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THESIS APPROVAL PAGE FOR MASTER OF SCIENCE IN ORAL BIOLOGY

Title of Thesis: "Bacterial Growth after Six Implantoplasty Protocols"

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Master of Science Degree

THESIS/MANUSCRIPT APPROVED: DATE:

SYNATZSKE.ANGELA.M.1403004681 Digitally signed by SYNATZSKE.ANGELA.M.1403004681 Date: 2022.07.29 14:16:48 -05'00'

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Bacterial Growth after Six Implantoplasty Protocols

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Disclaimers: No disclaimers.

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Word, Figure/Table, and Reference Count: 3285 words, 9 figures, 3 tables, 30 references

Short Running Title: Bacterial Growth after Implantoplasty

Summary (1 sentence): Brownie, Greenie sequence and Arkansas stone sequence will result in the least amount of bacterial growth in laboratory setting.

Abstract (427 words)

Background: Implantoplasty of a dental implant surface may prevent or decrease the formation of early bacterial colonizers on the altered surface and could potentially provide the opportunity for long term implant success and cleansability. The aim of this in vitro study was to evaluate the amount of bacterial growth on the implant surface following different implantoplasty protocols.

Materials and Methods: Sixty implants were treated with six different implantoplasty sequences. Sequence 1: Brownie, Greenie sequence (BG) (Diamond burs coarse, medium, fine, Brownie, Greenie); Sequence 2: Arkansas stone sequence (AS) (Diamond burs coarse, medium, fine, Arkansas stone); Sequence 3: Short diamond sequence (SD) (Diamond burs coarse, medium, ultra-fine); Sequence 4: Short diamond sequence with Greenie (SDG) (Diamond burs coarse, medium, ultra-fine, Greenie); Sequence 5: Complete diamond sequence (CD) (Diamond burs coarse, medium, fine, extra-fine, ultra-fine); Complete diamond sequence with Greenie (CDG) (Diamond burs coarse, medium, fine, extra-fine, ultra-fine, Greenie). Five untreated implants served as a control. Each implant was scanned with Keyence 3D Laser Scanning Confocal Profilometer rendering Sq values as a measure of surface roughness. 6mm of treated surfaces from test groups and non-treated surfaces from control groups were then exposed to *Streptococcusgordonii* and cultured for the presence of bacteria. The number of CFU/mL recovered were determined per group. Analysis of variance (ANOVA) testing with a Tukey's post hoc test was performed to determine significant differences among the different bur sequences.

Results: Mean surface roughness (root mean square value, Sq) amounted to 1.92 ± 0.72 μm (BG), 2.15 ± 0.69 μm (AS), 2.20 ± 0.45 μm (SDG), 3.24 ± 0.99 μm (SD), 2.54 ± 0.71 μm (CDG), 3.32 ± 1.04 μm (CD), and 15.65 ± 4.12 μm (control). The differences in surface roughness were statistically significant between control and test groups and also between BG/AS groups and SD/CD groups ($P<0.05$). Mean bacterial growth for each group was 1190 ± 363 CFU/ml (BG), 1353 ± 708 CFU/ml (AS), 1608 ± 1021 CFU/ml (SDG), 2836 ± 1874 CFU/ml (SD), 2130 ± 851 CFU/ml (CDG), 2942 ± 706 CFU/ml (CD), and 16560 ± 6693 CFU/ml (control). The differences in bacterial growth were statistically significant between control and test groups and also between BG/AS groups and SD/CD groups ($P<0.05$).

Conclusion: The BG sequence resulted in the smoothest implant surface and the least amount of bacterial growth. Considering the number of burs utilized and production of debris from the silicone polishers, the AS sequence might provide a more favorable result in a clinical setting since there was no statistical difference between the BG sequence and the AS sequence in terms of bacterial growth.

Key Words

Implantoplasty, peri-implant disease, peri-implantitis, controlled laboratory study, implantoplasty protocol, bur sequence.

