나노컴포지트에서 Acidulated Phosphate Fluoride 적용에 따른 *Streptococcus mutans* 부착량 변화

정영정 · 김영재 · 김정욱 · 장기택

서울대학교 치과대학 소아치과학교실 및 치학연구소

─── 국문초록 ───

복합레진 표면에 대한 APF gel 도포는 표면을 거칠게 하고 세균 부착을 증가시킨다. 본 연구는 Filtek Z250(FZ), Filtek Supreme Universal(FS)과, 실험적으로 나노충전재를 각각 0%, 3%, 6% 포함시켜 만든 복합레진(E0, E3, E6)으로 제작한 레진 시편을, APF gel을 적용한 군과 적용하지 않은 군으로 나누어 *Streptococcus mutans*의 부착량과 표면 조도를 측정하고 비교, 평가하여 다음과 같은 결과를 얻었다.

1. APF gel을 적용하지 않은 레진 시편에 대한 *S. mutans* 부착량은 FS에서 가장 적었고, FZ, E3, E6에 대한 부착량보다는 유의하게 낮았다($p < 0.05$).
2. 모든 레진군에서 APF gel을 적용한 레진 시편에 대한 *S. mutans* 부착량은 APF gel을 적용하지 않은 시편에 대한 부착량보다 유의하게 높았다($p < 0.05$).
3. APF gel을 적용한 레진 시편에 대한 *S. mutans* 부착량은 레진군간 유의한 차이를 보였으며($p < 0.05$), FS, FZ, E0, E3, E6의 순서로 높은 값을 보였다.
4. APF gel 적용 전이나 후에, 레진군간 표면조도의 차이는 유의하지 않았다($p > 0.05$).

주요어 : 나노충전재, APF gel, *Streptococcus mutans* 부착, 표면조도

Ⅰ. INTRODUCTION

In the last decades the size of the filler particles in dental composites has decreased considerably[1]. With the emergence of nanotechnology, nanofillers and nanocomposites have developed[2-4]. Nanofiller has several advantages. Particle size of nanofiller is below the range of wavelengths of visible light and thus they do not scatter or absorb visible light. Nanofillers offer a means of incorporating radiopacifiers that do not interfere with esthetic properties. The extremely small sizes of nanofillers allow the particles to fit into spaces between other particles in a composite and effectively increase the overall filler level. Nanofillers may permit higher filler levels by weight that will significantly reduce the effect of polymerization shrinkage and dramatically improve physical and esthetic properties[5].

One of the main reasons for replacement of resin composite restorations is secondary caries. The bacterial accumulation on the materials may cause the formation of secondary caries. Bacterial adhesion to composite surfaces is determined by several factors.

교신저자 : **장 기 택**

서울시 종로구 연건동 275-1
서울대학교 치과대학 소아치과학교실
Tel : 02-2072-2682
E-mail : jangkt@snu.ac.kr

Of those factors, the surface roughness of composite surfaces is of clinical importance in the process of bacterial retention[6-8]. Changes in this variable may, therefore, facilitate the prevention of caries and periodontitis. Surface roughness of composites depends on the type, size, amount of filler and the method of polishing[9-11]. Moreover, repeated application of acidulated fluoride to composites can make a pitted surface[12-19].

Frequently patients with composite resin restorations receive preventive treatment based on fluoride-containing dentifrice, mouthrinses and topical application of fluoride agents including acidulated phosphate fluoride(APF)[20]. One of the problems associated with intraoral APF application is its etching effect on inorganic substances incorporated in restorative materials[12-15]. Roughened surface may contribute to plaque accumulation and may result in surface staining of the materials, secondary caries and gingivitis[16,21].

The purpose of this *in vitro* study was to evaluate the changes in adhesion of *Streptococcus mutans* to composites containing nanofillers and surface roughness after application of acidulated phosphate fluoride gel.

II. MATERIALS AND METHODS

Table 1 lists the composite materials evaluated in this study. They include two proprietary resin composites, minifilled Filtek Z250[FZ](3M, St Paul, USA) and nanofilled Filtek Supreme Universal [FS](3M, St Paul, USA), and a series of experimental resin composites [E0, E3, E6] containing different amounts of nanofillers. The resin matrix of experimental composites was composed of BIS-GMA(70%) and TEGDMA(30%). The fillers of experimental resins were: barium glasses 1 ㎛ in diameter(type I), silica particles 40 nm in diameter(type II), and silica particles 7 nm in diameter(nanofiller, type III). The total contents of fillers and the weight of type I filler were held constant at 76 wt% and 70 wt%, since the ratio of type II to type III was changed. The minifilled composites FZ and the experimental composite E0 were included as control since they don't contain nanofillers.

