https://doi.org/10.5933/JKAPD.2020.47.2.188
J Korean Acad Pediatr Dent 47(2) 2020
ISSN (print) 1226-8496 ISSN (online) 2288-3819

Surface Roughness and Microbial Adhesion After Finishing of Alkasite Restorative Material

Choa Park[1], Howon Park[1], Juhyun Lee[1], Hyunwoo Seo[1], Siyoung Lee[2]

[1]*Department of Pediatric Dentistry, Oral Science Research Center, College of Dentistry, Gangneung-Wonju National University*
[2]*Department of Oral Microbiology, Oral Science Research Center, College of Dentistry, Gangneung-Wonju National University*

Abstract

This study is aimed to evaluate and compare the surface roughness and microbial adhesion to alkasite restorative material (Cention N), resin-modified glass ionomer (RMGI), and composite resin. And to examine the correlation between bacterial adhesion and surface roughness by different finishing systems.

Specimens were fabricated in disk shapes and divided into four groups by finishing methods (control, carbide bur, fine grit diamond bur, and white stone bur). Surface roughness was tested by atomic force microscope and surface observation was performed by scanning electron microscope. Colony forming units were measured after incubating *Streptococcus mutans* biofilm on specimens using CDC biofilm reactor.

Cention N surface roughness was less than 0.2 μm after finishing procedure. Control specimens of resin and Cention N specimens were significantly (p = 0.01) rougher. Pearson correlation coefficient (PCC = 0.13) indicated a weak correlation between surface roughness and *S. mutans* adhesion to the specimens.

Compared with resin specimens, RMGI and Cention N showed lower microbial adhesion. Surface roughness and bacterial adhesion were not significantly different, regardless of the finishing systems.

Key words : Cention N, Alkasite restorative, Surface roughness, Bacterial adhesion

Ⅰ. Introduction

In the field of operative dentistry, many posterior restorative materials are available for treating dental caries. Amalgam is the restorative material most widely used for more than 150 years[1]. European and international authorities are concerned mainly with the toxicological burden on the environment caused by mercury and less with patient safety issues[2]. However, amalgam has good mechanical properties, and its economic costs are reasonable[3,4].

For the replacement of amalgam, an alkasite restorative material is recently introduced. Cention N (Ivoclar Vivadent, Schaan, Liechtenstein), provides high flexural strength and tooth color aesthetics[5,6]. Alkasite is a new category of filling material and is a type of composite resin[5]. It is intended for restoring deciduous teeth and for permanent restorations for class I, II, or V caries. Cention N's high flexural strength is derived from a highly cross-linked polymer structure[7]. A

Corresponding author : Juhyun Lee
Department of Pediatric Dentistry, College of Dentistry, Gangneung-Wonju National University, 7 Jukheon-gil, Gangneung, 25457, Republic of Korea
Tel: +82-33-640-2452 / Fax: +82-33-640-3113 / E-mail: ljh55@gwnu.ac.kr
Received September 8, 2019 / Revised November 22, 2019 / Accepted November 18, 2019

previous study reported that Cention N has higher fracture resistance than does amalgam or other composite resins[8].

Alkasite material is made with an alkaline filler which is capable of releasing acid-neutralizing ions, prevents demineralization[9]. Significant large amounts of fluoride and calcium ions are also released, which enables enamel remineralization[10]. According to Samanta *et al*.[5] Cention N, in comparison with glass ionomer and composite resin, exhibited the lowest amount of microleakage. Many recent studies have stated that the restoration of Cention N is less time consuming than the placement of other restorations. Cention N restorations can provide economic and high-quality benefits[9-12].

A smooth surface is crucial for successful restorations. Because the surface roughness of the restorative material results in plaque deposition, discoloration of the restoration, microleakage, secondary caries, and other adverse developments, obtaining a polished surface in the finishing step is a prerequisite for successful restoration[9-10]. Bacterial adhesion is affected by various factors, including the smoothness of the restoration surface. Bollen *et al*.[11] reported that more bacterial adhesion occurs in materials with a surface roughness more than 0.2 μm. However, the correlation between bacterial adhesion and the surface roughness remains controversial.

The surface roughness of Cention N has been investigated previously[10], however, no previous study has focused on correlations between surface roughness and microbial adhesion for Cention N. The purposes of this study were (1) to analyze the surface roughness of and microbial adhesion to Cention N by a comparison with resin-modified glass ionomer (RMGI) and composite bulk fill resin and (2) to examine the correlations between bacterial adhesion and surface roughness by different finishing systems.

