sanitization of sports mouthguards. RSBO 2013;10:72-5.
4. Glass R, Bullard J, Conrad R. The contamination of protective mouthguards: A characterization of the microbiota fund in football players' protective mouthguards as compared to oral micro biota fund in first-year medical students. Am Dent Inst Con Educ J 2006;93:23-8.
5. Yoshida Y, Churei H, Takeuchi Y, Wada T, Uo M, Izumi Y, *et al*. Novel antibacterial mouthguard material manufactured using silver-nanoparticle embedded ethylene-vinyl acetate copolymer master batch. Dent Mater J 2018;37:437-44.
6. Glass RT, Conrad RS, Köhler GA, Warren AJ, Bullard JW. Microbiota found in protective athletic mouthguards. Sports Health 2011;3:244-8.
7. Suzuki H, Kawahara M, Hirata R, Matsubara Y, Takahashi S, Kuroki S, *et al*. Research on development of mouthguard cleaning solution. J Sports Dent 2010;13:92-8.
8. Ogawa T, Yamasaki S, Honda M, Terao Y, Kawabata S, Maeda Y. Long-term survival of salivary streptococci on dental devices made of ethylene vinyl acetate. Int J Oral Sci 2012;4:14-8.
9. Mańka-Malara K, Panasiewicz P, Kacprzyk M, Gawryszewska M, Mierzwińska-Nastalska E, Gawlak D. The effect of decontamination procedures on elastic polymeric materials used in dental mouthguards fabrication. Acta Bioeng Biomech 2019;21:63-71.
10. Atsumi Y, Sawaguchi M, Takahashi F, Nishikawa Y, Totsukasa T, Morita O. Cleaning effects of mouthguards used denture cleansers. J Sports Dent 2003;6:46-50.
11. Godfrey KM. Cationic emulsifiers in cosmetics. J Soc Cosmetic 1966;17:17-27.
12. Yamaguchi R, Takayama K. Disinfectants: Practices and background knowledge. J Environ Infect 2017;32:330-6.
13. Conrads G, De Soet JJ, Song L, Henne K, Sztajer H, Wagner-Döbler I, *et al.* Comparing the cariogenic species *Streptococcus sobrinus* and *S. mutans* on whole genome level. J Oral Microbiol 2014;6:26189.
14. International Organization for Standardization. ISO 22196:2011. Available from: https://www.iso.org/standard/54431.html. [Last accessed on 2011 Aug 15].
15. Asakawa T, Kanasaki A, Suzuki K, Iwata Y, Yasuda A, Asano T, *et al*. A survey of dental injuries and dental health among amateur kickboxing athletes. J Sports Dent 2017;21:1-10.
16. D'Ercole S, Martinelli D, Tripodi D. Influence of sport mouthguards on the ecological factors of the children oral cavity. BMC Oral Health 2014;14:97.
17. de Soet JJ, van Loveren C, Lammens AJ, Pavicić MJ, Homburg CH, ten Cate JM, *et al*. Differences in cariogenicity between fresh isolates of *Streptococcus sobrinus* and *Streptococcus mutans*. Caries Res 1991;25:116-22.
18. Inquimbert C, Bourgeois D, Bravo M, Viennot S, Tramini P, Llodra JC, *et al*. The oral bacterial microbiome of interdental surfaces in adolescents according to carious risk. Microorganisms 2019;7:319.
19. Gross EL, Beall CJ, Kutsch SR, Firestone ND, Leys EJ, Griffen AL. Beyond *Streptococcus mutans*: Dental caries onset linked to multiple species by 16SrRNA community analysis. PLoS One 2012;7:e47722.
20. Okada M, Kawamura M, Oda Y, Yasuda R, Kojima T, Kurihara H. Caries prevalence associated with *Streptococcus mutans* and *Streptococcus sobrinus* in Japanese schoolchildren. Int J Paediatr Dent 2012;22:342-8.