INTRODUCTION

A recent systematic review showed that a 10-year implant survival rate was 96.4% at the implant level (1). The presence of biofilm initiates a host immune response and can cause a destructive inflammatory process around implants that are in function. This will lead to pocket formation, bleeding on probing, suppuration and eventually bone loss around the implants. This progressive loss of supporting bone around the implant is termed peri-implantitis (2, 3). The prevalence of peri-implantitis is highly variable depending on the study and definition of peri-implantitis. In but a study by Shimchuk et al it was determined that the prevalence of peri-implantitis at the patient level was 15.2% and at the implant level was 8.7% based on the definitions from 2017 American Academy of Periodontology (AAP) workshop (4). When it comes to treatment success for peri-implantitis, there are limited treatment options proving to be successful. One of the most important factors to consider in peri-implantitis prevention and treatment is the constant battle that we face with microbial colonization (5). Early intervention for peri-implantitis is critical. The initiation of this destructive inflammatory process is closely associated with accumulation and formation of biofilm and microorganism colonies on the implant surface (6). Studies have shown that there is no single method that is superior to others when it comes to surface decontamination and treatment protocols for peri-implantitis (7, 8). Among different treatment modalities available, an osseous resective approach has been shown from case series and clinical trials to be a beneficial and an effective treatment option for peri-implantitis (9). A recent systematic review on treatment of peri-implantitis also has shown that the use of implantoplasty can improve clinical parameters such as bleeding on probing and plaque scores either when it was used alone or when it was used in combination with other treatment modalities (10). Implantoplasty focuses on reduction of local bacterial

accumulation and biofilm formation by removing implant threads and smoothing rough implant surfaces to create less plaque-retentive areas. The most frequently used techniques for implantoplasty in a clinical setting is to utilize diamond burs or carbide burs to remove exposed threads. Following implant thread removal, silicone polishers have also been used to further polish and smooth the implant surface (11). Different techniques with varying bur sequences will result in alteration of the implant surface with the goal to reduce the chance for bacterial accumulation. *Streptococcus gordonii* (*S. gordonii*) has been identified as one of the early bacterial colonizers which has a critical role in initiating the adhesion of middle and late bacterial colonizers. Treatment methods that can prevent or decrease the formation of this early colonizer on the surface of dental implants might provide the opportunity for long term implant success by prevention of biofilm formation (12). The present in vitro study aims to evaluate the surface roughness on dental implants following six different implantoplasty modalities and its relation to the amount of bacterial growth on the dental implant surface.

MATERIALS AND METHODS

Implants

For the test groups, the coronal six millimeters of sixty implants (3i T3 ® with DCD ® Tapered Implant; 5.0 Ø mm, length 11.5 mm) were treated with six different implantoplasty sequences using diamond burs of different grit sizes and different polishing burs. This coronal six millimeters of implantoplasty was done to resemble treatment of horizontal peri-implant bone loss with a supracrestal defect. Five untreated implants that received no implantoplasty served as the control group (Fig. 1).

Burs

The following burs were utilized with copious water irrigation.

- Diamond Instruments; Brassler, USA: Coarse (125 micron grit), medium (100 micron grit), fine (30 micron grit), extra-fine (15 micron girt), ultra-fine (8 micron grit)
- Polishers: Brownie®, Greenie®; Shofu Dental, Germany and Arkansas Stone; Brassler, USA

Implantoplasty procedures

Six different implantoplasty protocols were designed (Table 1) with modification based off of a previous study by Ramel et al (11).

1. BG = Brownie, Greenie sequence (Diamond burs coarse, medium, fine, Brownie, Greenie)
2. AS = Arkansas stone sequence (Diamond burs coarse, medium, fine, Arkansas stone)
3. SD = Short diamond sequence (Diamond coarse, medium, ultra-fine)
4. Group SDG = Short diamond sequence with Greenie (Diamond burs coarse, medium, ultra-fine, Greenie)
5. Group CD = Complete diamond sequence (Diamond burs coarse, medium, fine, extra-fine, ultra-fine)
6. Group CDG = Complete diamond sequence with Greenie (Diamond burs coarse, medium, fine, extra-fine, ultra-fine, Greenie)

One calibrated individual performed all sixty implantoplasty procedures. For coarse (125

micron grit), medium (100 micron grit) and fine (30 micron grit) diamond burs, high speed hand piece with 200,000 rotations per min (rpm) was used with copious water irrigation. For extra-fine (15 micron girt) and ultra-fine (8 micron grit) diamonds and all the polishing burs, 40,000 rpm was used with copious water irrigation. The procedure was continued until the implant surface was polished in such a way that it had a machined like surface appearance. A new set of burs was utilized for each implant.