Ⅱ. Materials and Methods

1. Experimental design

Specimens were prepared with 3 restorative materials: Cention N, RMGI, and composite resin. Table 1 lists the characteristics of the 3 materials. For each material, 54 specimens were constructed: 48 specimens were used for microbial colony forming unit, and 6 specimens were used for surface analysis.

2. Specimen preparation

Every specimen was prepared in a teflon mold with a diameter of 5.0 mm and a height of 2.0 mm. In the bottom part, a Mylar strip was placed above the glass slab, and the top surface was flattened with a resin applicator. Each specimen in all 3 groups was subjected to photopolymerization for 9 seconds; the light sources was a blue light-emitting diode (VALO™; Ultradent, USA) with an output power of 1800 mW (with a round tip of 10.0 mm in diameter). To minimize the variation according to operator, one operator made all the cylindrical specimens on the same day.

3. Finishing method

Group I remained unpolished. In accordance with the manufacturer's instructions for Cention N, fine diamond bur and tungsten carbide finishing bur was selected for group II and III respectively. In group IV, white stone bur was selected. Which is thought to be ideal for the contouring and finishing of enamel, composites, and glass ionomer restoratives. Table 2

Table 1. Dental restorative materials used in this study

Category (n = 54)	Product Name	Manufacturer	Batch no.
Alkasite	Cention® N	Ivoclar Vivadent, Schaan, Liechtenstein	X49425
RMGI	GC Fuji™ II	GC Corporation, Tokyo, Japan	261131
Composite resin	3M ESPE Filtek™ bulk-fill flowable restorative	3M ESPE, St. Paul, MN, USA	N998324

RMGI = Resin modified glass ionomer

Table 2. Finishing burs used in this study

Group	Material	Manufacturer	ISO code	Grit sizes
I	Control	-	-	-
II	Diamond bur	Komet, Stuttgard, Germany	8379 314 023	Fine (46.0 μm)
III	Tungsten carbide bur	Komet, Stuttgart, German	H379 314 023	8/12 Blades
IV	White stone bur	Shofu, Kyoto, Japan	F0090244J	Micrograined aluminum oxide grit

lists the details of 3 finishing systems used in this study.

All specimens in groups II, III, and IV went through the finishing process, performed in one direction for 30 seconds at 200,000 rpm in a high-speed handpiece. To prevent operator variability, the same operator performed all the finishing procedures on the same day.

4. Surface roughness measurement

4 specimens of each groups were used to test surface roughness measurement. An atomic force microscope (PSIA XE-100, Park Systems, Suwon, Korea) was used to measure the surface roughness of 4 specimens per group. The roughness was measured at 3 random points at the center of each specimen, and the measurements were averaged to calculate the roughness (R_a).

To assess the qualitative surface roughness of two specimens per group, surface scanning electron microscope (Inspect F, FEI, Hillsboro, OR, USA) was performed.

5. Bacterial strains and culture conditions

Streptococcus mutans ATCC 25175 was inoculated into a liquid medium of brain heart infusion (BHI) broth (Becton, Dickinson and Company, Sparks, USA) and cultured in a 5% CO_2 incubator for 24 hours. The optical density of the bacterial suspension was adjusted to 0.55 (600 nm) before inoculation of the biofilm reactor.

For the biofilm formation, a CDC Biofilm Reactor® (Biosurface Technologies, Bozeman, MT, USA) was used to grow the *S. mutans* biofilm for a total of 5 days, according to a validated protocol. The CDC Biofilm Reactor and the BHI liquid medium were subjected to high-pressure steam sterilization at 121°C for 15 minutes. The specimens were mounted on a CDC Biofilm Reactor rod with putty-type vinyl polysiloxane impression material (Exafine putty type; GC Corp, Tokyo, Japan). 9 specimen disks were fixed on 1 rods each. Total 8 rods were fixed on a CDC Biofilm Reactor. The specimen-loaded rod was subjected to ethylene oxide sterilization. The sterilized specimens were installed on the CDC Biofilm Reactor and exposed to 120 mL of artificial saliva (Xerova solution; Kolmar Korea, Sejong, Korea) for 4 hours to form an acquired pellicle coating. 100 mL of *S. mutans* suspension was fed of and 300 mL of BHI liquid medium into the interior of the CDC Biofilm Reactor. The CDC Biofilm Reactor was placed into a magnetic stirrer in a 37°C incubator. The magnetic stirrer was set to 50.0 RPM so that shear stress could be applied to the biofilm. During the initial 24 hours, only the vortex was formed without influx of the medium to induce the growth of the biofilms while the shear stress was maintained. After 24 hours, the inflow and outflow of BHI liquid medium were induced at a rate of 18.6 mL/h with the use of a peristaltic pump (JWSE100, JenieWell, Seoul, South Korea).

