Surface roughness and Streptococcus mutans adhesion on surface sealant agent coupled interim crown materials after dynamic loading by simulated chewing forces

onur şahin (sonurs60@hotmail.com)
Alanya Alaaddin Keykubat University: Alanya Alaaddin Keykubat Universitesi https://orcid.org/0000-0002-8018-6946
Eda Topçu
Ankara Sehir Hastanesi
Ayşegül Köroğlu
Zonguldak Bülent Ecevit Üniversitesi: Zonguldak Bulent Ecevit Universitesi
Füsun Cömert
Zonguldak Karaelmas Universitesi Tip Fakultesi: Zonguldak Bulent Ecevit Universitesi Tip Fakultesi
Burak Yılmaz
University of Bern: Universitat Bern

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Abstract

Background: Surface sealant agents have been developed as an alternative to conventional polishing methods. These agents can achieve smoother surfaces in less time than conventional methods, studies on the condition of these agents against the chewing forces are lacking. The purpose of this study is to evaluate the surface roughness and the adhesion of Streptococcus mutans adhesion after chewing simulation of surface sealent coupled interim prosthetic materials.

Methods: One hundred and twelve specimens were fabricated from two poly(methyl methacrylate) (Tab 2000, Dentalon Plus) and two bis-acryl (Tempofit, Protemp 4) interim crown materials and divided into 4 groups (n=7) according to applied surface treatment procedures: conventional polishing (control) and 3 surface sealant (Palaseal, Optiglaze, Biscover) coupling methods. The surface roughness values were measured with a profilometer before (Ra0) and after aging process through dynamic loading in a multifunctional chewing simulator for 10,000 cycles at 50 N load combined with integral thermocycling (between 5°C and 55°C) (Ra1). Specimens were incubated with Streptococcus mutans suspension and bacterial colonizations were evaluated.

Results: Except the use of Optiglaze and BisCover LV surface sealant agents on Protemp 4 and the use of Palaseal and Biscover LV on Tempofit; surface sealant agent application significantly decreased the surface roughness compared with the conventionally polished specimens. Statistically, surface roughness after dynamic loading showed a significant increase in all groups, except Tab 2000 and Protemp 4 control groups. A positive correlation was found between surface roughness values of interim prosthodontic materials and the quantitiy of Streptococcus Mutans.

Conclusions: Even though surface sealant agent application significantly decreased the surface roughness compared with the conventionally polished specimens, dynamic loading significantly increased the surface roughness of all surface sealant coupled groups. The Ra values of all test groups were higher than the plaque accumulation threshold (0.20 μm). Streptococcus mutans adhered more on rougher surfaces.

Background

Interim crowns provide function, aesthetics, protection of the pulp, prevent undesired tooth movements and gingival growth [1]. They may also be used to improve occlusal relationships in patients with non-ideal occlusion, when the vertical dimension is planned to be changed prior to permanent restorations, and to change the gingival shape, size, contour and localization [2].

Polyethyl methacrylate (PEMA), poly (methyl methacrylate) (PMMA), urethane dimethacrylate (UDMA), polyvinyl methacrylate (PVMA), bis-acryl composite resin, and composite resin can be used with direct and indirect techniques to fabricate interim crowns [1–3, 4]. Acrylic-based and composite-based materials, which have been used for many years, are still the most commonly used interim crown materials currently [2, 5].

The surface smoothness of restorations is essential for aesthetics and oral hygiene [6, 7]. Interim crowns should be biocompatible and have surface properties that prevent bacterial adhesion and discoloration. Several studies have indicated that increased surface roughness promoted the increase in bacterial adherence and plaque accumulation [7–17].

In order to minimize the surface roughness of dental materials, polishing processes are carried out in several stages in laboratory conditions or chairside. Recently, surface sealant agents have been developed by manufacturers as an alternative to conventional polishing methods [18–21]. Although it has been reported that these agents can achieve smoother surfaces in less time than conventional methods, studies on the condition of these agents against the chewing forces are lacking [18]. In addition, how different sealant agents interact with interim crown materials in varied compositions under loading is not known.

The present study aimed to evaluate the effect of different surface treatment methods on the surface roughness and *Streptococcus mutans* adherence on interim crown materials before and after dynamic loading in a multifunctional chewing simulator [22]. The first null hypothesis of this study was that surface sealant agent coupling would not affect the surface roughness of interim crown materials. The second null hypothesis was that dynamic loading would not affect the surface roughness and *Streptococcus mutans* adherence on tested materials.

Material And Methods

Specimen preparation and surface treatments

In the present study, two bis-acryl composite resin-based (Protempt 4 (Prt), Tempofit (Tmp)) and two auto-polymerized polymethyl methacrylate (Tab 2000 (Tab), Dentalon Plus (Dnt)) interim crown materials were evaluated. By using stainless steel molds, twenty-eight disc shaped (10 mm in diameter and 2 mm in thickness) specimens were prepared for each resin in accordance with the manufacturers' instructions and divided into 4 groups (n=7) for surface treatment procedures; conventional laboratory polishing (control) and application of 3 different surface sealant agents (Palaseal (Ps), Optiglaze (Og), Biscover LV (Bc)). The surface sealant agents and interim crown materials used in the present study are shown in Table 1.

Table 1

Surface sealant materials and interim crown materials used.

