An investigation of the effect of scaling-induced surface roughness on bacterial adhesion in common fixed dental restorative materials

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Statement of problem. Bacterial plaque must be routinely removed from teeth, adjacent structures, and prostheses. However, the removal of this plaque can inadvertently increase the risk of future bacterial adhesion.

Purpose. The purpose of this investigation was to assess the change in the surface roughness of 3 different surfaces after dental prophylactic instrumentation and how this influenced bacterial adhesion.

Material and methods. Forty specimens each of Type III gold alloy, lithium disilicate, and zirconia were fabricated in the same dimensions. The specimens were divided into 4 groups: ultrasonic scaler, stainless steel curette, prophylaxis cup, and control. Pretreatment surface roughness measurements were made with a profilometer. Surface treatments in each group were performed with a custom mechanical scaler. Posttreatment surface roughness values were measured. In turn, the specimens were inoculated with *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Actinomyces viscosus*. Bacterial adhesion was assessed by rinsing the specimens with sterile saline to remove unattached cells. The specimens were then placed in sterile tubes with 1 mL of sterile saline. The solution was plated and quantified. Scanning electron microscopy was performed. The statistical analysis of surface roughness was completed by using repeated-measures single-factor ANOVA with a Bonferroni correction.

Results. The surface roughness values for gold alloy specimens increased as a result of prophylaxis cup treatment (0.221 to 0.346 Ra) ($P<.01$) and stainless steel curette treatment (0.264 to 1.835 Ra) ($P<.01$). The results for bacterial adhesion to gold alloy proved inconclusive. A quantitative comparison indicated no statistically significant differences in pretreatment and posttreatment surface roughness values for lithium disilicate and zirconia specimens. In spite of these similarities, the overall bacterial adherence values for lithium disilicate were significantly greater than those recorded for gold alloy or zirconia ($P<.05$). Instrumentation of the lithium disilicate and zirconia with the stainless steel curette significantly increased bacterial adhesion compared with the control ($P<.05$).

Conclusions. The results of this investigation indicate that Type III gold alloy exhibited increased surface roughness values after stainless steel curette and prophylaxis cup treatments. Zirconia was less susceptible to bacterial adhesion than lithium disilicate, and greater bacterial adhesion was found for the stainless steel curette than the other instrumentation methods. (J Prosthet Dent 2014;112:1265-1270)

Clinical Implications

To prevent disease and ensure high esthetic quality, every effort must be made to avoid instrumentation of restorative margins, especially gold alloys. Zirconia demonstrated superior resistance to scaling-induced surface roughness and bacterial adhesion compared with Type III gold and lithium disilicate.

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Prosthodontic treatment requires restoration margins where a tooth or implant meets the restorative material. Type III gold alloy (GA), lithium disilicate (LD), and zirconia (ZR) are commonly used materials for fixed prosthodontics.[1-3] Bacteria can populate almost any surface.[4] The nature of the restorative margin provides a niche for bacteria not present on sound enamel.[5] Many factors influence bacterial adhesion; however, surface roughness (Ra) has been determined to be the predominant factor.[5-11] Surface roughness greatly facilitates the adhesion process, because the bacteria are sheltered from removal forces.[8]

If the bacteria are not removed regularly, their prolonged presence on teeth as well as restorative materials becomes the primary cause of oral diseases, including gingivitis and dental caries.[5,8,9,12,13] *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Actinomyces viscosus* have been implicated as the main causative organisms of dental caries.[14,15] Infected areas should be routinely assessed and treated to minimize the risk of such diseases.[16] Routine dental hygiene therapy consists of using stainless steel curettes (SSCs), an ultrasonic scaler (US), and a dental prophylaxis with a prophylaxis cup (PC) and prophylaxis paste.[17,18] Unfortunately, these treatments can often induce some degree of surface roughness on the tooth or implant, as well as on the restoration.[19,20]

Many authors have evaluated the influence of these instruments on the Ra value of a variety of restorative materials. Most tend to evaluate the effects of multiple types of instruments on 1 individual material rather than multiple materials. There are conflicting opinions as to whether or not a PC roughens the surface of GA.[21,22] Treatment with an SSC and a US can easily damage the surface of GA.[19] In contrast, treatment with the same instruments has not consistently yielded an increase in the Ra values of dental ceramics.[17] Dental investigations are inconsistent with regard to the effect on ZR. One investigation found that the Ra value was increased by treatment with both an SSC and US, whereas another found no change after treatment with the US.[23,24]

Several investigations have evaluated bacterial adhesion to multiple restorative materials.[6,18,25-30] GA and LD showed more bacterial adhesion compared with ZR.[25,27] However, few authors have evaluated the correlation of scaling-induced surface roughness of fixed dental restorative materials and its influence on bacterial adhesion.

