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Antibacterial Properties of Pit and Fissure Sealant Containing S-PRG filler on *Streptococcus mutans*

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Abstract

The purpose of this study was to evaluate the antibacterial properties of a sealant containing S-PRG filler compared to those of two contemporary commercial sealants to determine the inhibition of bacterial growth in broth culture and biofilm formation using the CDC Biofilm Reactor. The BeautiSealant containing S-PRG filler, the fluoride releasing Clinpro™ sealant, which are known to have higher antibacterial effects, and the non-fluoride releasing Concise™ sealant were selected for this study. A *Streptococcus mutans* culture in BHI broth without sealant served as a negative control in the planktonic growth inhibition test. As a result, bacterial growth was inhibited in all three sealant groups compared to that in the control. The Clinpro™ sealant showed a significantly reduced number of CFUs compared to those of the BeautiSealant and Concise™ sealants. However, no significant difference was detected between the BeautiSealant and Concise™ sealants. The Clinpro™ sealant significantly decreased biofilm formation compared to that by the BeautiSealant and Concise™ sealants. No significant difference was observed between the BeautiSealant and Concise™ sealants. In conclusion, the sealant containing S-PRG filler had a less potent anti-bacterial property and increased biofilm formation capacity compared to those of the fluoride releasing Clinpro™ sealant.

Key words : S-PRG filler, BeautiSealant, Pit and fissure sealants, *Streptococcus mutans*, Fluoride

Ⅰ. Introduction

Dental caries develop when bacterial plaque cannot be removed from the tooth surface. Approximately 90% of carious lesions are found in the pits and fissures of permanent molar teeth[1)]. Sealants have been used for decades as a preventive measure against caries developing in susceptible pits and fissures by forming a physical barrier between the oral environment and deep fissures[2-4)]. Once the pit and fissure are covered with a sealant, the bacteria are isolated, and the number of cariogenic bacteria (including *Streptococcus mutans*) decrease to 50%[1)].

This positive effect can be enhanced by adding some antibacterial agents to the sealant material[5)]. In recent years, chlorhexidine[6)], bioactive glass[7)], silver and zinc oxide nanoparticles[8)], fluoride compounds[9)], and S-PRG filler[10)] have been added to sealants as antibacterial agents.

S-PRG fillers are prepared via an acid-base reaction (of a traditional glass ionomer) between fluoroaluminosilicate glass (base) and a polyacrylic acid in the presence of water, whereby the preliminary product is a stable glass ionomer phase within the glass particles[11,12)]. Upon freeze-drying, the desiccated xero gel is further milled and silane-treated to form an S-PRG filler of a

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specific size range[13]. This filler has fluoride release and recharge potential[14], inhibits dentin demineralization[15], prevents demineralization of surrounding orthodontic brackets[16] and reduces plaque formation[17,18]. This positive effects may be due to the ability of the S-PRG filler to release various ion species (fluoride, strontium, aluminum, sodium, etc.) as well as its capacity as an acid buffer[19].

Resin composites that include S-PRG filler particles have antibacterial effects compared to those of conventional resin composite materials. Saku *et al.*[17] reported less plaque accumulation in resin containing S-PRG filler than in other composite resin restorations. Kimyai *et al.*[20] reported that bacterial adherence in a resin containing S-PRG filler is lower than that to a microfilled composite resin not containing S-PRG filler regardless of the prophylaxis technique and the generated surface roughness. However, the antibacterial effects of a sealant containing less filler than a composite resin containing S-PRG filler have not been reported.

The purpose of this study was to evaluate the antibacterial properties of a sealant containing S-PRG filler compared to those of two contemporary commercial sealants to determine the inhibition of bacterial growth in broth culture and biofilm formation using the CDC Biofilm Reactor (BioSurface Technologies Corp., Bozeman, MT, USA)[21].

Ⅱ. Materials and methods

1. Materials

The BeautiSealant containing S-PRG filler, the fluoride releasing Clinpro™ sealant, which are known to have higher antibacterial effects[9], and the non-fluoride releasing Concise™ sealant were selected for this study. Table 1 lists the materials selected for this investigation and their manufacturers.