Product	Code	Component	Manufacturer
Palaseal	Ps	Methyl methacrylate, tris(2-hydroxyethyl)-isocyanurate-triacrylate, acrylizedepoxyoligomer, acrylates, acrylizedpolysiloxane	Heraeus Kulzer GmbH
Optiglaze	Og	Methyl methacrylate, multifunctional acrylate, silica filler, photo inhibitor	GC Corp
Biscover LV	Bc	Dipentaerythritolpentaacrylate, ethanol	Bisco Inc
Tab 2000	Tab	Methyl methacrylate, n-butylmethacrylate	Kerr Corp
Dentalon Plus	Dnt	Methacrylate, copolymer, peroxide, initiator, pigment	Heraeus Kulzer GmbH
Protempt 4	Prt	Ethanol,2,2'-[(1-methylethylidene bis(4,1 phenyleneoxy)])] bis-diacetate,benzyl-phenyl- barbituric acid silane treated silica, tert-butyl	3M ESPE
Tempofit	Tmp	Ethoxylated bisphenol A dimethacrylate	Detax

The surfaces of all specimens were finished with a tungsten carbide bur (S274 190 060, Horico) and wet-ground by a sanding machine (Phoenix Beta, Buehler) for 100 rev/min during 15 seconds, using 400 grit silicon carbide abrasive paper (Atlas Waterproof Sheet, Saint-Gobain). Control group specimens of each material were first polished using a slurry of coarse pumice (Isler Pomza, Isler Dental) and water with a bristle brush on a polishing lathe (P1000, Zubler) under standard pressure (for 90 seconds at a rate of 1500 rpm), then, fine–polished using a polishing paste (Universal Polishing Paste, Ivoclar Vivadent) and a lathe flannel wheel (Blaudent, Anka Dental) for 90 seconds. After the specimens were coated with Palaseal, Optiglaze, or BisCover LV in a thin layer, they were polymerized for 90, 40 and 30 seconds, respectively, in a light-polymerizing unit (Dentacolor XS, Heraeus Kulzer GmbH) at a reading of 750 mW/cm^2.

Chewing simulation and surface roughness assesments

Surface roughness assessments were performed by using a contact profilometer (Perthometer M2, Mahr). Three measurements were made for each specimen by moving the diamond stylus (NHT- 6) of the device 5 mm in 7 seconds across the specimen's surface under constant pressure. The mean value of the measurements obtained for each specimen was recorded as R_a0 value in μm.

All specimens were subjected to an aging process consisting computerized dynamic loading test in a multifunctional chewing simulator (Mod Chewing Simulator, Esetron) for 10,000 cycles at 50 N load combined with integral thermocycling (between 5°C and 55°C). The descending and ascending velocities were 60 mm/s and the loading cycle frequency was 1.6 Hz. The antagonist tooth was simulated by stainless steel spherical ball, 6 mm in diameter. Following the chewing simulation, surface roughness of the specimens were remeasured. The measurements were repeated three times for each specimen and the means obtained for each specimen were recorded as R_a1.

Streptococcus mutans adhesion

Before bacterial adhesion, the specimens were cleaned with an ultrasonic cleaner (BioSonic; Coltène/Whaledent)for 15 minutes and sterilized in an autoclave at 121 °C for 15 minutes. Artificial saliva was prepared according to Fusayama formula: 0.4 g NaCl, 0.4 g KCl, 0.795 g $CaCl_2$ ($2H_2O$), 0.695 g $Na_2H_2PO_4$ (H_2O), 0.005 g Na_2S ($9H_2O$), 1g CH_4N_2O [23]. Specimens were covered with artificial saliva and mucin suspension (M2378, Mucin from porcine stomach, Type II, Sigma Aldrich) (140 mg/100 ml) (5 ml) in a petri dish and left for 1 hour to produce a pellicle [24,25]. *Streptococcus mutans*NCTC 10449 was used in this study. After rehydrate of*Streptococcusmutans*strain in Tryptic Soy Broth (TSB, Oxoid), 100 µL of broth was transferred on to blood agar (Oxoid) and incubated in 5% CO_2 ambient air at 37 °C for 24 hours. Then, tubes containing 2 ml of*Streptococcusmutans*suspension with 0.5 of McFarland turbidity (10^8 colony forming units/milliliter (CFU/mL)) were prepared in TSB (5% sucrose supplemented) and the incubated specimens in artificial saliva were transferred to those tubes. Tubes were incubated in the same ambient conditions for 24 hours. After incubation, the specimes were washed in sterile phosphate buffered saline (PBS) solution (8 gr NaCl, 0.2 g KCl, 1.44 g $Na_2HPO_4$and 0.24 g KH_2PO_4in 1000 ml of H_2O) by centrifuge at 1000 g for 3 minutes. After centrifugation, each specimen was placed in new glass tubes containing 1 ml of sterile PBS. The bacterial adhesion was evaluated by measuring colony-forming units per mL (CFU/mL). The tubes were treated for 6 minutes in an ultrasonic bath (50 kHz and 150 W), thereby the adherent bacteria cells were allowed to pass into the PBS.Three 1/10 serial dilutions were made in order to obtain the lower quantity of bacteria in the sample. A 100 µl of diluted PBS samples was sealded on blood agar and incubated in 5% CO_2 ambient at 37 °C for 24 hours. At the end of the incubation, the colonies observed were counted and the total number of adherent bacteria was calculated by multiplying with the dilution coefficient.

Scanning electron microscopy (SEM) analysis

The surfaces of all resin materials after dynamic loading were examined with a scanning electron microscope (SEM) (Nova Nano SEM 450, FEI Co.). The acceleration voltage of cathode was set to 14 kV. The images were obtained at ×200, ×2000 and ×5000 magnifications.

Statistical analysis

The data were statistically analyzed by using a software program (SPSS version 19.0; SPSS Inc.). Kolmorogov-Smirov test of homogeneity was used to evaluate the distribution of the variables. Surface roughness and bacterial adhesion data were analyzed with a 2-way ANOVA to evaluate the effects of surface treatment, resin type, and their interactions. The means were compared with Tukey HSD test(α=.05). Pearson correlation coefficient test was used to investigate the correlation between R_a1 and bacterial adhesion values after dynamic loading, and *P*<.05 was considered significant.