6. Measurement of bacterial colonization units

After induction of biofilm formation, the rod was separated from the CDC Biofilm Reactor. The specimens were washed 3 times with phosphate buffered saline (PBS) to remove bacteria loosely attached to the specimen.

The specimen was carefully separated from the silicone impression material and placed on a well plate containing 1.0 mL of PBS. The well plate containing the specimen was sonicated for 20 seconds to separate the biofilms from the specimen. Then 50.0 μL of the bacterial suspension diluted 1000-fold with PBS was spread on a blood agar plate in duplicate. After culturing in an incubator at 37°C with 5% CO_2 for 72 hours, the bacterial colonies were counted in a Colony counter (Flash & Go, IUL Instruments, Barcelona, Spain) to measure colony-forming units (CFU) per milliliter. In this study, bacterial adhesion test using CDC biofilm reactor were independently repeated on different days.

7. Statistical analysis

The results were statistically analyzed with IBM SPSS Statistics version 25.0 (SPSS Inc., IBM, Chicago, IL, USA). Data were calculated as means and standard deviations for each group. Data variables were subjected to analysis of variance, followed by Tukey's honestly significant difference test. The Pearson correlation coefficient was used to evaluate the potential association between surface roughness and bacterial adhesion to the surfaces of each specimens.

Ⅲ. Results

1. Surface roughness

Fig. 1 displays finished surface of the scanning electron microscope photomicrographs of the specimens in each group.

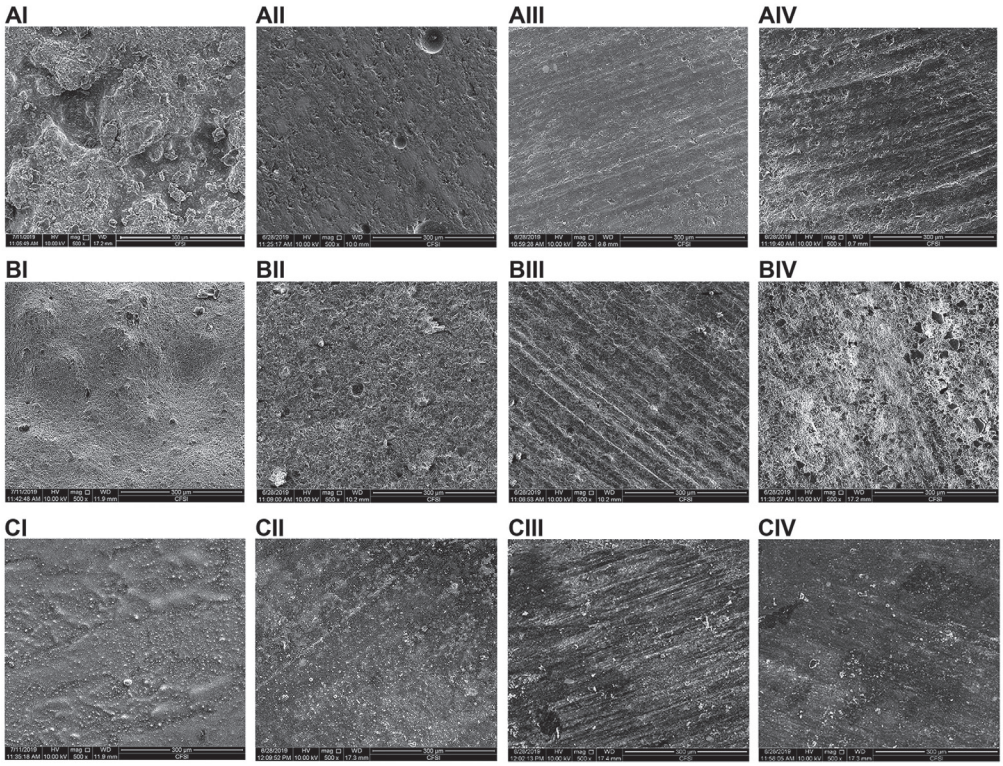

Fig. 1. Two dimensional scanning electron microscope images (original magnification, 500) of specimen surfaces with different finishing. A : Cention N specimens, B : RMGI specimens, C : Composite resin specimens, I : Group I (no finishing), II : Group II (finishing with diamond bur), III : Group III (finishing with carbide tungsten bur), IV : Group IV (finishing with white stone bur).

