Cell Attachment Following Instrumentation with Titanium and Plastic Instruments, Diode Laser, and Titanium Brush on Titanium, Titanium-Zirconium, and Zirconia Surfaces

Melissa S. Lang, DDS, MS $1/D$. Roselyn Cerutis, BS, PhD $2/$ Takanari Miyamoto, DDS, PhD, CAGS, MS, MBA³/Martha E. Nunn, DDS, MS, PhD⁴

Purpose: The aim of this study was to evaluate the surface characteristics and gingival fibroblast adhesion of disks composed of implant and abutment materials following brief and repeated instrumentation with instruments commonly used in procedures for implant maintenance, stage-two implant surgery, and periimplantitis treatment. Materials and Methods: One hundred twenty disks (40 titanium, 40 titaniumzirconium, 40 zirconia) were grouped into treatment categories of instrumentation by plastic curette, titanium curette, diode microlaser, rotary titanium brush, and no treatment. Twenty strokes were applied to half of the disks in the plastic and titanium curette treatment categories, while half of the disks received 100 strokes each to simulate implant maintenance occurring on a *repetitive basis. Following analysis of the disks by optical laser profilometry, disks were cultured with human gingival fibroblasts. Cell counts were conducted from scanning electron microscopy (SEM) images. Results: Differences in surface roughness across all instruments tested for zirconia disks were negligible, while both titanium disks and titaniumzirconium disks showed large differences in surface roughness across the spectrum of instruments tested. The rotary titanium brush and the titanium curette yielded the greatest overall mean surface roughness, while the plastic curette yielded the lowest mean surface roughness. The greatest mean cell counts for each disk type were as follows: titanium disks with plastic curettes, titanium-zirconium disks with titanium curettes, and zirconia disks with the diode microlaser. Conclusion: Repeated instrumentation did not result in cumulative changes in surface roughness of implant materials made of titanium, titanium-zirconium, or zirconia. Instrumentation with plastic implant curettes on titanium and zirconia surfaces appeared to be more favorable than titanium implant curettes in terms of gingival fibroblast attachment on these surfaces.* Int J Oral Maxillofac Implants 2016;31:799-806. doi: 10.11607/jomi.4440

Keywords: *cell attachment, dental implants, fibroblasts, maintenance, surface roughness, titanium*

E osseointegrated dental implants is a notable merging evidence confirms that the efficacy of benchmark.¹ The goal of professional dental implant treatment and of personal oral health is to prevent peri-implant mucositis and/or peri-implantitis, and to

School of Dentistry, Omaha, Nebraska, USA.

Correspondence to: Dr Melissa S. Lang, Department of Periodontics, Creighton University School of Dentistry, 2802 Webster Street, Omaha, NE 68178. Fax: (402) 280-5094. Email: MelissaLang@creighton.edu

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maintain the implant-supported restoration in a state of comfort and function with optimal esthetics.^{2,3} The accomplishment of such a goal is prefaced by proper periodontal maintenance on dental implants,⁴ including the removal of periodontal biofilms without scratching the implant-abutment surfaces.²⁻⁸

According to the American Academy of Periodontology consensus report,⁹ peri-implant diseases present in two forms, peri-implant mucositis and peri-implantitis. Peri-implant mucositis has been described as "a disease in which the presence of inflammation is confined to the soft tissues surrounding a dental implant with no signs of loss of supporting bone following initial bone remodeling during healing." Peri-implantitis has been described as "an inflammatory process around an implant, which includes both soft tissue inflammation and progressive loss of supporting bone beyond biological bone remodeling."9 It should be noted that the abutment surface damage produced by the different types of instruments for the removal of plaque,

¹Associate Professor, Department of Periodontics, Creighton University School of Dentistry, Omaha, Nebraska, USA.

Associate Professor, Department of Oral Biology, Creighton University School of Dentistry, Omaha, Nebraska, USA.