Results

According to the 2-way ANOVA, for R_a, the effect of interim material and interim material-surface treatment interaction was statistically significant (*P*< .05) (Table 2). Mean R_a0 and R_a1 values and standard deviations for interim material-surface treatment combinations are shown in Table 3. When conventionally polished material groups were compared, significant differences were observed for R_a0 values of Tab and Dnt groups, and for R_a1 values of Dnt group (*P*< .05).

Table 2
Two-way ANOVA results for comparison of surface roughness (R_a) and bacterial adhesion (CFU/mL).

Parameter	Source	SS	df	MS	F	*p*
R_a	Interim material (A)	12.775	3	4.258	8.950	< .001*
	Surface treatment (B)	1.650	3	.550	1.56	.331
	A x B	14.919	9	1.658	3.484	.001*
	Error	45.675	96	.476		
	Total	468.137	112			
CFU/mL	Interim material (A)	60067676.670	3	20022558.890	152.179	< .001*
	Surface treatment (B)	2594065.955	3	864688.652	6.572	< .001*
	A x B	7002976.509	9	778108.501	5.914	< .001*
	Error	12630959.143	96	131572.491		
	Total	217765083.000	112			

SS, sum of squares; df, degrees of freedom; MS, mean square. * *P*< .05 indicates statistical significance.

Table 3
Mean/SD R_a0 and R_a1 values (μm) and statistical summaries of test groups.

Interim Material	Surface Treatment	R_a0		R_a1		t-test**
		Mean (SD)	**Tamhane***	**Mean (SD)**	**Tamhane***	(*P* values)
Tab	Con	1.66 (0.39)	Cb	1.83 (0.37)	Aa	.433
	Ps	0.31 (0.26)	Aa	1.35 (0.58)	Aa	.002
	Og	0.52 (0.08)	ABa	2.29 (1.00)	Aa	.004
	Bc	0.53 (0.15)	Aa	1.60 (0.44)	Aa	.001
Dnt	Con	1.08 (0.26)	Bb	3.18 (0.64)	Bb	.001
	Ps	0.32 (0.11)	Aa	1.91 (0.86)	Aa	.003
	Og	0.40 (0.14)	ABa	2.50 (0.53)	Aab	.001
	Bc	0.56 (0.18)	Aa	1.95 (0.46)	Aa	.001
Prt	Con	0.74 (0.18)	Ab	0.91 (0.13)	Aa	.066
	Ps	0.22 (0.09)	Aa	1.41 (0.64)	Aab	.003
	Og	0.68 (0.24)	Bb	1.34 (0.71)	Aab	.049
	Bc	0.47 (0.12)	Aab	2.12 (0.99)	Ab	.004
Tmp	Con	0.81 (0.14)	Ab	1.88 (1.01)	Aa	.030
	Ps	0.51 (0.18)	Aab	2.09 (0.54)	Aa	.001

*Statistical comparisons between interim material/surface treatment groups were shown as letters and values having same letters are not significantly different for Tamhane test ($p > 0.05$). The capital letters indicates the comparisons between same surface treatment applied interim material groups and the small caps indicates the differences between surface treatment groups for the same interim material. **The pairwise comparisons of R_a0 and R_a1 values with independent sample t-test ($P < .05$ indicates statistical significance).

	Og	0.22 (0.11)	Aa	1.91 (0.53)	Aa	.001
	Bc	0.48 (0.19)	Aab	1.70 (0.88)	Aa	.010

*Statistical comparisons between interim material/surface treatment groups were shown as letters and values having same letters are not significantly different for Tamhane test ($p > 0.05$). The capital letters indicates the comparisons between same surface treatment applied interim material groups and the small caps indicates the differences between surface treatment groups for the same interim material. **The pairwise comparisons of R_a0 and R_a1 values with independent sample t-test ($P < .05$ indicates statistical significance).

For R_a0 values of all interim material groups, statistically significant differences were observed between the control and surface sealant applied specimen groups, except for Prt_Og, Prt_Bc, Tmp_Ps and Tmp_Bc ($P < .05$). Following dynamical loading, for R_a1 values, statistically significant differences were found between Dnt control and Ps or Bc coupled groups, and between Prt control and Prt_Bc ($P < .05$). Except for the control groups of Tab and Prt, the differences between the R_a0 and R_a1 values were statistically significant in all groups ($P < .05$). The R_a0 values (0.22 to 1.66 μm) and the R_a1 values (1.34 to 3.18 μm) for all groups were higher than the plaque accumulation threshold (0.20 μm). SEM images of the surfaces of Tab, Dnt, Prt, and Tmp after dynamic loading are shown in Figs. 1–4.

The interim material, surface treatment, and their interaction were statistically significant for bacterial adhesion ($P < .001$). Mean CFU/mL values, standard deviations (SD) and the statistical summaries for the interim material-surface treatment technique combinations are shown in Table 4. For all interim material groups, no statistically significant differences were observed between the control group and the surface sealant agent-coupled groups, except for Dnt_Bc ($P > .05$) (Fig. 5).

Table 4
Mean/SD CFU/mL values and statistical summaries of test groups.