2. Specimen preparation

The specimens were 7 mm in diameter and 2 mm thick and prepared with a metallic mold. Each sealant was packed into the mold, pressed between two Mylar strips sandwiched with two glass slides, and polymerized for 20 sec from both ends of the molds with a LED light curing unit (Valo, Ultradent Products Inc, South Jordan, UT, USA). All specimens were sterilized by autoclaving at 121℃ at 15 lbs pressure for 15 min.

3. Bacterial strain and culture conditions

The bacterial strain used for this study was *S. mutans* (KPSK-2), which was obtained from Department of Oral Microbiology, Gangneung-Wonju National University. Bacterial cells were incubated in brain heart infusion broth (BHI) (Becton-Dickinson and Co., Sparks, MD, USA) under aerobic conditions supplemented with 5% CO_2 at 37℃ for 24 h. Turbidity of the bacterial suspensions was measured with a spectrophotometer (Smart Plus 2700, Young-woo Institute, Seoul, Korea). A standard curve comparing culture turbidity and bacterial cell number was established and utilized. The bacteria were diluted to 2×10^9 colony forming units (CFU)/mL with BHI broth.

4. Planktonic growth inhibition test

Two experimental sealant blocks of each group were crushed to a powder with a ceramic mortar and pestle to extend the surface area. The ground powder was filtered through a 500-mesh sieve (Standard sieve, SAEHAN Lab, Seoul, Korea) to obtain < 25 ㎛ sized particles and sterilized by autoclaving at 121℃ at 15 lbs pressure for 15 min. The bacterial culture was prepared as described above. The bacterial suspension was adjusted to 1.5×10^3 CFU/mL with BHI broth.

Table 1. Sealant used in this study

Group	Material	Composition	Manufacturer
I	BeautiSealant (S-PRG filler containing sealant)	TEGDMA, UDMA, Fluoroboroaluminosilicate glass, Micro fumed silica	Shofu Inc., Japan
II	Clinpro™sealant (Fluoride releasing sealant)	TEGDMA, Bis-GMA, Tetrabutyl-ammoniumtetrafluoroborate, Silane-treated silica	3M ESPE, USA
III	Concise™sealant (Non-fluoride releasing sealant)	TEGDMA, Bis-GMA	3M ESPE, USA

TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate; Bis-GMA, bisphenol A glycidylmethacrylate

A 0.2 g aliquot of powder from each group was immersed in 2 mL of bacterial suspension and incubated at 37℃ in a CO_2 incubator for 24 h. A bacterial suspension without sealant served as a negative control. A 0.05 mL aliquot of the bacterial suspension was serially diluted with PBS and plated onto blood agar plates. Colonies were counted after incubating for 24 h at 37℃ in a CO_2 incubator. The antibacterial activities of the tested materials were measured as inhibition of bacterial growth compared to the negative control. Results are CFU/mL.

5. *S. mutans* biofilm assay using the CDC Biofilm Reactor

The CDC Biofilm Reactor was used to prepare the *S. mutans* biofilm. The sealant blocks for growing the biofilm were mounted into eight rods (each rod held three discs) that can be removed and replaced aseptically through the lid (Fig. 1).

The size of each coupon was 1.27 cm in diameter and 0.3 cm in height. As the size of the sealant block (0.7 cm diameter and 0.2 cm height) was smaller than the coupon holder, the remainder was wrapped in hydrophilic vinyl polysiloxane. The blocks wrapped in vinyl polysiloxane were placed in the CDC Biofilm Reactor, and the reactor was sterilized by autoclaving at 121℃ at 15 lbs pressure for 15 min.

The CDC Biofilm Reactor was filled with 100 mL *S. mutans* suspension (2×10^9 CFU/mL) and 300 mL BHI broth, and placed on a stir plate at 50 rpm. After inoculation, the reactor was incubated under shear conditions, but no media flow, for 24 h. BHI broth was then pumped through the reactor at a flow rate of 18.6 mL/min for 72 h.

