Original Article

Comparative Evaluation of Antimicrobial Efficacy of Fluoride-Based and Self-Assembling Peptide P_{11}-4-based Tooth Remineralization Agents on *Streptococcus mutans*: A Microbiological Study

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Abstract

Background: Dental caries is a biofilm-related oral disease that continues to afflict the majority of the world's population. The disease results from an interaction between specific bacteria and dietary constituents within a biofilm known as dental plaque. Among the cariogenic microorganisms, *Streptococcus mutans* (*S. mutans*) plays pivotal role in caries-inducing processes. **Objectives:** Evaluate and compare the antimicrobial efficacy of self-assembling peptide P_{11}-4-based tooth remineralization agents on *S. mutans*. **Materials and Methods:** An *in vitro* microbiological study. The antibacterial efficacy of self-assembling peptide P_{11}-4 gel (Group 1), fluoride enhanced hydroxyapatite gel (Group 2), acidulated phosphate fluoride gel (Group 3), chlorhexidine gluconate gel 1.0% w/w (Group 4; positive control), and normal saline (Group 5; negative control) was performed using time-kill assay over a period of 24 h and the number of *S. mutans* colony-forming units (CFUs) were calculated. **Statistical Analysis:** Statistical analysis was done using Kruskal–Wallis test and Mann–Whitney *post hoc* Test. The level of significance was set at $P < 0.05$. **Results:** Group 1 showed mean CFUs ($\times 10^3$) of 841.33 ± 3.51, Group 2 showed 10035.33 ± 60.68, Group 3 showed 1058.00 ± 56.96, Group 4 showed 0.00 ± 0.00, and Group 5 showed mean CFUs with 15226.67 ± 96.67. The difference in the mean CFUs ($\times 10^3$) between different groups was statistically significant at $P < 0.001$. **Conclusion:** The self-assembling peptide P_{11}-4-based tooth remineralization agent exhibited an inhibitory influence on *S. mutans* and hence formation of cariogenic bacteria dominant biofilm can thus be affected by its application.

Keywords: *Dental caries, fluorides, remineralizing agents, self-assembling peptides P_{11}-4, Streptococcus mutans*

Introduction

Dental caries persists as one of the most common oral diseases afflicting mankind. It adversely affects the physical and psychological well-being of an individual and is associated with an alteration in the oral health-related quality of life and an additional financial burden. The white spot lesions represent the earliest macroscopic evidence of enamel caries that mark the process of caries initiation with most of the mineral loss from the subsurface area and a relatively intact superficial layer.[1] As the collapse and irreversible breakage of the mineralized surface occurs following approximately 30% demineralization, early diagnosis and treatment of the white spot lesion with remineralizing agents is recommended.[1] Over the years, an array of strategies have evolved to intercept the progression of white spot lesions into frank cavitation.

Since the 1950s, fluoride has been widely used in caries prevention due to its strong anticaries action. Its effect on dental hard tissues is extensively documented.[2] One of the mechanisms of action of fluoride is related to its ability to bond with hydroxyapatite of enamel forming fluorhydroxyapatite and increase the resistance to demineralization and promote remineralization.

Recently, the biomimetic remineralization promoted by self-assembling peptide P_{11}-4 has been proven *in vitro* as an effective therapy for initial caries. P_{11}-4 has rationally been designed to facilitate formation of hydroxyapatite on its surface. The formulation is optimized to ensure the ability of monomeric P_{11}-4 to penetrate past

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the subsurface lesions and assemble into a biomatrix within. Furthermore, P_{11}-4 has shown that it assembles into fibers within carious lesions and promotes the remineralization.[3]

Dental caries being a complex, multifactorial biofilm-induced oral disease where *Streptococcus mutans (S. mutans)* plays a pivotal role in the development of virulent cariogenic biofilms,[4] use of tooth remineralizing agents with inhibitory influence on the bacterial growth is of paramount importance. Hence, as reducing the bacterial load of the oral cavity is one of the cardinal biological goals in preventing dental caries, the present study was designed to evaluate the effect of self-assembling peptide (P_{11}-4)-based tooth remineralization agents on the *S. mutans* count.