Associate Professor and Chair, Department of Periodontics, Creighton University School of Dentistry, Omaha, Nebraska,

USA. 4Professor, Department of Periodontics, Creighton University

residual cements, and/or calculus may significantly impact the formation of periodontal biofilm,¹⁰ impair the adhesion of fibroblasts,¹¹ and jeopardize the biocompatibility of the implant abutment system.12

Serino et al evaluated the outcome of conventional periodontal maintenance therapy on patients surgically treated for peri-implantitis.¹³ In this study, subgingival instrumentation was performed using an ultrasonic instrument with metal tips under irrigation with 0.12% chlorhexidine for 27 patients who received periodontal maintenance every 6 months for 5 years. It was revealed that, of the 28 implants with residual probing pocket depth, 12 implants showed further increase in probing depth during the 5-year followup.¹³ Data suggest that the repeated abutment surface damage over 5 years produced by the metal tip ultrasonic instruments may play a significant role in the progression of peri-implant disease.

Selection of proper instruments for the removal of biofilms during implant maintenance is critical for the success of long-term peri-implantitis management.5-8-13 Debridement of titanium implant surfaces with titanium-alloy and stainless steel curettes has been shown to scratch the titanium surface, and thus reduce the attachment of fibroblasts in vitro.¹¹ Subsequently, pure titanium curettes were found to leave slight working traces and alter the implant surface.¹⁴ Furthermore, diode lasers are commonly used to remove peri-implant soft tissue during the stage-two uncovering surgical phase or to recontour gingiva adjacent to an implant abutment.¹⁵ Moreover, various techniques in surgical debridement of a contaminated implant surface affected by peri-implantitis have been described.16 The use of rotary or mechanical instruments with surgical access has been advocated to increase the removal of plaque and calculus from the contaminated implant surface.17

To the authors' knowledge, there is a paucity of evidence regarding the *(1)* effect of pure titanium curettes on implant and abutment surfaces following repeated instrumentation and the subsequent fibroblast attachment, *(2)* effect of instrumentation on surface characteristics of zirconia abutments and the titanium-zirconium implant (Roxolid, Straumann), *(3)* information regarding subsequent fibroblast attachment following diode laser use on the surface of implants and abutments, and *(4)* information regarding the subsequent fibroblast attachment to implant and abutment surfaces following instrumentation with a rotary titanium brush (TiBrush, Straumann) used in open-flap debridement of a contaminated implant surface.

Therefore, the null hypothesis for this study is that the surface characteristics of and fibroblast attachment to titanium disks will not be significantly altered by instrumentation with plastic curettes, the diode

laser, or the rotary titanium brush, but will be altered by instrumentation with titanium curettes, while zirconia and titanium-zirconium disks will not be significantly altered by any instrumentation.

The primary aim of this study was to evaluate the surface characteristics of disks made of titanium, zirconia, and titanium-zirconium via scanning electron microscopy (SEM) and profilometer after repeated instrumentation with instruments commonly used during implant uncovering and maintenance procedures, and for the surgical treatment of peri-implantitis. The secondary aim of this study was to evaluate if adhesion of human gingival fibroblasts in vitro to titanium, zirconia, and titanium-zirconium disks could be changed following instrumentation with various instruments used for implant uncovering, implant maintenance, and the surgical treatment of peri-implantitis.

MATERIALS AND METHODS

A power analysis was conducted to determine the number of disks necessary for this study. The power analysis indicated that eight disks in each study group of each disk type are necessary, for a total of 120 disks. Sterile titanium Grade 2 disks with a machined surface, 5 mm in diameter (Straumann), zirconia disks (Straumann), and titanium-zirconium disks (Roxolid, Straumann) were used in this study.

Three disk types (groups) were each treated with five instrumentation techniques per group: Group 1: titanium disks; Group 2: titanium-zirconium disks; Group 3: zirconia disks. The instrumentation techniques were as follows: plastic curette (Hu-Friedy Implacare 4R/4L), titanium implant curette (Wingrove B5-6Ti R661, PDT), diode microlaser (NV Microlaser, Discus Dental), rotary titanium brush (TiBrush, Straumann), and no treatment (control). Zirconia disks were not instrumented with the titanium brush due to a contraindication for use of the titanium brush on this surface.