Surface roughness measurements

Keyence 3D laser scanning confocal profilometer was used to scan each implant surface. Each group of implants from the test group (six implantoplasty groups; ten implants per group) and control group (five untreated implants) were fixed into polyvinylsiloxane (PVS) impression material to stabilize the implants prior to evaluation of surface roughness (Fig. 2). Three locations (U: upper, M: middle and L: lower) within the coronal six millimeters of each implant surface were selected for the surface roughness measurements of root mean square roughness (Sq) and the mean value was calculated (Fig. 3). After surface roughness measurements, one random implant was selected from each group and scanning electron microscope (SEM) images were taken with 1000 x magnification.

Bacterial growth

Implants were then sterilized with a steam sterilizer (Amsco 400, Steris, Mentor, OH). A suspension of *S. gordonii* was prepared to inoculate the implant surfaces. *S. gordonii*, (ATCC 51656) was grown on Trypticase Soy Agar with 5% sheep blood (TSA II, BBL 221261) and incubated at 37 +/- 2° C for 48 hours in an anaerobic chamber. An inoculation suspension was

prepared by harvesting the growth of the organism from the TSA II and suspending it in sterile saline equal to 0.5 McFarland turbidity standard (approx. 1.5 x 10^8 CFU/mL), then a 1:10 dilution of the suspension was made with sterile saline resulting in an inoculation suspension of approximately 1.5 x 10^7 CFU/mL. For each group (six test groups and one control group), implants were secured in a tongue depressor with PVS impression material (Fig. 4). Flowable composite restorations were placed in the screw channels of each implant to prevent bacteria from invading the screw channel areas. Then implants were immersed in the inoculum suspension for 30 minutes in a way that only the coronal six millimeters of the implant surfaces were exposed (Fig. 5). Once inoculated, the implants were rinsed with deionized water and vortexed (Fisher Heavy Duty Vortex Mixer, Fisher Scientific, and Waltham, MA) for two minutes in sterile saline to remove the bacteria from the implants. The saline was serially diluted and plated on TSA II. The plates were incubated at 37 +/- 2°C for 48 hours in an anaerobic chamber. After incubation, the number of colony forming units (CFUs) on the plates were counted and CFU/mL recovered was calculated for each implant.

Statistical analysis

Analysis of variance (ANOVA) testing with a Tukey's post hoc test was performed to determine significant differences among different implantoplasty groups and untreated control group in terms of surface roughness and bacterial growth. The significance level was set to 0.05.

RESULTS

Surface roughness

All descriptive data for surface roughness are presented in Table 2 in μm. The control group, where no surface treatment was rendered, showed statistically higher surface roughness of 15.65 μm compared to any test groups. When comparing test groups only (Fig. 6), the BG group showed the smoothest surface with roughness of 1.92 μm, followed by the AS group with 2.15 μm. The SDG group showed 2.2 μm of surface roughness, CDG group 2.54 μm, SD group 3.24 μm and finally the CD group showed the highest value of surface roughness amongst all test groups with 3.32 μm. The BG, AS, SGD and CDG groups all showed statistically less surface roughness compared to the SD and CD groups. The BG group alone showed statistically less surface roughness compared to the CDG group while there was no statistical difference in terms of surface roughness between the BG, AS and SDG groups and between the AS, SDG and CDG groups.

Bacterial growth

All descriptive data for bacterial growth are presented in Table 3 in CFU/mL. The untreated control group showed statistically higher number of bacterial growth with 16560 CFU/mL compared to any of the test groups. When comparing test groups only (Fig. 7), the BG group showed the least amount of bacterial growth with 1190 CFU/mL followed by the AS group with 1354 CFU/mL. The SDG group had 1608 CFU/mL of bacterial growth, the CDG group had 2130 CFU/mL, the SD group had 2836 CFU/mL and finally the CD showed the greatest number of bacterial growth with 2942 CFU/mL. Amongst the BG, AS, SDG and CDG groups there were no statistical difference in terms of bacterial growth. Amongst the SDG, CDG, SD and CD groups there were also no statistical differences in terms of bacterial growth. The BG and AS groups showed significantly less bacterial growth compared to the SD and CD groups.