Materials and Methods

Isolation of *Streptococcus mutans*

The study was approved by the Ethical Committee of Sri Rajiv Gandhi College of Dental Sciences (No. SRGCDS/2021/127) on January 18, 2021. Unstimulated human saliva was obtained from a single healthy volunteer after obtaining written informed consent based on the Helsinki Declaration of 1975, as revised in 2000[5] who had refrained from eating, drinking, or tooth brushing for at least 2 h. The volunteer had not received any medication during the 3 months preceding the study and had no active gingival/periodontal disease or active caries.

S. mutans was isolated from the saliva on Mitis Salivarium Bacitracin agar (HIMEDIA, Mumbai) incubated at 37°C for 24 h in a candle extinction jar to provide 5%–10% carbon dioxide. The colonies were identified by gram staining reaction and biochemical tests. A colony of *S. mutans* was inoculated into 5 ml brain heart infusion (BHI) broth (HIMEDIA, Mumbai) and incubated at 37°C for 4 h. The culture suspension was adjusted to Mcfarland 0.5 opacity standard to obtain a bacterial suspension containing 1.5×10^8 organisms/ml.

The test materials were categorized as follows:

- Group 1: Self-assembling peptide P_{11}-4 gel (Curodont™ Protect; Credentis, Switzerland)
- Group 2: Fluoride enhanced hydroxyapatite gel (Remin Pro®; VOCO, Germany)
- Group 3: Acidulated phosphate fluoride gel (Insta Topical Gel; RRP, India)
- Group 4: Chlorhexidine gluconate gel 1.0% w/w (Hexigel®; ICPA, India) (Positive control)
- Group 5: Normal saline (Negative control).

Inoculation of *Streptococcus mutans* test materials

About 10 µl of the adjusted *S. mutans* culture was inoculated on the test materials placed in the Eppendorf tubes and incubated for 1 h at 37°C in a moist chamber inside the candle extinction jar in triplicates. About 1 ml of sterile BHI broth was added to the test materials and vortexed for 30 s; 10 µl of the suspension was then transferred to a sterile BHI agar plate (HIMEDIA, Mumbai) and spread uniformly using a sterile L spreader (Tarson, Kolkata). The agar plates were incubated at 37°C for 24 h in a candle extinction jar. The colonies were counted using a digital colony counter and the CFU/ml were calculated based on the dilution of the culture suspension done [Figure 1]. The study parameters were obtained in terms of triplicate for further consideration to perform statistical analysis.

Statistical analysis

Statistical analysis was done using Kruskal–Wallis test and Mann–Whitney *post hoc* test. The level of significance was set at $P < 0.05$.

Results

Table 1 compares the mean colony-forming units (CFUs) ($\times 10^3$) between different groups. The test results demonstrate that Group 1 showed mean CFUs ($\times 10^3$) of 841.33 ± 3.51, Group 2 showed 10035.33 ± 60.68, Group 3 showed 1058.00 ± 56.96, Group 4 showed 0.00 ± 0.00, and Group 5 showed mean CFUs with 15226.67 ± 96.67 [Graph 1]. This difference in the mean CFUs ($\times 10^3$) between different groups was statistically significant at $P < 0.001$.

Table 2 illustrates multiple comparisons of difference in mean CFUs ($\times 10^3$) between groups.

The test results demonstrate that the Group 4 showed significantly least mean CFUs ($\times 10^3$) as compared to other groups at $P < 0.001$. This was followed next by Group 1 showing significantly lesser mean CFU ($\times 10^3$) levels as compared to other groups at $P < 0.001$ and with Group 3 at $P = 0.007$. This was followed next by Group 3 showing significantly lesser mean CFU ($\times 10^3$) levels as compared to Group 2 and Group 5 at $P < 0.001$ and finally Group 2 showed significantly lesser mean CFUs as compared to Group 5 at $P < 0.001$. This infers that Group 4 showed significantly least CFUs levels, followed by Group 1, 3, 4 and highest in Group 5 [Graph 2].