Interim Material	Surface Treatment	Cfu/mL	
		Mean (SD)	**Tamhane***
Tab	Con	177.14 (55.37)	Aa
	Ps	111.43 (41.81)	Aa
	Og	150.00 (41.53)	Aa
	Bc	99.29 (60.72)	Aa
Dnt	Con	2487.86 (348.59)	Cbc
	Ps	2887.14 (459.19)	Cc
	Og	1912.29 (563.62)	Cab
	Bc	1480.57 (621.02)	Ba
Prt	Con	980.00 (486.21)	Ba
	Ps	927.14 (340.13)	Ba
	Og	734.86 (430.32	Ba
	Bc	1186.86 (638.34)	Ba
Tmp	Con	950.00 (92.60)	Ba
	Ps	1382.14 (172.60)	Ba
	Og	1114.29 (199.26)	Ba
	Bc	1015.71 (190.84)	Ba

*Statistical comparisons between interim material/surface treatment groups were shown as letters and values having same letters are not significantly different for Tamhane test (*P* > .05). The capital letters indicate the comparisons between same surface treatment applied interim material groups and the small letters indicate the differences between surface treatment groups for the same interim material.

According to the Pearson Correlation Analysis, the coefficient of correlation between R_a1 and Streptococcus mutans adhesion was statistically significant ($P < .001$, $r^2 = .323$) and indicated that these two variables were moderately correlated (Table 5). SEM images of *Streptococcus mutans* adhesion and proliferation on rough surfaces are shown in Fig. 6.

Table 5
Correlation between R_a1 and bacterial colonisation.

	R_a1	Colonisation
R_a1 Pearson correlation	1	,323**
Sig.	112	,001
N		112
Colonisation Pearson correlation	,323**	1
Sig.	,001	112
N	112	
**Correlation is significant for $P \leq .01$ level.		

Discussion

The first null hypothesis of this study was rejected because the effect of surface sealant agent coupling was significant on the surface roughness of some interim crown materials. Dynamic loading was significant on the surface roughness of interim crown materials except for the control groups of Tab and Prt, also *Streptococcus mutans* adherence on interim crown materials was affected by dynamic loading. Accordingly, the second null hypothesis was rejected.

In the present study, R_a0 values of interim crown materials ranged between 0.22 and 1.66 µm, which were below the clinical undetectability limit of 10 µm that Kaplan et al [26] reported. However, these values are above the threshold R_a of 0.20 µm that Bollen et al [27] indicated. Similar to the study findings of Ayuso-Montero et al [8], the control groups of PMMA resins showed higher surface roughness values compared with the control groups of bis-acryl composite resins. Contrarily, unlike to the findings of the present study, Şen et al [3] reported that due to the heterogeneous composition of bis-acrylate composite resins, higher surface roughness values were observed with to the filler particles extruding on the surface.

Surface sealant agents applied to the surfaces of materials in a single phase are more advantageous than conventional polishing processes in terms of application and time. Surface sealant agents contribute to surface smoothness by filling the surface defects and micro cracks after application. However, due to their high viscosity, there are disadvantages such as weak bonding to the underlying material, degradation of surface quality, and low resistance to abrasion [18, 29]. In the present study, when the R_a0 values were evaluated, similar to previous studies [18, 20], surface sealant agent application decreased the Ra values of all PMMA resin groups and bis-acryl resin groups, except Og- or Bc-coupled Prt and Pc- or Bc-coupled Tmp groups. Statistically significant differences observed in PMMA resins may have been due to increasing effect on the molecular weight of the components present on the surface of the methacrylate, and decreasing the surface roughness with the application of surface sealant agent. However, it has been shown that surface sealant agent application could remove surface particles that are not polymerized or adhered to the surface, causing surface irregularities. Also, application errors and the formation of air bubbles were reported to increase the R_a [19].

Due to intraoral conditions including chewing forces, the unpolymerized layer may be easily separated from the surface and micro-cracks may occur [21]. In the present study, dynamic loading was performed with multidirectional chewing simulator in order to evaluate the effects of oral environment on specimens prepared from interim crown materials. During dynamic loading, 6 mm diameter steatite, which has similar physical properties to enamel, was used as an antagonist [28]. It is recommended that 240,000 chewing cycles should be performed to simulate 1 year of clinical service [22]. In the present study, 10,000 cycles corresponding to 15 days of clinical use were applied and after the dynamic loading process, it was observed that the surface roughness increased in all surface sealant agent coupled specimens. Also, SEM images of all resin groups after dynamic loading were consistent with the surface roughness measurements. This result may be attributed to the surface defects that occurred due to easy removal of the layer potentially incompletely polymerized on the surface of the resin, and to the low resistance of sealant agents to abrasion [21].

Streptococcus mutans has been reported to be the most abundant bacteria on enamel and root plaque (77%) [14], has high adhesion to all surfaces in the mouth, and it is a bacteria that is virulent with its acidogenic and aciduric properties [11]. Although in vitro studies have shown that artificial saliva does not reflect all the features of natural saliva [29], its use is essential for standardization [30, 31]. In the present study, to provide bacterial adhesion on the surface of the specimens, artificial saliva was prepared in accordance with the equation of Fusayama [29].

Similar to the findings of the present study, Aykent et al [9] reported a positive correlation between the surface roughness and bacterial adhesion of restorative materials polished with different procedures. In the present study, the SEM images (Fig. 6) revealed bacterial aggregation in areas with high surface roughness. Haralur et al [32] compared stainless steel crowns, PMMA and bis-acryl resin interim crown materials, and the highest dental plaque accumulation was observed on PMMA specimens and the least was observed on the stainless-steel crown. Bacterial adhesion and proliferation on PMMA and bis acryl resin groups were reported to be due to hydrophilic polymer matrix and monomer structure. In the present study, the highest bacterial adhesion was found in the Dnt_Ps specimen group, while the least bacterial adhesion was in the Tab_Bc group.

Although surface roughness is an important feature in terms of bacterial adhesion, it is not a sufficient factor alone [6]. The effects of physical properties of materials such as surface electrical properties and free energy, hydrophobicity, fluoride release, as well as chemical properties have been previously studied [15]. Quirynen et al [16] reported more dental plaque deposition on hydrophilic surfaces than on hydrophobic surfaces. Olsson et al [15] stated that there was a critical limit on the hydrophobicity of surfaces in dental plaque deposition and the deposition below this limit would be minimal. Pellicle coating of the surfaces of dental materials changes the surface energy, which changes the bacteriostatic or bactericidal effect of the dental plaque [17]. Accordingly, these factors should be taken into consideration when interpreting the effects of varying factors and situations.