DISCUSSION

The primary etiology factors for peri-implantitis include poor oral hygiene (13) and certain periodontal pathogens such as *Prevotella intermedia*, *Tannerella forsythia* and *Fusobacterium nucleatum* (14). Therefore, successful elimination of plaque and detoxification of the contaminated implant surface is critical in resolving peri-implant disease. Implantoplasty has been proposed with the idea that a smoother implant surfacewill allow for proper decontamination of the implant surface (15). In fact, the rough implant surface characteristics stronglyinfluence the progression of peri-implantitis. Based on the ligature induced peri-implantitis model, the progression of peri-implantitis, if left untreated, is more pronounced and faster at implants with a rough surface compared to implants with a machined surface (16, 17, 18). Moreover, bacterial growth on implants with different surfaces in vitro showed that there was significantly more bacterial growth on rough surface implants compared with machined surface implants (19). Therefore removal of exposed threads and smoothening of the implant surface in cases with horizontal peri-implant bone loss with a supracrestal bony defect can potentially be beneficial in treating peri-implantitis. However, to date, there is no standardized protocol for implantoplasty procedures and the literature reports a wide variability of protocols. Many studies that looked at effectiveness of implantoplasty utilized different sequences to perform implantoplasty and some studies have shown better results than others. In fact, some studies did not even specify sequences of implantoplasty that was performed. For instance, a study done by Romeo and Sapata used sequences of different grit sizes of diamond burs and finished with an Arkansas stone and/or silicone polishers and both studies resulted in about three millimeters of probing depth reduction (20,21). Meanwhile, Dalago's study used diamond burs only and resulted in about two millimeters of probing depth reduction (22). In

contrast to Dalago's study, Lasserre's research utilized only 30 micron grit size of diamond burs and had four millimeters of probing depth reduction (23). Some studies however did not specify the drill sequence and had about 1.3 millimeters of probing depth reduction (24). Meanwhile, when sequences of three different diamond burs with different girt sizes and polishing burs were utilized, it resulted in a probing depth reduction of five millimeters (25). There are a wide variety of different burs that have been utilized with variable results. However, the most frequently used techniques for implantoplasty in a clinical setting is to utilize diamond burs to remove exposed threads. Following implant thread removal, silicone polishers have also been used for some studies to polish and smooth the rough implant surface. Unique techniques with different bur sequences will result in a variation of surface roughness and varying degrees of bacterial accumulation as well. In 2016, Ramel et al evaluated whether or not one of six implantoplasty sequences is superior at rendering a minimal final implant surface roughness. His conclusion was that considering the final surface roughness and treatment duration, the use of rotary diamond burs in decreasing roughness, followed by an Arkansas stone (AS), appears to be an optimal treatment option (26). However actual bacterial accumulation associated with the surface roughness following implantoplasty has not been evaluated. This in vitro study was conducted to assess which implantoplasty protocol is the most favorable to generate a surface with the least amount of bacterial growth.

The SEM images of implant surfaces after six different implantoplasty procedures (with 1000 x magnification) showed a smoother surface in the BG, AS and SDG groups compared to the CDG, SD and CD groups (Fig. 8). The control group, where no surface treatment was rendered, showed statistically higher surface roughness and greater bacterial growth than any test groups as would be expected. The bacterial growth for the control group was about 5.6

times higher than the CDG group, which has the highest bacterial growth in comparison to the other treated groups. Thus, any implantoplasty protocol will help in decreasing the amount of bacterial growth and could potentially provide the opportunity to reduce peri-implant inflammation in a clinical setting. Moreover, the amount of bacterial growth mirrored the degree of surface roughness. The BG group with the least surface roughness resulted in the least amount of bacterial growth and the CD group with the most surface roughness resulted in the most amount of bacterial growth. In terms of bacterial growth, the SD and CD groups are unfavorable groups because they resulted in statistically greater amounts of bacterial growth compared to the BG and AS groups. The SDG and CDG groups on the other hand are not statistically significant from any other groups in terms of bacterial growth. Although the BG and AS groups are not statistically significantly more favorable than the SDG and CDG groups, they are clearly more favorable than the SD and CD groups. The SDG and CDG groups are not significantly more favorable than the SD and CD groups (Fig. 9). The BG and AS groups used the exact same sequence of diamond burs but the BG group finished with two different silicone polishers (Brownie®, Greenie®) and the AS group finished with only one polishing bur (Arkansas Stone). This potentially can impact treatment duration due to the different number of burs utilized. Moreover, silicone polishers tend to create debris and wear out more easily than the Arkansas Stone bur. Some clinicians recommend the use of Arkansas Stone burs as polishing burs to avoid the potential for further contamination of the surgical site (11, 26).