Preparation of specimens

Specimens were 5 mm in diameter and 2 mm thick made with a polytetrafluoroethylene mold. Resin composite was packed into the mold, pressed between two Mylar strips sandwiched with two glass slides. The specimens were then polymerized for 40 seconds from both ends of the molds with a halogen light curing unit(Curing Light 2500; 3M, St Paul, USA). Sixty specimens of each material were fabricated and divided into two groups of the same number. Specimens in no treatment group received no further treatment after polymerization. For APF treatment group specimens, the entire surface was treated with 1.23% APF gel(60 SECOND TASTE® Gel; Pascal, Bellevue, USA) for 4 minutes. The specimens were washed with distilled water to remove any visible remnants of the gel, and dried.

Table 1. Resin composites used in this study

Materials	Codes	Filler contents (wt%)		Filler information	Resin type
Filtek Z250	FZ	78%		0.01-3.5㎛ zirconia/silica	BIS-GMA
Filtek Supreme	FS	78.5%		nanomer: 75 nm silica nanocluster: 0.6-1.4 ㎛ (5-20 nm zirconia/silica)	BIS-EMA UDMA
Experimental composites	E0		type II: 6%	type I: 1 ㎛ Ba glass type II: 40 nm silica type III: 7 nm silica	BIS-GMA TEGDMA
	E3	76% type I: 70%	type II: 3% type III: 3%		
	E6		type III: 6%		

Saliva samples

Stimulated whole saliva was collected from a healthy donor and kept at 4℃. It was clarified by centrifugation at 8000g for 15 minutes at 4℃. The supernatant was heated to 60℃ for 30 minutes to inactivate the degradative enzymes. Sodium azide, at a final concentration of 0.04%, was then added to prevent microbial growth. Samples of the saliva were kept at 4℃ and used the same and the next day.

Labeling of *S. mutans*

The bacterial strain used for this study was *S. mutans* Ingbritt, which was obtained from SNUCTC (Seoul National University Collection for Type Culture). Aliquots of frozen bacteria were grown overnight in 100 ml of Todd-Hewitt broth(THB; Difco, Detroit, USA) at 37℃ in 5% CO_2. Aliquots of the bacteria were added to a fresh THB. The bacterium was inoculated to THB which included 1 μCi of (6-[³H]-methyl)-thymidine(5mCi, Amersham, LC Buckinghamshire, UK) per 1 ml of THB and cultured overnight at 37℃. The cells were collected by centrifugation at 3000g for 20 minutes at 4℃. The washing procedure was repeated three times with phosphate-buffered saline(PBS). The bacteria were dechained and dissociated by means of sonication conducted in an ice bath. The dechaining process was verified by visual observation. Following sonication, the bacterial suspensions were washed again in PBS, centrifuged and re-suspended in PBS. The optical density(OD) of the labeled bacterial suspension was adjusted to OD of 1.0 at 660nm.

In vitro adherence of *S. mutans*

Twenty pairs of specimens from each material(no treatment group and APF treatment group) were stored overnight with saliva at 37℃. Composite specimens were washed with saline. They were then placed in scintillation vials and incubated with 1 ml of clarified saliva for 30 minutes at room temperature in an apparatus where the tubes were continuously inverted ten times per minute. The specimens were then washed twice with PBS. 1 ml samples of the ³H-labeled bacteria in PBS were added to each

specimen tube. After incubation for 2 hours at room temperature, the specimens were washed three times with PBS. The specimens were transferred to scintillation vials containing scintillation fluid. The amount of radioactive-labeled bacteria adhered to each specimen was measured by scintillation counter (Beckman; GMI, Ramsey, USA).

Measurement of surface roughness

The mean surface roughness(Ra:㎛) of 10 pairs of specimens from each material(no treatment group and APF treatment group) was measured with a profilometer(Accura 2000; INTEKPLUS, Daejeon, Korea). The mode of roughness measurement was non-contact, white-light scanning interferometry. Readings were taken at the center of each specimen and sampling areas of 0.44 mm by 0.28 mm were used.

Statistical analyses

Two-way ANOVA was used to determine significant interactions between materials and treatment groups. One-way ANOVA and Tukey's post-hoc tests were used to compare the amount of *S. mutans* adhesion and the mean surface roughness between materials for each treatment group. Independent Samples t-test and Mann-Whitney test were used to compare the amount of *S. mutans* adhesion and the surface roughness between treatment groups for each material.