To evaluate formation of the *S. mutans* biofilm on the blocks, the hydrophilic vinyl polysiloxane was removed from the blocks with sterilized tweezers. The blocks were washed twice with PBS to remove the non-attached bac-

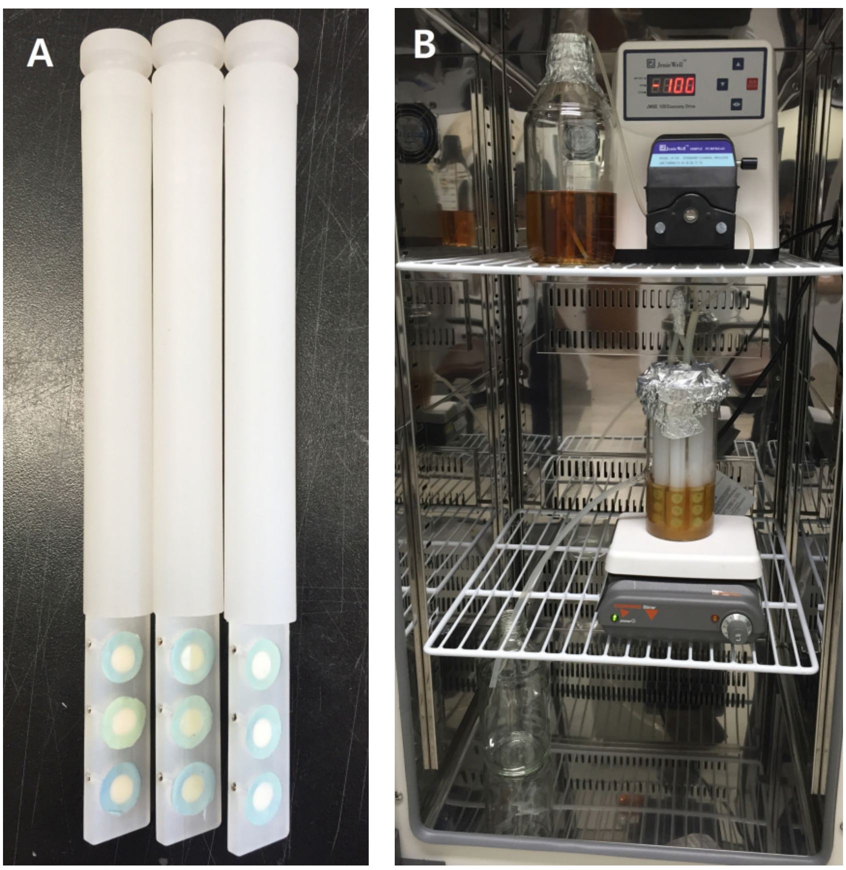

Fig. 1. (A) CDC Biofilm Reactor sampling rods with the BeautiSealant, Clinpro™ sealant, and Concise™ sealant discs. (B) Experimental set-up for the CDC Biofilm Reactor.

teria. Then, the blocks were transferred to 2 mL PBS and sonicated with an ultrasonic sonicator (VC 100, Sonics & Materials Inc., Danbury, CT, USA) for 15 s at 60 W to disperse the biofilm. A 0.1 mL aliquot of the dispersed solution was serially diluted with PBS and 0.05 mL of the diluted solution was plated onto blood agar plates. The colonies were counted after incubating for 24 h at 37℃ in a CO_2 incubator. The results are expressed as CFU/mL.

6. Scanning electron microscopic (SEM) observation of the *S. mutans* biofilm

The sealant blocks used for biofilm formation were pre-fixed with 4% glutaldehyde and 1% paraformaldehyde solution in 0.1 M cacodylate buffer at pH 7.4 for 4 h and then rinsed with 0.1 M cacodylate buffer three times for 10 min each. The blocks were dehydrated through an ethanol series (10, 60, 70, 80, 90, and 100%) for 20 min each with isoamyl acetate and dried with CO_2 using a critical point dryer (HPC-2 critical point dryer, Hitachi, Tokyo, Japan). The prepared blocks were observed under an SEM (VP-FEVP-FEVP-FE, SUPRA55VP, Zeiss, Zena, Germany).