Means and standard deviations of surface roughness (R_a) are listed in Table 3 and in Fig. 2. In the comparison between the materials of the specimens in group I, Cention N had the highest surface roughness, followed by RMGI and composite resin. All tested specimens showed lower surface roughness values after the finishing procedure, regardless of the restorative materials. Finishing of the Cention N specimens (groups II, III, and IV) caused a significant decrease in the surface roughness (p = 0.01).

The roughness of the finished composite resin specimens was significantly different from that of the control group (p = 0.04). After the finishing procedure, the lowest surface roughness value was observed in the composite resin specimens subjected to carbide bur finishing. Among RMGI specimens, there was no significant difference in roughness between the control group and groups II, III, and IV.

The difference in surface roughness between the three restorative materials, regardless of the finishing bur used, was not statistically significant.

Fig. 2 displays the scanning electron microscope photomicrographs of the surfaces of the specimens in each group.

Table 3. Ra values of tested specimens

Group	Finishing method	Mean ± Standard deviation (μm)		
		RMGI	Cention N	Composite resin
I	Control	0.309 ± 0.082	0.415 ± 0.015	0.235 ± 0.039
II	Carbide bur	0.304 ± 0.136	0.146 ± 0.024	0.083 ± 0.014
III	Diamond bur	0.246 ± 0.094	0.125 ± 0.039	0.151 ± 0.039
IV	White stone bur	0.278 ± 0.059	0.134 ± 0.028	0.148 ± 0.050

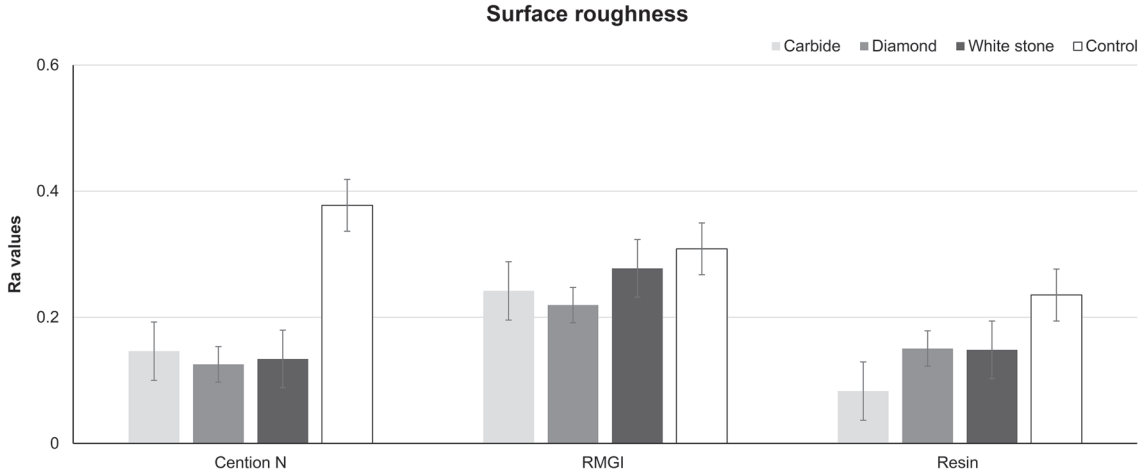

Fig. 2. Means and standard deviations of surface roughness values according to burs used in finishing.

2. Bacterial adhesion

Surface adhesion of *S. mutans* was assessed with the automatic colony counter. The number of CFU per milliliter ($\times 10^3$) in each group is listed in Table 4 and in Fig. 3. After the finishing procedure, the CFU value tended to decrease, but not significantly. Post hoc analysis revealed no significant difference in numbers of CFU between RMGI and Cention N specimens in groups I, III, IV, and V. In contrast, the finished composite resin specimens (groups II, III, and IV) showed significantly lower CFU than did the controls (p = 0.03).