Every peri-implant defect is different and there are multiple treatment options available that can be utilized. One of the main limitations of implantoplasty is that it is only applicable for the treatment of a supracrestal defect (horizontal bone loss) or a defect that is not amenable to

peri-implant regeneration. Another controversial topic related to implantoplasty is the possibility for titanium deposition in the surrounding tissue during the implantoplasty procedure. Controlling titanium debris can be challenging. It has been recommended to perform implantoplasty in the supracrestal aspect first even before the removal of the granulation tissue. This allows for removal of titanium particles and debris embedded in the granulation tissue at the same time (27). However, a recent systematic review by Stavropoulos et al concluded that titanium particles are less likely to initiate peri-implant disease with biological complications (28, 29, 30). However, the possibility cannot be completely excluded.

Lastly, this was an in vitro, controlled laboratory study. The actual process of peri-implant bone loss and the response to treatment is a complex process. The adhesion and colonization of bacterial plaque after different implantoplasty protocols in a clinical setting and its relation to improvement in clinical parameters such as probing depth changes and bleeding on probing after peri-implantitis treatment need to be further investigated.

CONCLUSION

The BG sequence (diamond burs coarse, medium, fine, Brownie, Greenie) resulted in the smoothest implant surface and the least amount of bacterial growth. However, considering the number of burs utilized and production of excess debris due to silicone polishers, the AS sequence (diamond burs coarse, medium, fine, Arkansas stone) might be more favorable in a clinical setting since there was no statistical difference between the BG and AS groups in terms of surface roughness and bacterial growth. Further investigation in a controlled clinical setting is needed to further evaluate if one implantoplasty protocolcan in fact result in more improved clinical parameters compared to other implantoplasty protocols.

ACKNNOWLEDGMENTS

The authors declare no conflicts of interest with this study.

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FIGURES AND TABLES

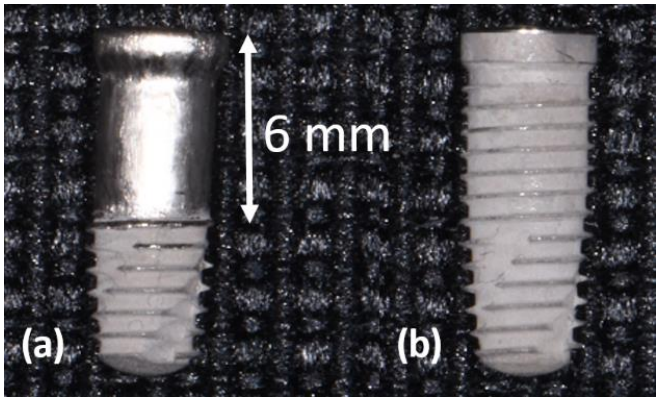

Fig. 1. (a): test group with coronal 6 mm treated with implantoplasty. (b): control group with untreated surface.

	coarse	medium	fine	extra-fine	ultra-fine	Brownie	Greenie	Arkansas
BG	x	x	x			x	x	
AS	x	x	x					x
SD	x	x			x			
SDG	x	x			x		x	
CD	x	x	x	x	x			
CDG	x	x	x	x	x		x	

Table 1. Six different implantoplasty protocols using different burs: BG = Brownie, Greenie sequence, AS = Arkansas stone sequence, SD = Short diamond sequence, SDG = Short diamond sequence with Greenie, CD = Complete diamond sequence, CDG = Complete diamond sequence with Greenie.