The purpose of this investigation was to assess the change in the surface roughness of GA, LD, and ZR after treatment with a US, an SSC, and a PC with fine prophylaxis paste. The influence of instrumentation with a US, an SSC, and a PC on bacterial adhesion was evaluated by inoculating the specimens with *S mutans*, *L acidophilus*, and *A viscosus*.

The null hypotheses for this investigation were that treatment with a US, an SSC, or a PC would not increase the Ra value of the specimen compared with a polished surface, regardless of material, and that treatment with a US, an SSC, or a PC would not influence bacterial adhesion to the research specimens.

MATERIAL AND METHODS

Specimen preparation

A total of 48 GA specimens (Firmilay II; Jelenko) in their ingot state as provided by the manufacturer (13×8×1 mm) were polished at 200 rpm with irrigation by using a polishing machine (MultiPrep System; Allied High Tech). The specimens were mounted individually with double-sided adhesive tape. A 30-μm lapping film was used until the specimen was flat, followed by a 6-μm lapping film for 10 minutes and then a 1-μm lapping film for 5 minutes. The specimen was removed from the mounting plate, acetone was used to remove the tape, new tape was applied, and the opposing side was polished. All specimens were gently steam cleaned after polishing to remove residual debris.

Also, 48 LD specimens (IPS e.max Press; Ivoclar Vivadent) were fabricated by investing (IPS PressVEST; Ivoclar Vivadent) base plate wax patterns (Neowax; Dentsply) with the same dimensions as the GA ingots and hot isostatically pressing them according to the manufacturer's instructions. Additionally, 48 ZR specimens were fabricated by cutting oversized ZR blanks (Everest ZS-Blank [B42/16]; KaVo) with a linear precision saw (IsoMet 5000; Buehler) to compensate for the shrinkage specified on each blank in their green state. Specimens were sintered according to the manufacturer's instructions (KaVo Everest Therm; KaVo) to create the same dimensions as for the GA ingots. Both the LD and ZR specimens were polished by following the same protocol as with the GA specimens.

Twelve specimens of each material were divided into 4 treatment groups corresponding to their designated treatment (control group [CTR], PC, SSC, or US). Ten of these specimens were identified as treatment specimens, and the other 2 served as scanning electron microscope (SEM) representative specimens.

Instrumentation, inoculation, and microscopy

The specimens were steam cleaned, then measured for pretreatment Ra value at 3 different locations along the y-axis (Y=3, 5, and 7) with a profilometer (Proscan 2000; Scantron). All 3 Ra values were recorded, and the average was calculated. Each treatment specimen was sequentially fixed in place on a custom device (Sabri Dental Enterprises) used to standardize the treatment rendered. Each instrument was fixed in an arm with weights to produce loads of 5.0 N for the SSC and PC and 1.0 N for the US.[31-33] The SSC (SG11/12 Gracey Curette; Hu-Friedy) and US (Cavitron Select SPS; Dentsply Intl) were attached with the tip engaged at a 15-degree angle to the specimen. Five even strokes were made with the SSC and US[24] in 3 locations spread

across the surface of each specimen. A new SSC tip was used for every 6 specimens treated.[34] The US was used at a low setting with the water-cooling system activated.[35] A new universal US tip (BioSonic Ultrasonic Scaler Insert, 30 kHz; Coltène/Whaledent) was used for each treatment group. The slow-speed handpiece (KaVo Dental) with PC was attached at a 90-degree angle to the specimen.[36] The handpiece with PC and fine prophylaxis paste (Nupro with Fluoride; Dentsply Intl) was operated for 20 seconds, moving slowly back and forth across the specimen at 5000 rpm.[37] A new disposable PC (Rinn Crescent Prophy Cup; Dentsply Intl) was used for each specimen. The same calibrated operator completed all instrumentation. All specimens were gently steam cleaned to remove residual debris. The posttreatment Ra measurements were made by following the same protocol as was used for the pretreatment Ra. The specimens were then placed in individual sterile tubes and sterilized in a low-temperature sterilization system (Sterrad NX; Advanced Sterilization Products).