Discussion

Dental caries is a multifactorial disease caused by an interplay of various factors such as a susceptible host,

Table 1: Comparison of mean CFUs ($\times 10^3$) between different groups using Kruskal-Wallis test

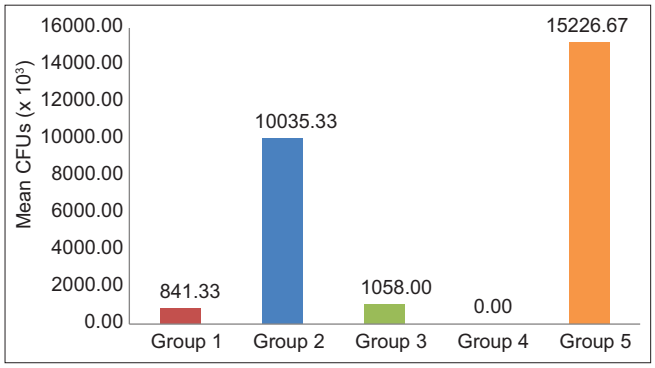
Groups**	*n*	Mean±SD	Minimum	Maximum	*P*
Group 1	3	841.33±3.51	838	845	<0.001*
Group 2	3	10,035.33±60.68	9994	10,105	
Group 3	3	1058.00±56.96	996	1108	
Group 4	3	0.00±0.00	0	0	
Group 5	3	15,226.67±98.69	15,138	15,333	

*Statistically significant; **Group 1-Self-assembling peptide P_{11}-4 gel, Group 2-Fluoride enhanced hydroxyapatite gel, Group 3-Acidulated phosphate fluoride gel, Group 4-Chlorhexidine gluconate gel and Group 5-Saline. SD: Standard deviation

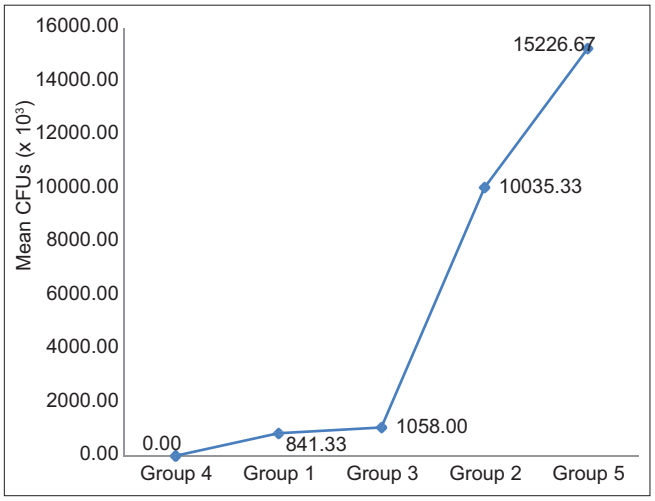
Table 2: Multiple comparisons of difference in CFUs (×10³) between groups using Mann–Whitney *post hoc* test

Groups (I)	Groups (J)	Mean difference (I-J)	95% CI for difference		*P*
			Lower	Upper	
Group 1	Group 2	−9194.00	−9349.20	−9038.80	<0.001*
	Group 3	−216.67	−371.87	−61.47	0.007*
	Group 4	841.33	686.13	996.53	<0.001*
	Group 5	−14,385.33	−14,540.53	−14,230.13	<0.001*
Group 2	Group 3	8977.33	8822.13	9132.53	<0.001*
	Group 4	10,035.33	9880.13	10,190.53	<0.001*
	Group 5	−5191.33	−5346.53	−5036.13	<0.001*
Group 3	Group 4	1058.00	902.80	1213.20	<0.001*
	Group 5	−14,168.67	−14,323.87	−14,013.47	<0.001*
Group 4	Group 5	−15,226.67	−15,381.87	−15,071.47	<0.001*