7. Statistical analysis

Data are presented as means and standard deviations (SD). Intergroup differences were estimated by one-way analysis of variance (ANOVA), followed by a post-hoc multiple comparison (Tukey's test) to compare means. A *p*-value < 0.05 was considered significant.

Ⅲ. Results

1. Planktonic growth inhibition test

Table 2 and Figure 2 show the planktonic CFUs after incubation with the experimental materials. All materials showed significantly reduced planktonic CFUs ($p < 0.05$) compared to that of the negative control. The ANOVA showed significant differences among the three experimental groups. Tukey's post-hoc test indicated a significantly reduced number of CFUs by the Clinpro™ sealant ($p < 0.05$) compared to those of the BeautiSealant and Concise™ sealants.

Table 2. *Streptococcus mutans* cell count in the planktonic growth inhibition test (mean ± standard deviation CFU/mL)

Group (n = 4)	Bacterial count (CFU/mL × 10^5)
I (BeautiSealant)	1.143 ± 0.257[c]
II (Clinpro™ sealant)	0.234 ± 0.097[a,b,c]
III (Concise™ sealant)	1.418 ± 0.136[c]
IV (No sealant)	2.051 ± 0.285

The one-way ANOVA test, Tukey
[a] Compared to group I, statistically significant at $p < 0.05$
[b] Compared to group III, statistically significant at $p < 0.05$
[c] Compared to group IV, statistically significant at $p < 0.05$
No significant difference between groups I and III

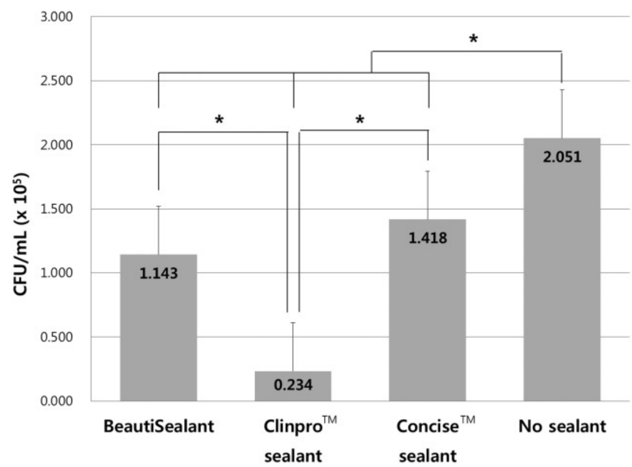

Fig. 2. *Streptococcus mutans* cell count in the planktonic growth inhibition test. Data are mean and standard deviation (SD) (mean ± SD, CFU/mL × 10^5) (* : $p < 0.05$).

2. Biofilm assay using the CDC Biofilm Reactor

The results of CFU values of *S. mutans* in biofilm are represented in Table 3 and Figure 3. The ANOVA showed significant differences among the three groups. Tukey's post-hoc test indicated a significantly reduced number of CFUs by the Clinpro™ sealant ($p < 0.05$) compared to those of the BeautiSealant and Concise™ sealants.

Table 3. *Streptococcus mutans* cell count in biofilm assay (mean ± standard deviation, CFU/mL)

Group (n = 9)	Bacterial count (CFU/mL × 10^5)
I (BeautiSealant)	1.416 ± 0.626
II (Clinpro™ sealant)	0.631 ± 0.309[a,b]
III (Concise™ sealant)	1.738 ± 0.767