All in vitro scaling was performed by a single investigator (M.L.) utilizing a sterile technique. Although not measured or calibrated, forces exerted in instrumentation with curettes were consistent with those that would be used to remove adherent calculus deposits from a root as described by Dmytryk et al.¹¹ To simulate maintenance during the first year of follow-up, 20 strokes (5 strokes \times 4 visits) were applied to four disks in each group with the titanium curette and the plastic curette.To simulate multiyear maintenance intervals occurring four times per year, 100 strokes (5 strokes \times 4 visits \times 5 years) were performed on four of the disks in each group with the titanium curette and the plastic curette.

Disks were instrumented with a diode microlaser as follows: The initiated laser tip was applied for 60

seconds as described by Castro et al¹⁸ using the manufacturers' recommended setting for implant recovery (1.4 Watts, continuous mode).

Disks instrumented with the rotary titanium brush were treated as follows: The rotary titanium brush was inserted in an oscillating handpiece (NSK). The activated titanium brush was applied for 60 seconds to the disk surface. Zirconia disks were not instrumented with the titanium brush.

The disks were scanned and analyzed for surface roughness average (Ra) by an optical laser profilometer (Proscan 2100, Scantron Industrial Products). The scan length was 4.4 mm. Roughness average (Ra) values were computed three times per disk using the Proscan 2100 software (Proscan 2100, Scantron Industrial Products).

Following analysis of the disks via the profilometer, the disks were washed with Liquinox (Alconox) to remove any debris or contaminants. The disks were steam autoclaved at 120°C for 20 minutes. Then, the disks were cultured with human gingival fibroblasts as described by Dmytryk et al.¹¹

The disks were placed in 96-well tissue culture plates and overlaid with 0.3 mL of a human gingival fibroblast suspension at a density of 104 cells/mL of cell culture media. The units were incubated for 24 hours at 37 $^{\circ}$ C in a humidified CO₂ incubator. The units were removed from the 96-well dishes, rinsed in saline solution, fixed by immersion in 95% ethanol for 5 minutes, and air-dried.

The number of cells attached to three independent 0.5-mm² areas/unit were analyzed qualitatively for the number of attached cells using SEM. Each disk was placed on a specimen holder with carbon tape (PELCO Tabs, 12 mm OD, TED PELLA). The disks were examined with a TM3000 Tabletop Microscope (Hitachi-High Technologies) using an accelerated voltage of 15 kV and magnification of 30x to 150x. Photomicrographs

were taken of three regions (upper, center, and lower) on each disk. Two independent examiners (L.H.V. and J.K.) counted the cells.

Statistical Analysis

The differences among the groups were investigated using software (SPSS, version 22.0 for Windows, IBM). Statistical significance was set at *P <* .05. Summary statistics were calculated by instrument for each disk for surface roughness and mean cell counts. One-way analysis of variance (ANOVA) was conducted for surface roughness and mean cell counts to compare instruments for each disk type. Summary statistics were also calculated for plastic and titanium instruments by number of strokes (20 versus 100) for each disk type with comparisons made using two-sample t tests for both surface roughness and mean cell counts. Twoway ANOVA was conducted to test the simultaneous effect of disk type and instrument on surface roughness and on mean cell counts. Two-way ANOVA was also conducted to test the simultaneous effect of disk type and instrument, while taking into consideration number of strokes on plastic and titanium instruments. Post hoc multiple comparisons were also conducted using the Student-Newman-Keuls test for both surface roughness and mean cell counts.

Summary statistics with corresponding *P* values comparing surface roughness by instrument type and disk type are presented in Table 1. Summary statistics with corresponding *P* values comparing surface roughness and the number of strokes for plastic and titanium curettes for each disk type are given in Table 2. Summary statistics with corresponding *P* values for mean cell count by instrument type and disk type are presented in Table 3. Summary statistics with corresponding *P* values comparing cell count and the number of strokes for plastic and titanium curettes for each disk type are given in Table 4.

Table 3 Summary Statistics for Mean Cell Count (Mean Number of Cells Across Three Areas) by Instrument Type and Disk Type

Table 4 Summary Statistics for Mean Cell Count by Instrument Type and Disk Type with Division by Strokes for Plastic and Titanium Curettes (n = **3 Per Group)**

RESULTS

Surface Roughness

Table 1 shows significant differences in surface roughness by instrument type for titanium disks and the titanium-zirconium disks ($P < .001$ for both types of disk), while no difference in surface roughness was noted for zirconia disks ($P = .782$).