The specimens used in the current study were prepared in disc form containing flat surfaces, however, the recesses and protrusions on the tooth morphology may not allow an effective polishing process and the roughness and bacterial adhesion may be affected. Clinical studies are needed to corroborate the findings of the present study. Also, further in vitro and in vivo research is needed to evaluate other factors affecting bacterial adhesion to interim materials particularly when surface sealants are used.

Conclusions

Within the limitations of this study, the following conclusions were drawn:

Even though surface sealant agent application significantly decreased the surface roughness compared with the conventionally polished specimens, dynamic loading significantly increased the surface roughness of all surface sealant coupled groups. The Ra values of all test groups were higher than the plaque accumulation threshold (0.20 μm). Streptococcus mutans adhered more on rougher surfaces. Although tested surface sealant agents enabled smoother surfaces, their use on the occlusal surfaces of tested interim crown materials may lead to increased roughness compared with conventional polishing. When Dentalon is used, Biscover LV application can be recommended for smoother surfaces with less bacterial adhesion,

Declarations

Availability of data and materials

The datasets supporting the findings of this article are available from the corresponding author.

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Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

For this type of study, formal consent is not required.

Conflict of interest

The authors declare no competing interests.

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Figures

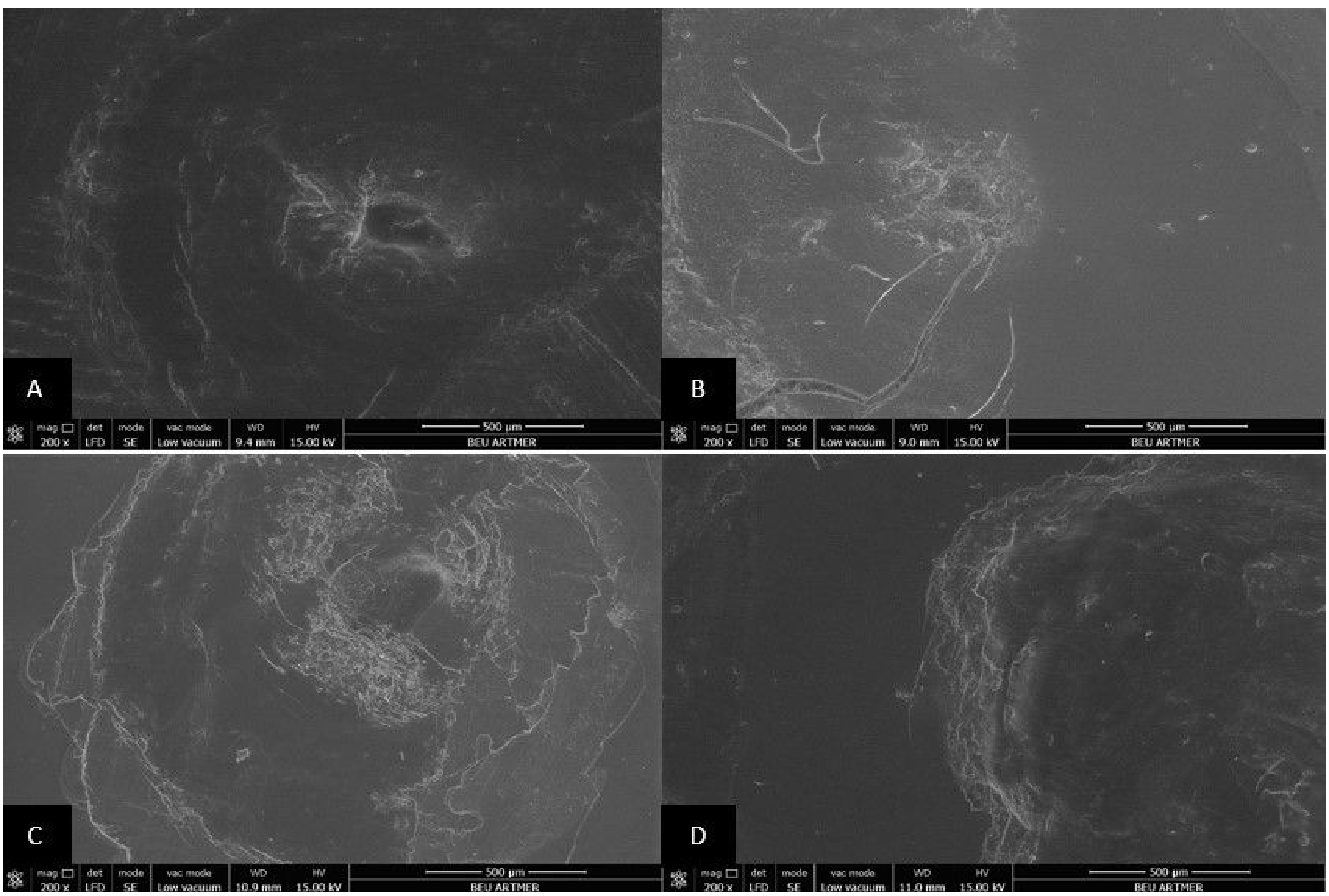


Figure 1

Scanning electron micrograph analysis after dynamic loading process (×200 magnification). A, Conventionally polished B, Palaseal. C, Optiglaze. D, BisCover LV coupled with Tab 2000 interim crown material.