*Statistically significant. CI: Confidence interval



Graph 1: Mean CFUs (×10³) between different groups. CFUs: Colony-forming units



Graph 2: Mean CFUs (×10³) between different groups. CFUs: Colony-forming units

cariogenic microorganisms, and a conducive environment. *Mutans streptococci* regarded as the primary etiologic agent of dental caries, through adhesion attaches to the dental pellicle and breaks down sugars for energy to produce lactic acid, causing an acidic environment around the tooth and thereby resulting in the demineralization of the enamel and dentin.[6] Early diagnosis and management of the incipient white spot carious lesion will play an important role in preventing the progression of the lesion and subsequent breakdown of the tooth structure. A wide variety of products that facilitate the remineralization of the incipient caries lesions are currently available. In addition to the remineralization potential, demonstration of antibacterial efficacy by these agents is highly desirable. The present study thus aimed to evaluate the effect of self-assembling peptide (P_{11}-4)-based tooth remineralization agents on the *S. mutans* count.

In the present study it was observed that the self-assembling peptide P_{11}-4-based remineralization agent (Curodont™ Protect; Credentis, Switzerland) exhibited lower *S. mutans* colony count followed by acidulated phosphate fluoride gel (Insta Topical Gel; RRP, India) and fluoride enhanced hydroxyapatite gel (Remin Pro®; VOCO, Germany), thereby demonstrating antimicrobial effect against *S. mutans*.

Fluorides are well known for their antimicrobial effects, they affect the enrichment of *S. mutans* by reducing environmental acidification in dental biofilms. Studies done by Pandit *et al*.[7] and Neilands *et al*.[8] support the idea that fluoride can affect the acidogenicity, acidurity, and extracellular polysaccharide formation of dental biofilms. However, fluorides are effective against *S. mutans* at specific concentrations and further increase in it can lead to toxicity.

Self-assembling β-sheet-forming peptides have shown to form three-dimensional fiber networks supporting tissue regeneration. In particular, the self-assembling peptide P_{11}-4 has shown potential in the treatment and prevention of dental caries. Application of monomeric P_{11}-4 solution to early carious lesions can increase net mineral gain by forming *de novo* hydroxyapatite crystals. Peptide P_{11}-4 diffuses into the subsurface lesion body and assembles therein into higher order fibrils, facilitating mineralization of the subsurface volume by mimicking the natural biomineralization of the tooth enamel, and it remains within the lesion body as a scaffold built-in