The one-way ANOVA test, Tukey

[a] Compared to group I, statistically significant at $p < 0.05$

[b] Compared to group III, statistically significant at $p < 0.05$

No significant difference between groups I and III

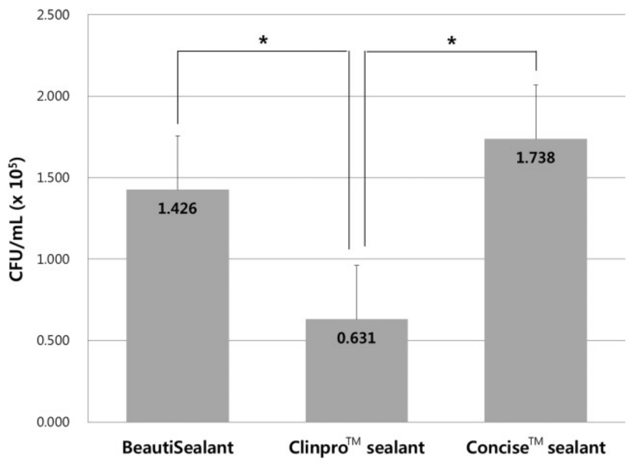

Fig. 3. *Streptococcus mutans* cell count in biofilm assay. Data are mean and standard deviation (SD) (mean ± SD, CFU/mL) (* : $p < 0.05$).

3. SEM observations of adherent bacteria

To confirm our results, we observed the *S. mutans* biofilm by SEM. Figure 4 shows SEM photographs of *S. mutans* that adhered to the respective material. It was observed by an SEM that the number of *S. mutans* that adhered to the surface of the Clinpro™ sealant (B1 and B2) was significantly lower than that to the BeautiSealant (A1 and A2) and the Concise™ sealant (C1 and C2).

Ⅳ. Discussion

S. mutans was chosen as a representative cariogenic oral bacterium because it is one of the most important microorganisms in the etiology of dental caries and is particularly found in early plaque. *S. mutans* produces glucosyltransferase that enable glucose to be transferred from sucrose for synthesis of glucans (cellulose-like polymers), which increase cariogenicity[22].

A review of comparative studies examining bacterial levels in sealed permanent teeth showed that sealants reduce bacteria in caries lesions, but some studies re-

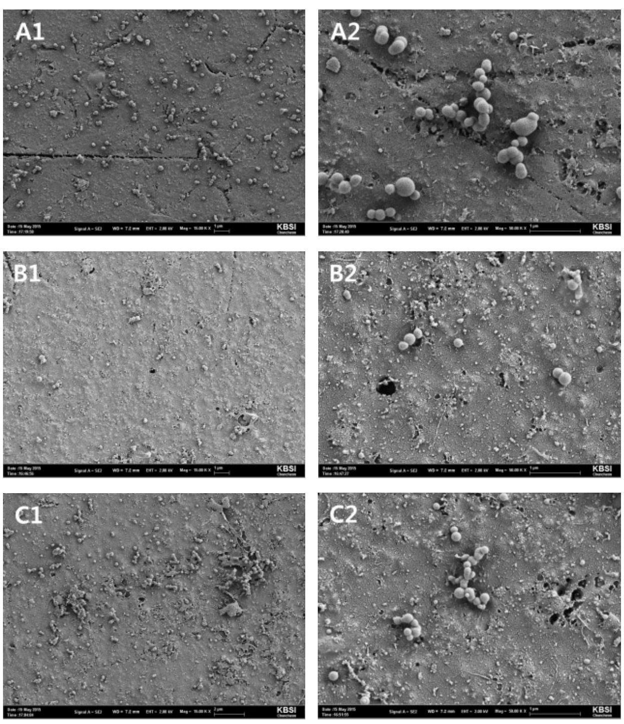

Fig. 4. Scanning electron microscopic image of sealant colonized with *Streptococcus mutans* after 72 h in the CDC Biofilm Reactor. (A) BeautiSealant, (B) Clinpro™ sealant, and (C) Concise™ sealant. (1) Magnification (× 15,000), (2) magnification (× 50,000).

ported that low levels of bacteria persist[23]. As microleakage of sealant cannot be avoided, antibacterial properties of fissure sealant materials may contribute to prevent occlusal caries.