Table 4. *Streptococcus mutans* bacterial counts

Group	Finishing method	Mean ± Standard deviation ($\times 10^3$ CFU/mL)		
		RMGI	Cention N	Composite resin
I	Control	60.78 ± 76.60	45.96 ± 44.16	96.36 ± 77.72
II	Carbide bur	54.47 ± 51.18	25.09 ± 25.78	29.01 ± 37.67
III	Diamond bur	45.3 ± 39.99	28.18 ± 31.29	50.42 ± 21.61
IV	White stone bur	38.86 ± 32.82	24.05 ± 15.96	32.32 ± 17.27

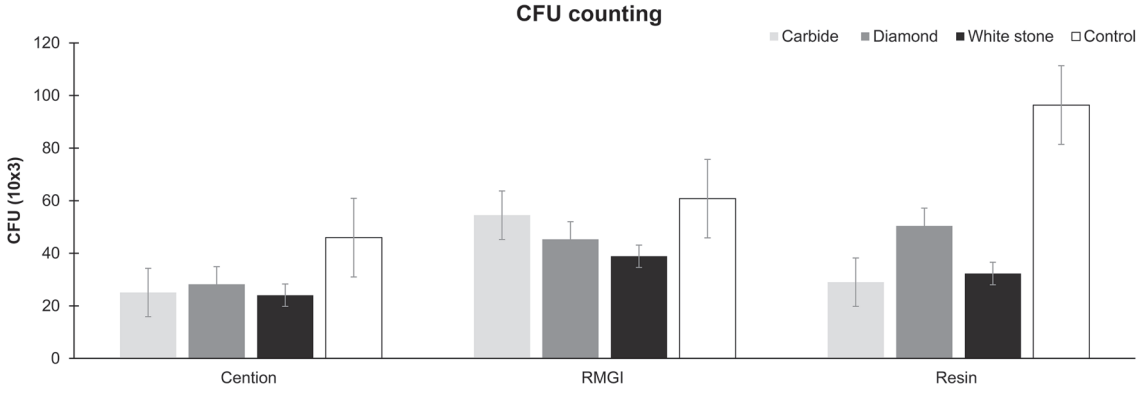

Fig. 3. Means and standard deviations of bacterial adhesion according to burs used in finishing.

3. Correlation between surface roughness and bacterial adhesion

The Pearson correlation coefficient (PCC = 0.13) revealed a negative correlation between surface roughness and bacterial adhesion. There was a tendency for specimens with higher surface roughness to exhibit higher bacterial adhesion, but no statistical significance was found.

IV. Discussion

In this study, composite resin and RMGI specimens were compared with alkasite specimens. Composite resins have been available for nearly 50 years[8]. Despite their promising mechanical properties, the critical disadvantage of composite resins is shrinkage, which results in marginal microleakage, postoperative sensitivity, and secondary caries[9]. Bulk fill composite resins are well known to minimize shrinkage. Less shrinkage and bulk-filling capacity were the reason for the comparative setting. RMGI has advantages of fluoride release, chemical bonding to tooth structure, and good biocompatibility[10]. RMGI was selected for comparison because of its capacity for bulk-fill capacity with photopolymerization and ion release.

Caries lesions result from the colonization and infection of the tooth surface with bacteria[11]. Surface roughness has a well-known close correlation with biofilm formation. Thus, it is very important to conduct proper finishing after the restoration. The final surface quality of the material depends on various factors: filler size and shape, filler loading, surface hardness, finishing procedures, and the structure of resin matrix. Traditionally, composite resins with larger filler particles were thought to have higher surface roughness after finishing[12]. In this study, the finishing method was conducted using carbide, diamond, and white stone burs, but the differences in surface roughness were not statistically significant.

Setty *et al*.[10] observed that the surface of Cention N was rougher than that of Filtek Z350 XT Restorative. In accordance with their study, Cention N had the roughest surface in the control group, but after the finishing procedure, the R_a value was below the threshold of 0.2 μm in all cases (Table 3).

Resin specimens showed the lowest surface roughness among all groups. The control surface roughness was 0.235 μm, which was close to the threshold value. Values lower than the threshold value (0.2 μm) were observed in all groups after the finishing process. The R_a values were lower because the filler content of the resin was similar to that of the microhybrid composite[12].