Fig. 2. Each group was fixed into PVS impression material to stablilize the implants

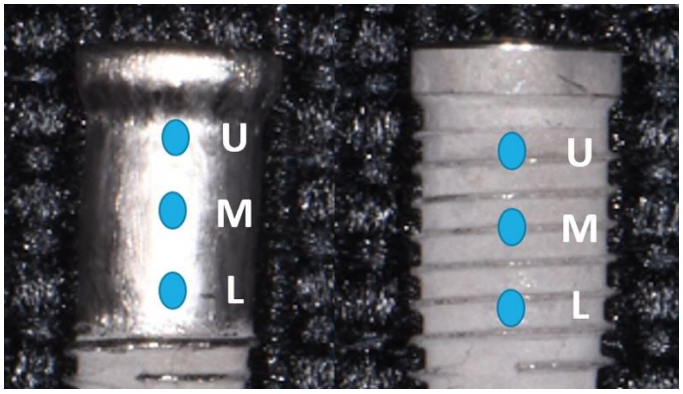

Fig. 3. Three locations (L: lower, M: middle, U: upper) within the coronal 6 mm of the implant surface were selected to measure the surface roughness and the mean value was calculated.

Fig. 4. Implants were secured on a tongue depressor with PVS impression material to facilitate immersion in inoculum suspension (see Fig. 5).

Fig. 5. Implants were immersed in the inoculum suspension in a way that only the coronal 6 mm of the implant surfaces were exposed.

Group	μm (Mean +/- SD)	Median
BG	1.92 +/- 0.72	1.97
AS	2.20 +/- 0.49	2.14
SD	3.24 +/- 0.99	3.16
SDG	2.15 +/- 0.69	1.96
CD	3.32 +/- 1.04	3.22
CDG	2.54 +/- 0.71	2.56
Control	15.65 +/- 4.11	14.87

Table 2. The mean value of root mean square roughness (Sq) values stated in μm, standard deviation (SD) and median for each test (implantoplsty) group and control group.

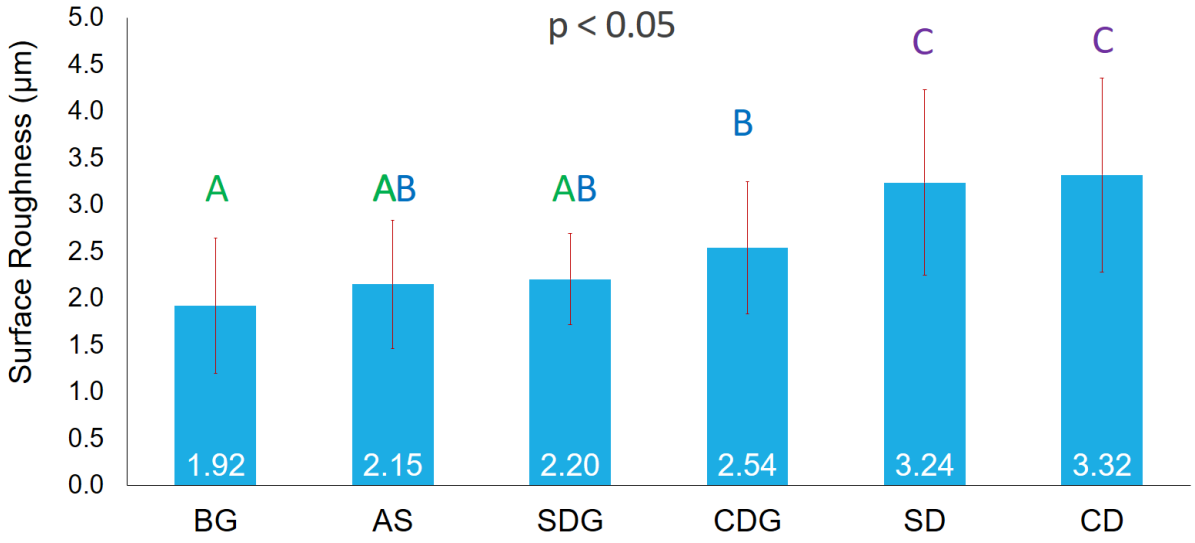

Fig. 6. Six different groups using different implantoplasty protocols were compared for surface roughness. Bars with the different letters are significantly different than each other ($p < 0.05$). SD and CD groups showed significantly higher surface roughness compared to rest of the groups while BG group showed significantly less surface roughness compared to CDG group.