Thus, we carried out this study to evaluate the antibacterial properties of sealants containing S-PRG filler compared to that of contemporary commercial sealants in vitro. The test materials included the S-PRG filler-containing sealant BeautiSealant, which has antibacterial effect by releasing a number of ions, the fluoride-releasing Clinpro™ sealant, and the non-fluoride releasing Concise™ sealant. Bacterial growth was inhibited in all three sealant groups compared to that in the control. Antibacterial effects would be attributed to low pH of sealants or ions released from sealants.

Song[24] reported that the degree of sealant conversion is 40-60%; therefore, unpolymerized monomers remain. These unpolymerized monomers could influence the lower pH environment and affect growth of *S. mutans*[25]. Because all three groups had monomers, they may have affected inhibition of bacterial growth.

More bacterial growth was observed in the BeautiSealant group than that in the Clinpro™ fluoride releasing sealant group. Similar bacterial growth was observed when compared to the non-fluoride releasing Concise™ sealant. Previous studies reported that S-PRG filler has an antibacterial effect by releasing a number of ions. In particular, the releasing and recharging ability of fluoride ions from the S-PRG filler is excellent[14,26]. Fluoride has several mechanisms for its antibacterial effect. Fluoride interferes with bacterial metabolism and dental plaque acidity, inhibits the glycolytic enzyme enolase and a proton-extruding ATPase, as well as the bacterial colonization and competition[4,27]. Furthermore, intracellular or plaque-associated enzymes, such as acid phosphatases, pyrophosphatases, peroxidases, and catalases may be affected by fluoride ions[28]. The S-PRG filler releases inorganic elements, such as Sr, Al, B, etc. Sr shows a synergistic antibacterial effect when combined with fluoride[29,30]. In addition, Al release is associated with enhanced fluoride release, which may lead to an increase in the number of alumino-fluoro complexes[31]. An in vivo study showed that B has antibacterial activity against periodontitis and inhibits bacterial and fungal quorum sensing[32]. Moreover, Sr, F, and B ions contribute to inhibit growth of oral bacteria[33]. Therefore, ions released from the S-PRG filler adjacent to the enamel would suppress bacterial growth and subsequent acid production in the oral environment. However, in this study, the antibacterial effect of BeautiSealant was not greater than we expected. Previous studies claiming the antibacterial properties of the S-PRG filler used the filler directly or a composite resin containing a number of fillers[18,34-36]. BeautiSealant contains a smaller amount of the filler than that used in previous studies. The filler content in BeautiSealant is approximately 40%, whereas filler content of composite resin, including the S-PRG filler, is about 70%. Several studies have reported that higher S-PRG filler content leads to higher antibacterial properties[35,37,38]. It is thought that BeautiSealant does not have enough S-PRG filler to have an antimicrobial effect.

The Clinpro™ sealant has an organic fluoride compound, tetrabutylammonium fluoride. The tetrabutylammonium ion forms a tight ion-pair with fluoride, and such ion-pairs leach out of the material, which may lead to higher water sorption and solubility[39]. As a result, a number of fluorides are released. Naorungroj *et al.*[9] reported that the Clinpro™ and Embrace sealants were the only materials to show discernible inhibition zones in an agar diffusion test, even though all of the tested sealants contained fluoride. Therefore, the Clinpro™ sealant seemed to have a greater antibacterial effect in this experiment.

The inhibition of planktonic streptococci does not reflect the situation in dental biofilms because biofilm bacteria are up to 500 times more resistant to antimicrobial agents than those of planktonic bacteria[40]. Therefore, we also performed the biofilm assay using the CDC Biofilm Reactor. This reactor allows biofilm to form on the surfaces of experimental substrate in a highly reproducible manner. The system was developed to grow biofilms under slow laminar flow close to the air-liquid interface. Biofilms form occurs in hydrodynamic stressed conditions very similar to in vivo conditions. The CDC Biofilm Reactor avoids most of the disadvantages of static reactors based on bacterial sedimentation rather than attachment that do not allow biofilm formation using a clinically realistic method[41].