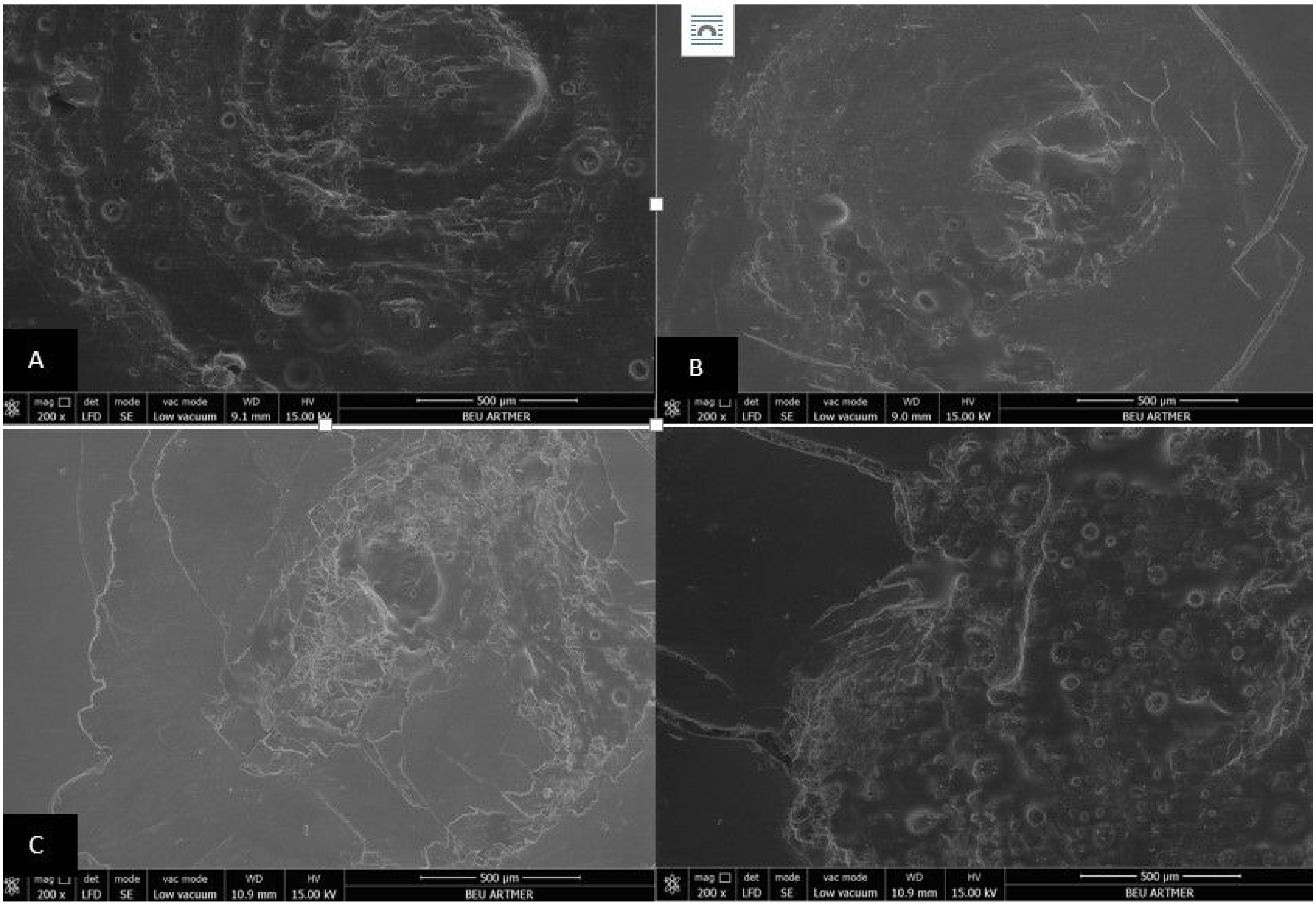


Figure 2

Scanning electron micrograph analysis after dynamic loading process (×200 magnification). A, Conventionally polished B, Palaseal. C, Optiglaze. D, BisCover LV with Dentalon Plus interim crown **material.**