S mutans (ATCC 25175), *L acidophilus* (ATCC 4356), and *A viscosus* (ATCC 43146) were cultured on trypticase soy agar with 5% sheep blood (TSA II, BB 221239/221261) and incubated at 35 ±2°C in an anaerobic environment. A suspension containing approximately 1×10^8 colony-forming units per milliliter (CFU/mL) of each organism was prepared by suspending the growth harvested from TSA II plates in sterile saline. The suspensions were adjusted so that the turbidity was equal to a 0.5 McFarland turbidity standard. Equal parts of the 3 suspensions were combined (approximately 1.5×10^8 CFU/mL) and then diluted 1:100 in brain-heart infusion broth (Bacto 237400). This resulted in an inoculum suspension containing approximately 1.5×10^6 CFU/mL total bacteria. Each specimen was covered with 1.0 mL of the inoculation suspension. The specimens were then incubated for 18 hours at 35 ±2°C in an anaerobic environment. After incubation, the culture broth was removed, and the specimens were washed with 1.5 mL sterile water 3 times. Next, 1 mL of sterile saline was added to each specimen in sterile tubes, and the specimens were vigorously mixed for 2 minutes to remove attached cells. The saline solution was serially diluted 1:10 (n=4 for controls and n=5 for treatment specimens) and plated on TSA II. Plates were incubated 35 ±2°C in an anaerobic environment for 4 days. The numbers of CFU on the plates were counted, and the CFU/mL recovered was calculated.[34]

The 24 SEM representative specimens were evaluated under SEM (JSM 6610; JEOL) by a trained technician to visually document both surface roughness induced by treatment and bacterial adhesion to the specimens. The CTR specimens served as a baseline representation for each material. Photographs were made of representative specimens to compare the visual characteristics of the respective surfaces.

Statistical analysis

The data were analyzed with univariate and descriptive methods. The pretreatment Ra and posttreatment Ra values were compared by using repeated-measures ANOVA with 2 factors (treatment and material). Because of significant interaction terms, the data were reanalyzed with repeated-measures single-factor ANOVA, and a Bonferroni correction was applied to allow for multiple comparisons (α=.01).

Bacterial counts were analyzed with both parametric and nonparametric methods (Kruskal-Wallis nonparametric ANOVA on ranks) because of the nonnormality of the data. Post hoc analysis was accomplished by using Mann-Whitney *U* tests for pairwise comparisons (α=.05).

RESULTS

The mean pretreatment Ra values for all specimens ranged from 0.215 to 0.248. The difference between pretreatment and posttreatment Ra values for GA specimens instrumented with the SSC and PC was statistically significant (0.221 to 0.346 Ra) (F=8.4; df= 2; P<.01). The SSC induced an increase in Ra of 0.264 to 1.835 (F=10.3; df=2; P<.01), nearly 13 times that of the PC. All other differences were not statistically significant (F=4.6; df=2; P>.01) (Table I).

The LD specimens, regardless of treatment, showed statistically significantly higher CFU/mL counts than GA and ZR (F=6.4; df=2; P<.05). No statistically significant differences in CFU/mL counts were found between GA and ZR, regardless of treatment (F=1.3; df=4; P>.05). Additionally, with respect to the ZR and LD specimens, the nonparametric testing showed no statistically significant differences in the CFU/mL counts of the CTR and PC, the PC and the US, and the SSC and US specimens (F=1.9; df=2; P>.05).

However, the ZR and LD specimens treated with SSC demonstrated statistically significantly higher CFU/mL counts compared with the CTR specimens (F=6.6; df=2; P<.05). Only the LD specimens showed significantly different CFU/mL counts between the CTR and US and the PC and SSC specimens (F=6.5; df=2; P<.05). The LD specimens treated with the US exhibited the greatest CFU/mL counts, followed by LD treated with SSC, ZR treated with SSC, and LD treated with PC. The CTR specimens in both LD and ZR showed the lowest CFU/mL within their material. The GA specimens yielded inconclusive results with regard to treatment and CFU/mL counts (see Table I).