Ⅲ. RESULTS

In vitro adherence of *S. mutans*

The amount of *S. mutans* that adhered to the respective materials was summarized in Table 2 and Fig. 1. Two-way ANOVA run on the amount of *S. mutans* adhesion revealed that the interaction between materials and treatment groups was significant(F=209.3; p⟨0.05). The amount of *S. mutans* adhered to materials was therefore treatment group dependent. Treatment factor showed higher F-value(F=4276.6) than material factor(F=366.2); both factors were significant(p⟨0.05).

In no treatment group, the greatest number of *S. mutans* were adhered to E6. The smallest number of *S. mutans* were adhered to FS in both groups. In APF treatment group, the amount of *S. mutans* adhesion was significantly different between materials($p<0.05$), and increased in order of FS, FZ, E0, E3 and E6. For all materials, the amount of *S. mutans* adhesion in APF treatment group was significantly greater than that in no treatment group ($p<0.05$).

Surface roughness

Table 3 shows the mean surface roughness observed for the different materials. Mean Ra values ranged from 0.21 to 0.29 ㎛ for specimens in no treatment group, and from 0.30 to 0.42 ㎛ in APF treatment group. Two-way ANOVA run on the Ra value revealed that the interaction between materials and treatment groups was not significant ($p>0.05$). In both no treatment group and APF treatment group, no significant difference was found in the Ra values among the materials($p>0.05$). For FZ and FS, the Ra values observed in APF treatment group were significantly greater than those

in no treatment group($p<0.05$). For E0, E3 and E6, the Ra values observed in APF treatment group were greater than those observed in no treatment group, but the differences were not statistically significant ($p>0.05$).

Ⅳ. DISCUSSION

Clinically, topical application of APF gel can accelerate the degradation of surface of restorative materials and increase the surface roughness[22]. The roughened surfaces may allow increased bacterial accumulation and surface staining. There may be three major interaction pathways among resin composites and fluoride agents[23,24]. Interactions exist with organic matrix, filler-matrix coupling agent and reinforcing filler. The organic matrixes of the composites evaluated are mostly organic esters of methyl methacrylate derivatives. Organic esters undergo hydrolytic cleavage of the ester group in low pH. Fluoride ion has been implicated in depolymerization reactions of the matrix-filler interface[25]. Phosphoric acid and hydrofluoric acid in APF gel may be responsible for degradation of the filler particles and the latter is known to be potentially more destructive[12]. All these mechanisms might weaken the filler-matrix interface, resulting in filler loss[23,24], increased surface

Table 2. Amount of *S. mutans* adhered on the respective materials(cpm)

Materials (codes)	No treatment group		Statistical category	APF treatment group		Statistical category
	Mean	SD		Mean	SD	
FZ	2332.17	520.87	a, b	6357.17	272.23	d
FS	1612.50	318.99	c	3567.00	271.80	e
E0	2184.17	498.25	a, c	7526.50	140.94	f
E3	2422.00	515.46	a, b	10147.48	229.10	g
E6	2939.83	738.52	a	11684.33	391.55	h

* Identical letters indicate that the values are not statistically different($p>0.05$).

Table 3. Mean surface roughness(Ra: ㎛)

Materials (codes)	No treatment group		APF treatment group	
	Mean	SD	Mean	SD
FZ	0.209	0.089	0.389	0.148
FS	0.222	0.124	0.418	0.098
E0	0.269	0.162	0.387	0.151
E3	0.290	0.080	0.336	0.146
E6	0.272	0.178	0.296	0.143

roughness[26-28], weight loss[20,29] and decreased hardness[27,30].

Degradation of filler particles by APF agents appeared to be related to their composition and size[12,15,22,23]. Sposetti et al.[31] suggested that the silicone dioxide is susceptible to the hydrofluoric acid. The filler particles present in resin composites are usually composed partially or totally of silica. It has been observed that strontium, barium, boroaluminosilicate, silicate, and zinc glass exhibited extended degradation on acid attack, whereas quartz, silica, lithium aluminosilicates showed less involvement[15,23]. Colloidal silica is hydrolyzed at low pH, too[12,15,23,29]. The pattern of filler degradation and surface attack is more apparent on larger particles[12,14,23]. Soeno et al.[17,18] found that the influence of APF agents on composite surface is apparent for macro-inorganic filled composites whereas the microfilled and submicron hybrid composites containing inorganic fillers not greater than 1 ㎛ were not sensitive to APF agents. Therefore, It is recommended to use the microfilled or submicron hybrid composite, considering the size of the defects produced by APF agents, as well as the possibility of staining of roughened area[12,17,21].