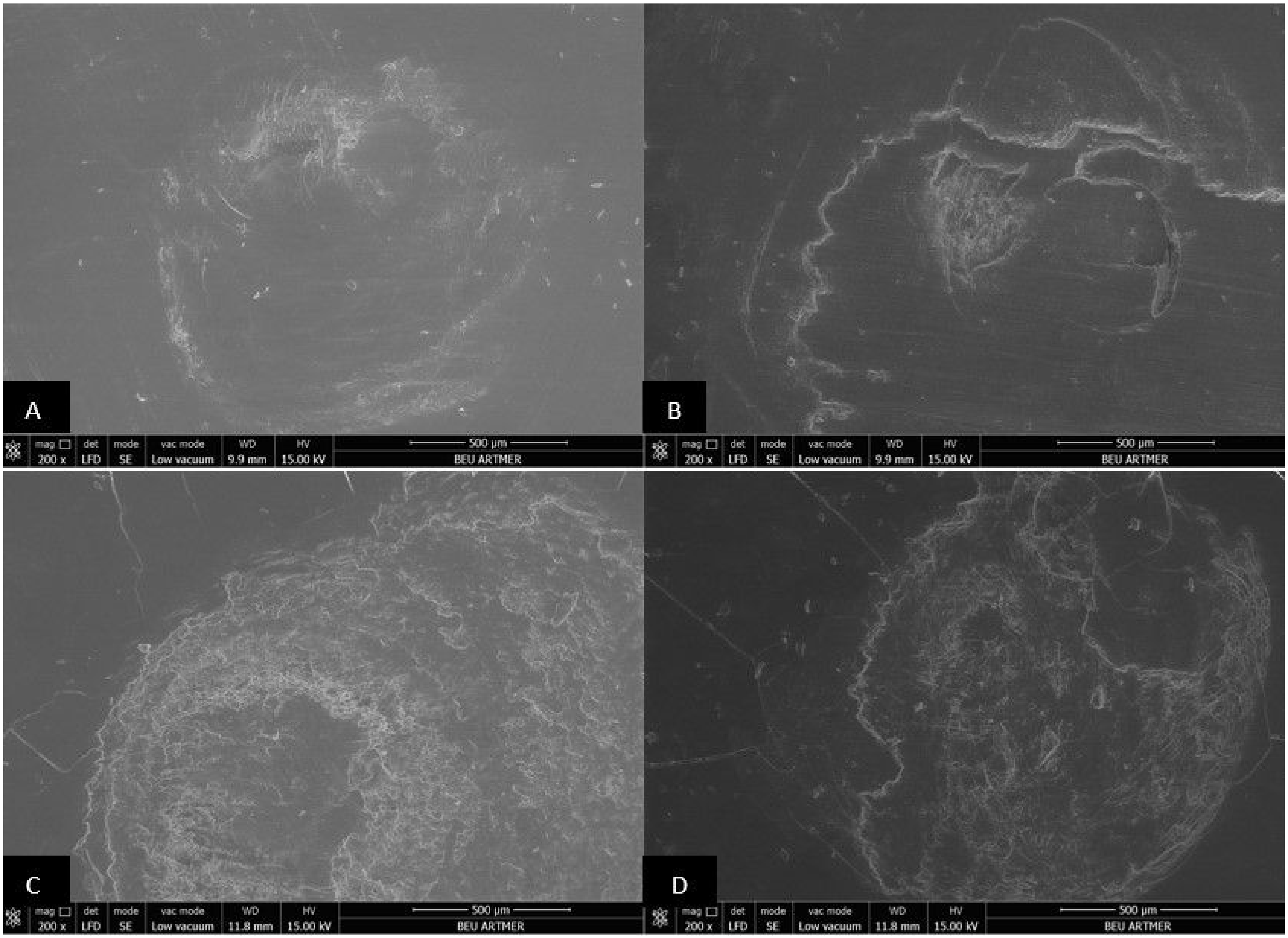


Figure 3

Scanning electron micrograph analysis after dynamic loading process (×200 magnification). A, Conventionally polished B, Palaseal. C, Optiglaze. D, BisCover LV with Protemp 4 interim crown material.

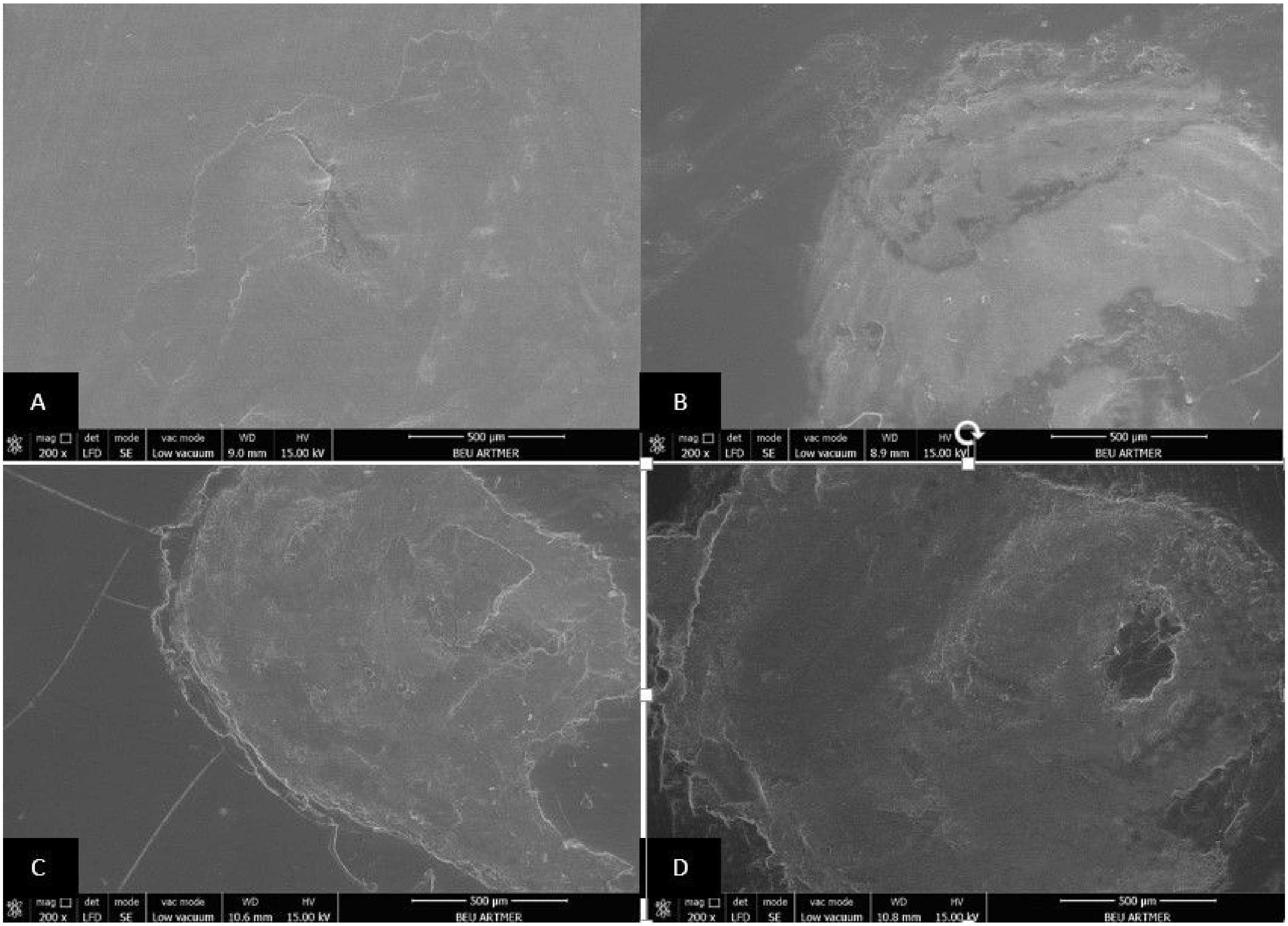
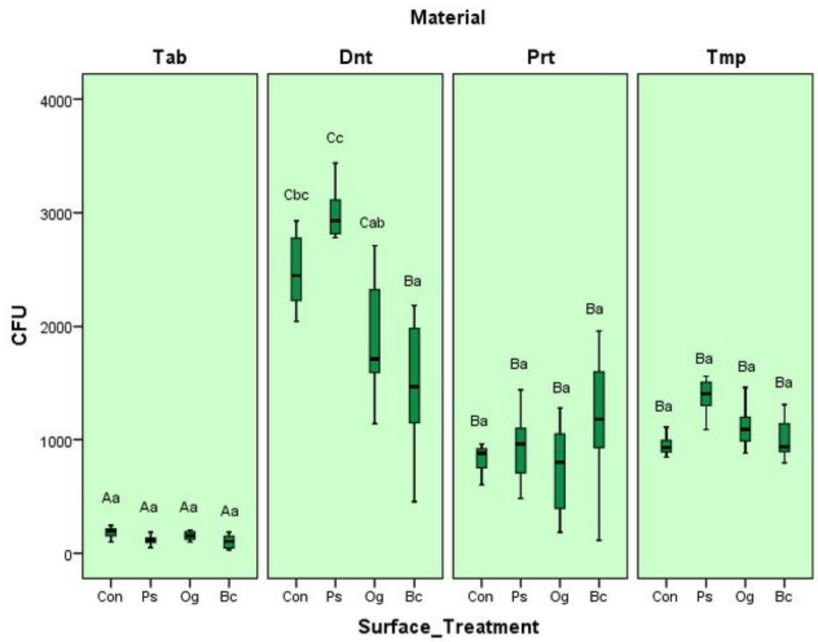