Group	CFU / mL (Mean +/- SD)	Median
BG	1190 +/- 364	14200
AS	1354 +/- 708	1230
SD	2836 +/- 1875	1970
SDG	1608 +/- 1021	1300
CD	2942 +/- 707	2810
CDG	2130 +/- 851	2110
Control	16560 +/- 6693	14200

Table 3. The mean value of bacterial growth stated in CFU / mL (Sq), standard deviation (SD) and median for each test (implantoplasty) group and control group.

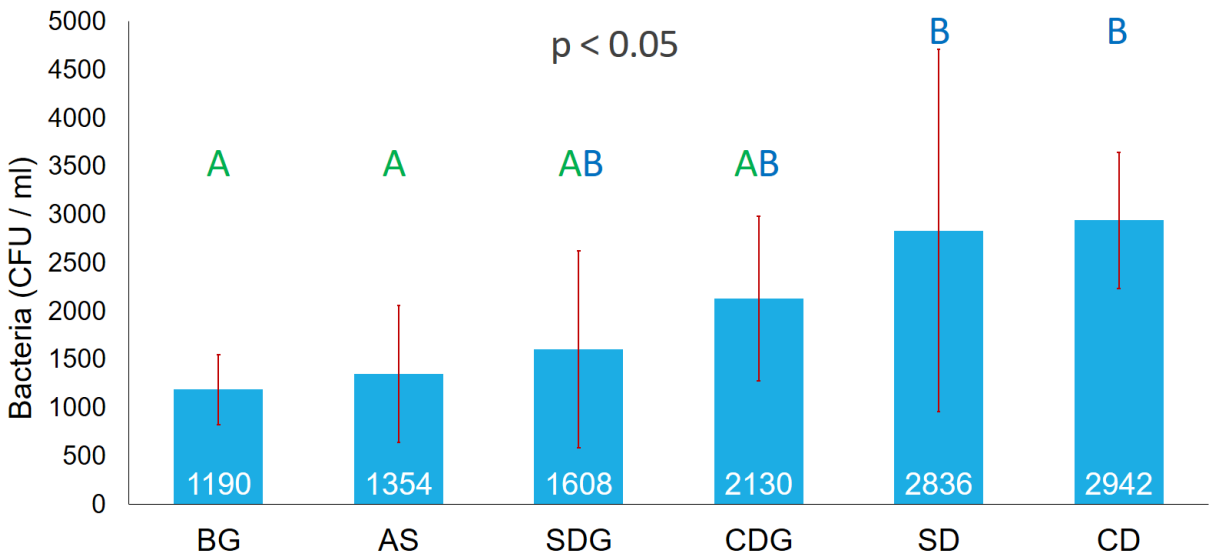

Fig. 7. Six different groups using different implantoplasty protocols were compared for bacterial growth. Bars with the different letters are significantly different than each other ($p < 0.05$). SD and CD groups showed significantly greater amount of bacterial growth compared to BG and AS groups.