GA specimens showed obvious surface alterations at 50× magnification. The PC produced shallow circular patterns. The US produced moderately deep scratches, and the SSC produced even deeper scratches. The LD specimens treated with the US displayed minor surface alterations at 50× magnification. At 300× magnification, mild surface roughness induced by the SSC became visible. No signs of roughness were apparent on the LD

TABLE I. Results for mean Ra and CFU/mL values (n=10 for all groups)

Type III Gold					Lithium Disilicate					Zirconia				
Group	Initial Ra	Post Ra	Change	CFU/mL	Group	Initial Ra	Post Ra	Change	CFU/mL	Group	Initial Ra	Post Ra	Change	CFU/mL
CTR	0.220			79 889	CTR	0.203			170 778	CTR	0.220			40 600
SD	0.000			79 570	SD	0.000			113 371	SD	0.000			24 981
PC	0.221	0.346	0.125	120 860	PC	0.258	0.241	−0.017	221 111	PC	0.225	0.226	0.001	92 000
SD	0.024	0.069		101 860	SD	0.022	0.024		113 297	SD	0.016	0.018		71 940
SSC	0.264	1.835	1.571	64 750	SSC	0.236	0.246	0.010	512 222	SSC	0.211	0.200	−0.010	258 556
SD	0.034	0.657		31 892	SD	0.057	0.046		274 079	SD	0.013	0.007		250 693
US	0.282	0.270	−0.012	24 640	US	0.296	0.313	0.017	754 444	US	0.204	0.202	−0.002	46 522
SD	0.041	0.054		21 190	SD	0.068	0.085		784 699	SD	0.006	0.009		44 894

CFU, colony-forming units; CTR, control group; PC, prophylaxis cup; SSC, stainless steel curette; US, ultrasonic scaler.

specimens instrumented with the PC. The ZR specimens showed no signs of roughness at ×50 magnification. However, mild surface alterations caused by the US were evident at ×500 magnification, and those caused by the SSC were evident at ×5000 magnification. No bacteria were seen on any of the specimens.

Evidence of instrumentation with the PC was clearly visible on both the GA and LD specimens, but not on the ZR specimens. The same was true for the specimens instrumented with the SSC. However, a greater depth to the scratches was seen on the GA specimens compared with the LD specimens. The US showed visual signs of instrumentation on all 3 test materials. There appeared to be a fine scratch on the GA specimens, whereas the markings on the LD and ZR specimens were less precise and appeared to have little to no depth.

DISCUSSION

The null hypotheses that treatment with a US, an SSC, or a PC would not increase the Ra value of the research specimens nor influence bacterial adhesion were rejected. Multiple investigations have evaluated how the surface roughness of a specific material is affected by treatments with 1 or 2 periodontal instruments. Fewer investigations have been completed that evaluate the effects of several instruments on the surface roughness of several materials. Even fewer investigations combine these effects with the resultant influence on bacterial adhesion.

Roulet and Roulet-Mehrens[22] found that polishing GA with different pastes had no effect on surface roughness (Ra). However, the present investigation found that treatment with a PC and fine prophylaxis paste yielded a change in Ra values from 0.221 to 0.346. These results are supported by Heath and Wilson,[21] who found that polishing GA with fine and medium prophylaxis paste (Nupro green and red) caused a significant increase in Ra values. Both the present investigation and that of Heath and Wilson did not use any additional lubricant such as a saliva substitute. The presence of such a lubricant may decrease the abrasive quality of this treatment.