Figure 4

Scanning electron micrograph analysis after dynamic loading process (×200 magnification). A, conventionally polished B, Palaseal. C, Optiglaze. D, BisCover LV with Tempofit interim crown material.



*Statistical comparisons between interim material/surface treatment groups were shown as letters and values having same letters are not significantly different for Tamhane test (*P*>.05). The capital letters indicates the comparisions between same surface treatment applied interim material groups and the small caps indicates the differences between surface treatment groups for the same interim material.

Figure 5

Mean CFU values (Cfu/mm) of test groups.

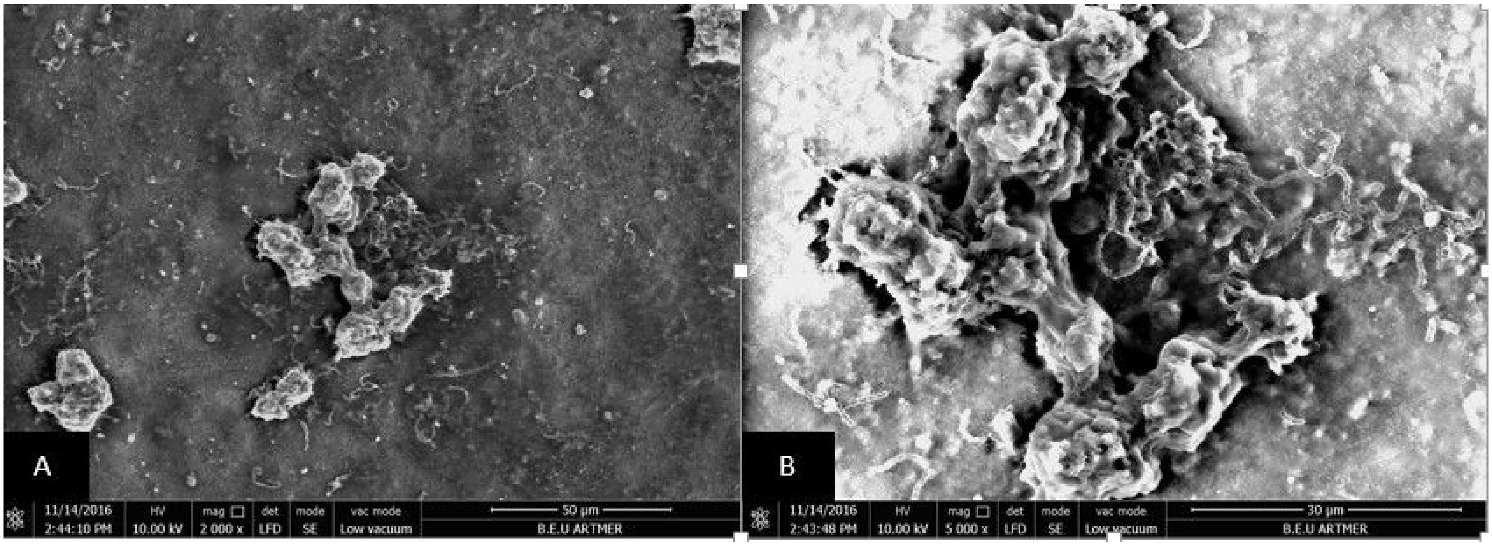


Figure 6

Scanning electron micrograph analysis after Streptococcus Mutans adhesion and proliferation (note aggregation on rough surface), A,2000×magnification, B,5000×magnification.