In this study, although all materials showed the increasing tendency of the surface roughness after APF gel application, the difference between no treatment group and APF treatment group was not great, and the differences in E0, E3, E6 were not statistically significant. These may be attributed to small filler size; Since the inorganic fillers of the composites used in this study are not greater than 1 ㎛, changes in surface roughness might not be distinct. Another reason for the lack of changes in surface roughness may derive from the detection limit of the surface analyzer. It can be explained by the fact that the amount of S. mutans adhesion increased significantly by APF application.

Caries attack is usually initiated by cariogenic bacterial adhesion to the tooth surface. Therefore, a plaque adhesive property might be an important index to predict the anticariogenicity of the materials[32]. Physically, bacterial adhesion and retention occur in four phases: transport of bacterium toward the surface, initial bacterial adhesion, attachment by specific interactions, colonization of the surfaces[6]. Surface properties are important determinants in bacterial adhesion[11]. Studies by Quirynen et al.[33] showed that an increase in the surface roughness of resin strips above an Ra values of 2 ㎛ resulted in a dramatic increase in the bacterial colonization of these surfaces in comparison to smooth strips(Ra=0.12 ㎛). The influence of surface roughening might be explained by the fact that the establishment of a reversible binding of bacteria preferentially occurs in the surface irregularities where microorganisms are protected against mechanical shear[7,8]. However, the initial colonization of resin composites was not only dependent on surface roughness[34-38]. Skjorland et al.[39] suggested that surface topography could not account for bacterial accumulation on composites. Barsotti et al.[34] stated that bacteria did not adhered in the same number on the composites with a comparable surface roughness. Yamamoto et al.[37] and Hosoya et al.[38] found that no relationship was observed between the surface roughness values and bacterial adhesion. The results of this study were consistent with those of previous studies: The amount of S. mutans adhesion on the composites used in this study was statistically different although the surface roughness was not. This result can be explained by the fact that the other factors such as electrical property(zeta potential)[40,41], hydrophobicity[40-42], contact angle[35,36], surface free energy[7,43] can also play roles in bacterial adhesion and retention. Chemical composition of the surface is also important for bacterial colonization, particularly when the surface possesses components which are either beneficial or detrimental to the adhering population[44].

All resin composites in this study were cured against the same kind of surface in order to eliminate the influence of manual polishing on surface properties. Difference in surface roughness of matrix finished surfaces may be attributed to inherent material properties such as filler particle sizes and their ability to form a homogeneous polymer-rich layer, as well as the flaws on the matrix strips[45].

One of the hypotheses in this study was that nanofillers would contribute to smoothening of the surface and reduction of the effect by APF agents, resulting in reduced S. mutans adhesion. But, the Ra value of nanofilled composite(FS) was not significantly different from that of minifilled composite(FZ) both before and after APF gel application. A possible

explanation for this result is that FS contains predominantly aggregated zirconia/silica nanoclusters, and the size of nanocluster fillers (0.6-1.4 ㎛) is similar to that of FZ although primary particle size of FS is 5 to 20 nm[45]. Despite of lack of the difference in surface roughness, the amount of *S. mutans* adhesion on FS was lesser significantly than that on FZ. This result indicated that effective primary particle might play an important role in bacterial adhesion, considering the fact that FS and FZ have the same composition of filler and resin matrix with the only exception of the size of effective primary particle. This possible explanation could be supported from the SEM finding by Yamamoto et al.[37] that bacteria adhered firmly to the filler particles of composite surfaces.

Pallav et al.[46] suggested that the structure becomes more homogeneous and surface smoothness is improved by admixing small amount of smaller particles in a composite. In this study, the experimental resin composites with nanofillers were expected to have improved surface structure with reduced roughness and bacterial adhesion. But, the Ra values were not different between experimental composites. This result might be due to the facts that the surface roughness of resin composite is principally determined by the presence of protruding filler particles above the resin matrix[9] and three experimental composites contained the same amounts of the same largest filler particles. As the content of nanofiller increased, the Ra values after APF application and the difference of the Ra values between before and after APF application showed decreasing trend although they were not statistically significant. Nanofillers might have a favorable effect on surface roughness although it is predominantly determined by the largest particles.