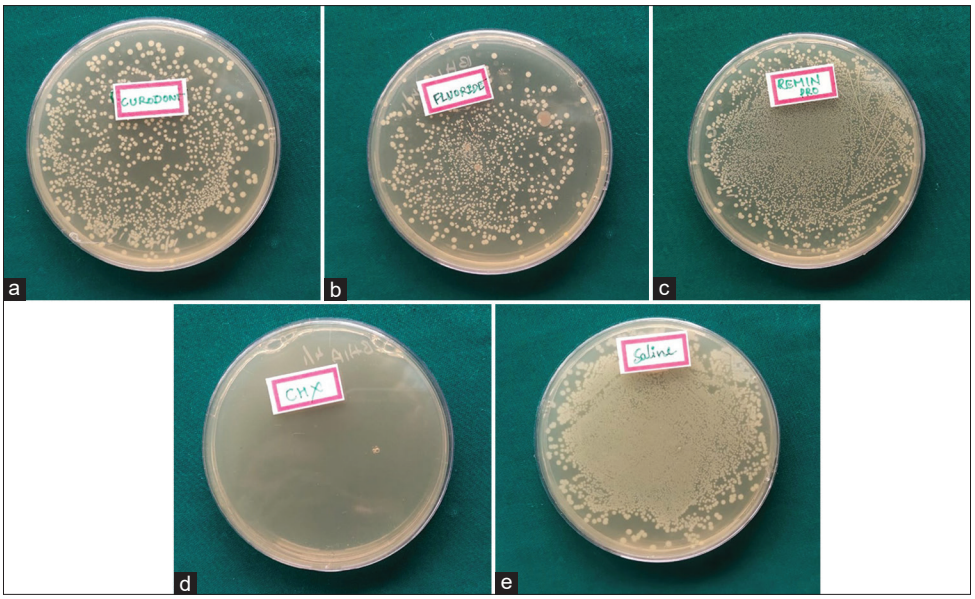


Figure 1: S. mutans colonies seen with: (a) Self-assembling peptide P^{11}-4 gel. (b) Fluoride enhanced hydroxyapatite gel. (c) Acidulated phosphate fluoride gel. (d) Chlorhexidine gluconate gel (positive control). (e) Saline (negative control). Streptococcus mutans: S. mutans

by the newly formed hydroxyapatite.[9] In the present study self-assembling peptide-based remineralization agent showed lesser *S. mutans* CFU/ml compared to the other test materials, thereby demonstrating antimicrobial efficacy. However, the self-assembling peptide P_{11}-4-based agent being a relatively newer material direct comparison of the antibacterial property could not be done due to paucity of literature.

Limitations of the study

The *in vitro* design of the present study does not reproduce the complex and heterogeneous intraoral conditions such as the volume and composition of saliva and tooth surface area experienced in *in vivo* de/remineralization. Furthermore, in the present study, the antibacterial influence of the agents was determined only on *S. mutans* as it plays a major role in initiation of dental caries. However, due to the polymicrobial etiology of dental caries, further research on the influence of these agents on other cariogenic bacteria is warranted. Furthermore, the antibacterial efficacy cannot be entirely attributed to self-assembling peptide P_{11}-4-based agent due to the presence of fluoride in Curodont™ Protect. Studies involving the use of self-assembling peptide P_{11}-4-based agents without the synergistic influence of fluoride or other therapeutic agents hold scope for further research.

Conclusion

The self-assembling peptide P_{11}-4-based remineralization agent exhibited an inhibitory influence on *S. mutans* and can be regarded as an alternative to conventional agents in the prevention and interception of progression of incipient caries lesions.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Sindhura V, Uloopi KS, Vinay C, Chandrasekhar R. Evaluation of enamel remineralizing potential of self-assembling peptide P (11)-4 on artificially induced enamel lesions *in vitro*. J Indian Soc Pedod Prev Dent 2018;36:352-6.
2. Buzalaf MA, Pessan JP, Honório HM, Ten Cate JM. Mechanisms of action of fluoride for caries control. Monogr Oral Sci 2011;22:97-114.
3. Alkilzy M, Santamaria RM, Schmoeckel J, Splieth CH. Treatment of carious lesions using self-assembling peptides. Adv Dent Res 2018;29:42-7.
4. Jeon JG, Rosalen PL, Falsetta ML, Koo H. Natural products in caries research: Current (limited) knowledge, challenges and future perspective. Caries Res 2011;45:243-63.
5. World Medical Association Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects. Edinburgh: The Declaration of Helsinki Revised by the World Medical Organisation; 2000.
6. Colak H, Dülgergil CT, Dalli M, Hamidi MM. Early childhood caries update: A review of causes, diagnoses, and treatments. J Nat Sci Biol Med 2013;4:29-38.
7. Pandit S, Kim HJ, Song KY, Jeon JG. Relationship between fluoride concentration and activity against virulence factors and viability of a cariogenic biofilm: *In vitro* study. Caries Res 2013;47:539-47.
8. Neilands J, Troedsson U, Sjödin T, Davies JR. The effect of delmopinol and fluoride on acid adaptation and acid production in dental plaque biofilms. Arch Oral Biol 2014;59:318-23.
9. Kind L, Stevanovic S, Wuttig S, Wimberger S, Hofer J, Müller B, *et al.* Biomimetic remineralization of carious lesions by self-assembling peptide. J Dent Res 2017;96:790-7.