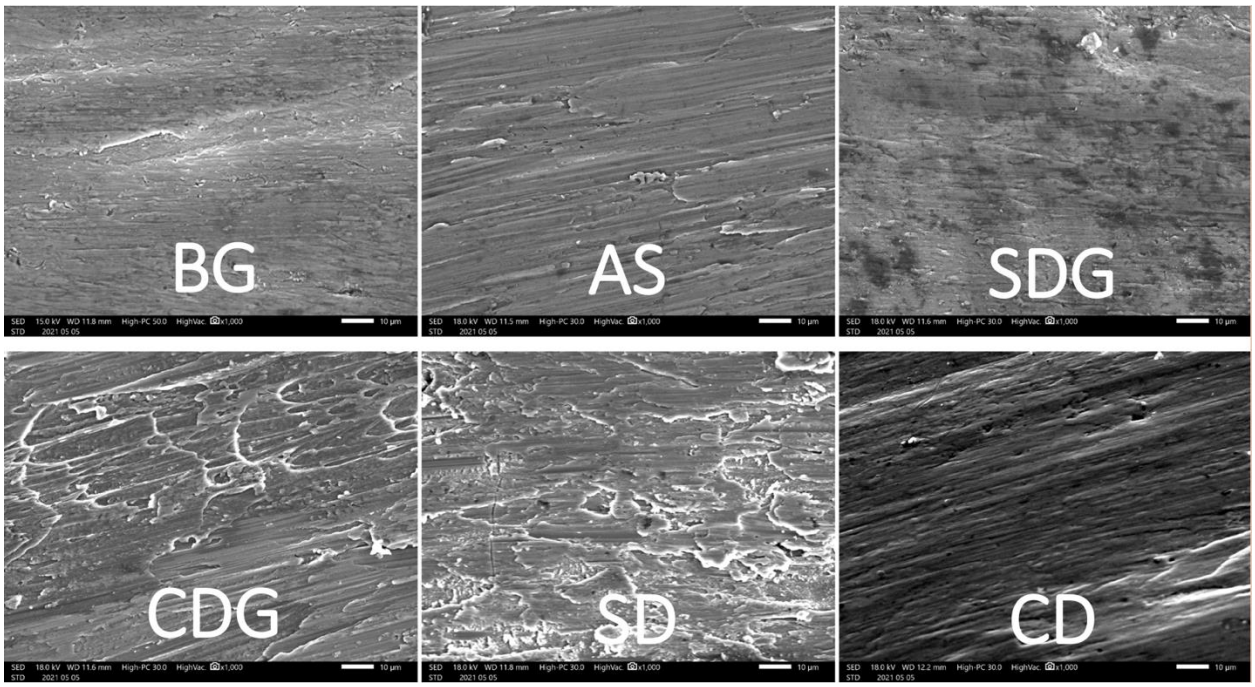

Fig 8. SEM images of implant surfaces after six different implantoplasty procedures (1000 x magnification).

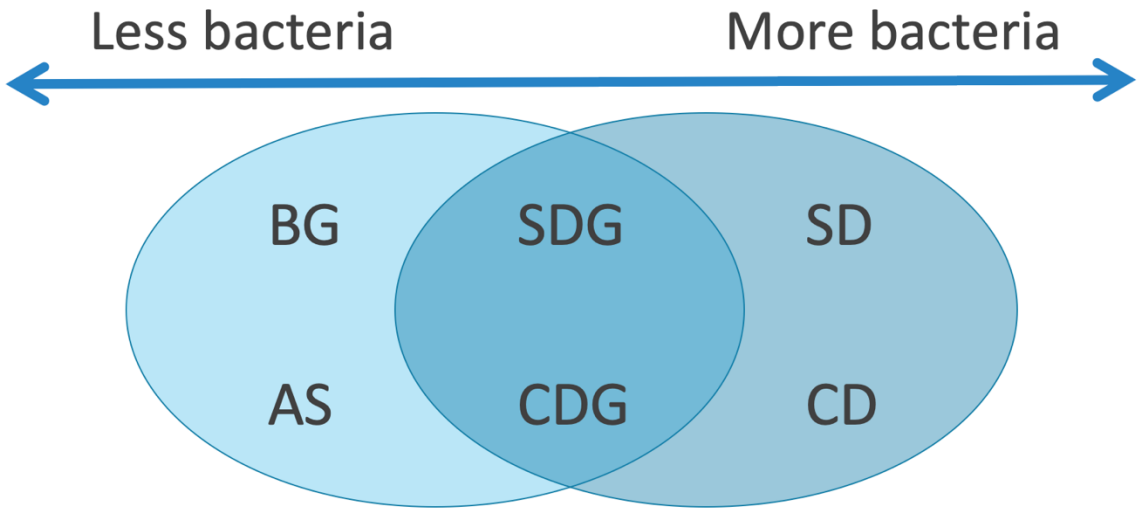

Fig 9. Venn diagram showing statistical analysis of bacterial growth after six different implantoplasty sequences. The left side is more favorable in terms of bacterial growth compared to the right side. SD and CD groups resulted in more bacterial accumulation and growth. SDG and CDG groups are in the middle and their results are not statistically significant from any other groups. BG and AS groups however, showed the least amount of bacterial growth. BG and AS are more favorable in terms of bacterial growth than SD and CD groups. However, they are not significantly better than SDG and CDG groups in terms of bacterial growth.