Cutler et al[19] found that alloys with high gold content are very susceptible to surface deterioration when treated with a variety of prophylactic instruments. These findings are logical, as an alloy with higher gold content will be softer than other metals with a lower gold content. In particular, the greatest damage was caused by a US. In contrast, the US created no detectable increase in Ra values in this investigation. Rather, the most significant increase in Ra values (difference of 1.571) was after treatment with the SSC. Both investigations engaged the tip of the US and used irrigation. The present investigation used a low setting. However, the setting used by Cutler et al was not indicated in the investigation. With regard to the SSC, Cutler et al performed the instrumentation on edge, whereas the present investigation engaged the tip of the instrument. Engaging the edge of the instrument is difficult when treating a surface that has been machined to be smooth and flat. Teeth rarely have flat surfaces such as those found in an in vitro study. Therefore, although engaging the tip of the SSC proved to be more destructive in nature, it is also more clinically relevant.

The results suggest that no significant change occurred in the Ra values of ZR or LD after treatment with the PC, the SSC, and the US. The hardness of these 2 materials greatly exceeds that of GA. Vigolo and Motterle[24] found that treating ZR with such instruments did yield significant increases in Ra values. However, the force they applied to the specimens exceeded those applied in the present investigation. The results are similar to those produced by Seol et al,[23] whereby the treatment of ZR with US tips yielded no significant change in Ra values. Likewise, Lee et al[17] found roughness results of LD unaltered by treatment with both a US and SSC. Nonetheless, they found minor scratching of the surfaces, which

can lead to compromised esthetic qualities and unsightly staining of the restorative surface. The present investigation supports these findings with respect to GA and LD. Additionally, the US was the only instrument to produce surface discoloration of the ZR specimens. These surface markings could be remnants of the tip of the instrument as it is abraded by the material being treated.

With regard to bacterial adhesion, the LD specimens yielded a significantly higher CFU/mL count compared with GA and ZR. This result was comparable with that of Bremer and Kohorst,[25] who found that of 5 different types of dental ceramics, the ZR specimens showed the least amount of bacterial adhesion after 24 hours of intraoral wear, whereas the LD specimens showed the most. Hahnel et al[26] found that in spite of a higher Ra value of LD compared with other dental ceramics, including ZR, no correlation was observed between Ra and bacterial adhesion. Another investigation comparing ZR and titanium with respect to bacterial adhesion found that ZR yielded significantly less bacterial adhesion with Ra values similar to those in the present investigation.[29] Although no statistical difference in bacterial adhesion was noted between GA and ZR during the present investigation, Kim et al[27] found that GA had more bacterial adhesion than ZR. These varying results with regard to bacterial adhesion reinforce the idea that multiple factors influence bacterial adhesion other than surface roughness.[7,8,10,11]

The results of this investigation indicated statistically significant differences in bacterial adhesion due to the instruments used on the materials tested. However, few comparable investigations are available. Future research in this area is recommended.

The SEM findings of the present investigation are supported well by other studies. Seol et al[23] found deep scratches on GA specimens after instrumentation with a US and no surface alterations of ZR at ×100 magnification. Likewise, Vigolo and Motterle[24] discovered deeper scratches on the ZR specimens instrumented with the US compared with the smaller scratches induced by the SSC. No bacteria were visualized, because the specimen preparation was inadequate for preserving the bacteria for imaging purposes.

The present investigation had several limiting factors: the minimal amount of bacterial varieties, the lack of saliva, and the clinical relevance of the specimen configuration. Including more than 1 species of bacteria to simulate the diverse group of organisms found within the oral cavity enhanced the clinical applicability of this investigation. Saliva was not included in this in vitro study. Saliva contains many proteins, glycoproteins, and other nutrients that can also affect adherence. Intraorally, these materials will have a tooth-to-restorative-material interface and thus greater potential for scaling-induced surface roughness and damage when passing over the interfaces. Future research is needed to confirm the adverse effect on the restoration margin of the instrumentation methods in this investigation.

Dental materials often exhibit a certain degree of porosity. This can be inherent or created during processing. Porosity can influence surface roughness and bacterial adhesion. The degree of porosity for each specimen was not considered in this investigation. Rather, strict standardization in processing and treatment was implemented to minimize its effect on the results obtained.

CONCLUSIONS

The following conclusions can be drawn from the results of this study. Type III gold exhibited increased surface roughness values after SSC and PC treatments. LD and ZR exhibited no change in surface roughness values after SSC, US, or PC treatments. ZR was less susceptible to bacterial adhesion than LD was. Greater bacterial adhesion was found in both LD and ZR for the SSC than for the other instrumentation methods.

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