In the current study, the biofilm assay results were similar to those of the planktonic growth inhibition test. Moreover, the SEM photographs of bacterial adherence on the S-PRG sealant and other sealants are presented in this report to visualize the topographical differences more clearly. In many studies, biofilm formation has been investigated in conjunction with several properties of these materials, such as surface roughness, surface free-energy, electrical property, hydrophobicity, and fluoride release[30,42,43]. Hanning[44] reported that plaque formation on solid surfaces is influenced predominantly by the oral environment rather than material-dependent parameters. The ion and unpolymerized monomers released from the sealant could change the surrounding environment[17]. Therefore, the difference in dental plaque accumulation among the three sealants could be due to the ion-releasing capability of the material and unpolymerized monomers as in our planktonic assay results.

Some limitations of this study should be mentioned. The present study investigated antibacterial ability of only the BeautiSealant block in the short term, and we did not consider other environmental elements such as saliva. Previous studies have reported an interaction between material containing the S-PRG filler and human saliva. Saku *et al.*[17] reported that a composite resin containing the S-PRG filler allows less *S. mutans* adherence when the samples were soaked in human saliva. Hotta *et al.*[45] found that saliva coating the S-PRG resin reduces the adherence of *S. mutans* to the resin. Hence, it is necessary to conduct long-term studies to evaluate the effects of other environmental elements such as saliva in the BeautiSealant on its antibacterial ability.

Ⅴ. Conclusion

We evaluated the antibacterial properties of a sealant containing S-PRG filler compared to those of two contemporary commercial sealants to determine inhibition of bacterial growth in broth culture and biofilm formation using the CDC Biofilm Reactor. The BeautiSealant containing S-PRG filler, the fluoride releasing Clinpro™ sealant, which are known to have higher antibacterial effects, and the non-fluoride releasing Concise™ sealant were selected for this study.

The Clinpro™ sealant showed a significantly reduced number of CFUs compared to those of the BeautiSealant and Concise™ sealant in planktonic growth inhibition test. The Clinpro™ sealant showed significantly less biofilm formation than those of the BeautiSealant and Concise™ sealant. However, no significant difference was observed between the BeautiSealant and the Concise™ sealant. The sealant containing the S-PRG filler had a weaker anti-bacterial property and increased biofilm forming capacity compared to those of the fluoride releasing Clinpro™ sealant.

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국문초록

S-PRG filler를 포함한 치면열구전색제의 *Streptococcus mutans*에 대한 항미생물 특성에 관한 연구

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본 연구는 Planktonic growth inhibition test와 CDC Biofilm Reactor를 사용한 Biofilm assay를 통해 S-PRG filler를 함유하는 치면열구전색제의 *S. mutans*에 대한 항미생물 효과를 기존의 치면열구전색제와 비교하고자 하였다.

S-PRG 필러를 함유하는 치면열구전색제인 BeautiSealant, 불소를 방출하는 치면열구전색제인 Clinpro™ sealant, 불소 미방출 치면열구전색제인 Concise™ sealant를 실험군으로 선정하였다.

성장억제평가를 위해 치면열구전색제를 사용하지 않은 군을 음성 대조군으로 설정하였으며, 3개의 실험군 모두 대조군보다 유의할 정도로 낮은 집락 형성 단위를 보였고, Clinpro™ sealant가 BeautiSealant와 Concise™ sealant보다 유의할 정도로 낮은 집락 형성 단위를 보였다. BeautiSealant와 Concise™ sealant 군간에는 유의한 차이가 관찰되지 않았다.

바이오 필름 평가에서도 Clinpro™ sealant군이 BeautiSealant와 Concise™ sealant군들에 비해 유의할 정도로 낮은 집락 형성을 보였으며, BeautiSealant와 Concise™ sealant 군간에 유의한 차이는 관찰되지 않았다.

본 연구 결과 S-PRG filler를 포함하는 치면열구전색제인 BeautiSealant는 기존의 불소방출 치면열구전색제에 비하여 낮은 항미생물 효과와 높은 바이오 필름 형성능을 보였다.

주요어: S-PRG filler, BeautiSealant, Pit and fissure sealants, *Streptococcus mutans*, Fluoride