The amount of *S. mutans* adhesion on the experimental composites, against my expectation, increased significantly with increasing nanofiller contents both before and after APF application. Willems et al.[47] suggested that the handling properties became poorer due to clustering of filler particles and increasing viscosity as the smaller filler particles increased. Experimental composites with more nanofillers, in fact, showed increased viscosity and more porosity during placement of the composites into the mold and the increased amount of *S. mutans*

adhesion might be attributed to this increased porosity. It appeared to be due to clustering of filler particles. Thus, the effort to overcome this problem should be made. The experimental composites including E0 showed more adhesion of *S. mutans* after APF application than proprietary composites FS and FZ, since the experimental composites contained large amount of barium glass that is susceptible to APF agents. Also, the monomer component of experimental composite may be associated with increased *S. mutans* adhesion. In the process of pellicle deposition by selective adsorption of salivary glycoproteins prior to bacterial colonization, compositional differences in composites may influence the nature of the protein deposited[48]. All these factors could have effects on the increased bacterial adhesion to experimental composites, but more studies about these factors should be continued.

Although the pellicle was simulated by salivary coating before bacterial adhesion, this study had several limitations of *in vitro* studies. Clinically, the effects of 1.23% APF gel on resin composites may be modified by many factors. Saliva may dilute or buffer the gel, thus reducing the surface changes, and a coating of salivary pellicle might have a protective effect[27]. However, reaction time is potentially increased *in vivo*, since only suction or expectoration is normally used to remove excess gel. In addition, polishing and normal abrasion may modify the *in vivo* results by smoothing roughened surfaces or by exposing fresh filler for subsequent attack[15]. With regard to *S. mutans* adherence to restorative materials, difference between *in vitro* and *in vivo* may reflect various factors that are not simulated *in vitro* (e.g., saliva and salivary proteins, pH, oral hygiene habits and self-cleansing)[49]. Indeed, inadequate oral hygiene seems to be the main cause of plaque accumulation on restorative composite *in vivo*. In addition, bacterial adherence and early plaque formation on pellicle-coated surfaces are influenced predominantly by the oral environment, e.g., intraoral shear forces originating from muscles, tongue, and salivary flow, rather than by material-dependent parameters[50]. Studies, therefore, are necessary to evaluate the effects of these variables *in vivo*.

Admixing of nanofiller to microhybrid composite did not have a favorable effect on the bacterial adhesion

after APF gel application. More studies about the ways to admix nanofillers are necessary to improve surface properties and physical properties without loss of good handling property.

Ⅴ. CONCLUSIONS

Changes of surface roughness and amount of *Streptococcus mutans* adhesion in composites with and without nanofillers by acidulated phosphate fluoride agents were assessed. The results were as follows;

1. In no treatment group, the amount of *S. mutans* adhered to FS was the smallest. It was significantly different from those of FZ, E3, E6($p<0.05$) although it was not significantly different from that of E0($p>0.05$).
2. For all resin composites used, the amount of *S. mutans* adhesion in APF treatment group was significantly greater than that in no treatment group ($p<0.05$).
3. In APF treatment group, the amount of *S. mutans* adhesion was significantly different between materials($p<0.05$), and increased in order of FS, FZ, E0, E3 and E6.
4. Difference of the surface roughness between materials was not statistically significant in both no treatment group and APF treatment group ($p>0.05$). For FZ and FS, the Ra values observed in APF treatment group were significantly greater than those observed in no treatment group ($p<0.05$).

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Abstract

CHANGES IN ADHESION OF *STREPTOCOCCUS MUTANS* TO NANOCOMPOSITE RESINS AFTER ACIDULATED PHOSPHATE FLUORIDE GEL APPLICATION

Young-Jung Jung, Young-Jae Kim, Jung-Wook Kim, Ki-Taeg Jang

Department of Pediatric Dentistry, College of Dentistry and Dental research Institute, Seoul National University

Topical application of APF gel can increase the surface roughness of resin composites and the roughened surfaces may allow increased bacterial accumulation and surface staining. Resin specimens of two proprietary resin composites, Filtek Z250(FZ) and Filtek Supreme Universal(FS), and experimental resin composites containing 0%, 3%, 6% nanofillers(E0, E3, E6) were fabricated and divided into two groups of the same number; APF treatment group and no treatment group. The amount of *S. mutans* adhered to specimens and the mean surface roughness(Ra) were measured. The results were as follows;

1. In no treatment group, the amount of *S. mutans* adhered to FS was the smallest. It was significantly different from those of FZ, E3, E6($p < 0.05$) although it was not significantly different from that of E0($p > 0.05$).
2. For all resin composites used, the amount of *S. mutans* adhesion in APF treatment group was significantly greater than that in no treatment group($p < 0.05$).
3. In APF treatment group, the amount of *S. mutans* adhesion was significantly different between materials($p < 0.05$), and increased in order of FS, FZ, E0, E3 and E6.
4. Difference of the surface roughness(Ra) between materials was not statistically significant in both no treatment group and APF treatment group($p > 0.05$)

Key words : Nanofiller, APF gel, *Streptococcus mutans* adhesion, Surface roughness