In this study, not only surface roughness but also microbial adhesion of cariogenic *S. mutans* was observed. The CDC Biofilm Reactor used to culture *S. mutans* dynamic biofilm was chosen because it reproduces continuous saline flow and nutrient supply to reflect the oral situation as much as possible in nonoral experiments. The primary strength of this study lies in reproducement of artificial oral biofilm in CDC biofilm reactor.

The Pearson correlation coefficient, as mentioned, indicated a weak correlation (PCC = 0.13) between surface roughness and *S. mutans* adhesion to the specimens. Number of studies demonstrated no correlation between the surface roughness and the number of CFU of *S. mutans*[13-15].

Resin composites are known to have thicker biofilm formation and accumulation than other restorative materials[16,17]. In this study, resin specimens showed significantly high CFU values among the control groups (p = 0.03).

Cention N and RMGI showed similar CFU values, with no statistical significance between groups. Previous studies suggested that fluoride could significantly decrease the *S. mutans*-levels in plaque by reducing the ability of S. mutans to ferment sucrose[18]. Another study of fluoride release in which Cention N and glass-ionomer cement showed fluoride ion release in both acidic and neutral pH at all time intervals[19]. The low CFU values, regardless of the rough surfaces, can be explained by ion release potentiation by these restoratives. Bayrak *et al*.[20] showed that polishing promoted significant increase of fluoride release on restorative materials. But future research of the relationship between ion release and microbial adhesion of Cention N is essential.

The results of this study indicate that bacterial adhesion differs significantly between restorative materials, according to the finishing techniques used. This study is the first to examine the correlation of surface roughness of Cention N with bacterial adhesion. The main strength of this study lies in CDC Biofilm Reactor, which were used to reproduce the dynamic oral situation outside the oral cavity. But due to the CDC biofilm reactor and only a limited number of specimens were used. Future research with an experimental design with positive control and negative control must be followed.

V. Conclusions

In this study, surface roughness was weakly correlated with *S. mutans* adhesion. Compared with smoother resin surfaces, rough surfaces of RMGI and Cention N showed lower microbial adhesion. Surface roughness and bacterial adhesion were not significantly different, regardless of the finishing systems.

Authors' Information

Choa Park *https://orcid.org/0000-0002-9228-595X*
Howon Park *https://orcid.org/0000-0001-5741-9804*
Juhyun Lee *https://orcid.org/0000-0002-7586-9478*
Hyunwoo Seo *https://orcid.org/0000-0001-6894-0099*
Siyoung Lee *https://orcid.org/0000-0001-8826-1413*

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국문초록

피니싱 처리 이후 알카자이트 수복재의 표면거칠기와 미생물 부착

박초아[1] 대학원생 • 박호원[1] 교수 • 이주현[1] 교수 • 서현우[1] 교수 • 이시영[2] 교수

[1]강릉원주대학교 치과대학 소아치과학교실 및 구강과학연구소,
[2]강릉원주대학교 미생물학 및 면역학교실 및 구강과학연구소

이 연구는 새롭게 개발된 알카자이트 재료인 Cention N에 finishing처리를 한뒤에 표면 거칠기와 박테리아 부착에 대하여 조사하고자 함이다.

레진강화형 글래스아이오노머와 컴포짓트 레진, 알카자이트 재료를 원통형의 디스크 형태로 제작하였다(n = 48). 이 후 대조군과 3가지 카바이드버, 미세다이아몬드 버, 화이트스톤버의 피니싱 버에 따른 4가지 하위군으로 분류하였다. 표면 거칠기는 atomic force microscope으로 조사하였으며 표면관찰은 scanning electron microscope을 이용하여 진행하였다. 우식원성 미생물인 *streptococcus mutans*의 시편 부착을 위하여 CDC biofilm reactor를 사용하여 바이오필름을 배양한후 집락형성단위를 측정하였다.

레진과 Cention N의 아무처리 하지 않은 컨트롤 군의 표면 거칠기는 통계적으로 유의하게 피니싱 처리된 시편들보다 거칠었다. 하지만 표면 거칠기와 미생물 부착사이의 상관관계는 매우 약했다(PCC = 0.13). RMGI와 Cention N은 레진시편에 비해 미생물 부착이 적게 일어났다.

Cention N은 피니싱만으로도 임상적으로 허용되는 수준인 0.2 μm 이하의 거칠기를 보였으며 이온 방출 성질로 미생물 부착이 레진과 비교시 적은 것을 확인할수 있었다.