For comparison of the number of strokes in Table 2, only titanium curettes with titanium disks even approached a difference ($P = .052$). Figure 1 shows the surface roughness interaction plot for each disk type

Fig 1 Surface roughness (Ra) interaction plot for instrument **Fig 2** Surface roughness (Ra) interaction plot for instrument type with number of strokes by disk type.

according to instrument type with number of strokes included. As can be seen in Fig 1, differences in surface roughness across all instruments tested for zirconia disks were negligible, while both titanium disks and the titanium-zirconium disks showed large differences in surface roughness across the spectrum of instruments tested. The only large increase in surface roughness with increased strokes for a curette and disk type in Fig 1 was noted for titanium curettes on titanium disks, although the difference, as noted earlier, failed to achieve statistical significance $(P = .052)$.

Figure 2 shows surface roughness for the different disk types over all instruments tested without including number of strokes in the figure. Again, the figure shows that surface roughness is much less for zirconia disks across all instrument types. ANOVA also reveals significant differences among instrument types, disk types, and the interaction of instrument type with disk type ($P < .001$ for all three elements).

Student-Neuman-Keuls multiple comparisons reveal that all disks are significantly different from each other. With instrument types, only titanium disks and titanium curettes and titanium brush were not significantly different from each other. These two (titanium brush and titanium curettes) also yielded the greatest overall mean surface roughness, while plastic curettes yielded the lowest mean surface roughness, with plastic curettes producing significantly lower surface roughness than the other groups, including the control group.

type without number of strokes by disk type.

Cell Count

Table 3 shows significant differences in mean cell count by instrument type for titanium disks $(P = .002)$ and zirconia disks ($P < .010$), while no difference in mean cell count was noted for the titanium-zirconium disks $(P=.117)$.

For comparison of the number of strokes in Table 4, the only statistically significant difference noted was for zirconia disks with titanium curettes ($P = .015$), with the higher number of strokes resulting in more than double the cell count. All other differences in cell count by number of strokes were not statistically significant, although the number of strokes for the titanium curette on the titanium-zirconium disks approached statistical significance ($P = .053$), with the higher number of strokes tending to yield greater cell count.

A two-way ANOVA was conducted for curette type with number of strokes included by disk type for cell count. Disk type, instrument type, and instrument type by disk type interaction were all significantly different $(P = .019$ for disk type, $P = .002$ for instrument type, and $P = 0.001$ for interaction between disk type and instrument type). Multiple comparisons using Student-Newman-Keuls were also conducted for disk type and instrument type. Mean cell counts for titanium and titanium-zirconium disks were not significantly different, although mean cell count for zirconia disks was significantly different from the other two disk types, with the mean cell count for zirconia disks being significantly lower.

Fig 3 Mean cell count interaction plot for instrument type with **Fig 4** Mean cell count interaction plot for instrument type with-
out number of strokes by disk type.

For instrument type, control, titanium with 20 strokes, and laser were not significantly different from each other, and titanium with 20 strokes, laser, titanium brush, titanium with 100 strokes, plastic with 20 strokes, and plastic with 100 strokes were not significantly different from each other. The greatest mean cell count for titanium disks occurred with plastic curettes with 20 strokes (mean cell count $= 20.9$). The greatest mean cell count for titanium-zirconium disks was found with titanium curettes with 100 strokes. For both titanium disks and titanium-zirconium disks, the lowest mean cell counts were obtained for the control group.

For the zirconia disks, the greatest mean cell count was obtained with the microlaser, while the lowest mean cell count was obtained with titanium curettes with 20 strokes. Figure 3 shows the interaction between disk type and instrument type with strokes included. As can be seen from the interaction plot, mean cell count varies widely by instrument type, depending on the disk type under investigation. Figure 4 shows the interaction plot for disk type with instrument type without strokes included. Similarly to the two-way ANOVA that included strokes, the greatest cell count was obtained for plastic curettes for titanium disks, titanium curettes for the titanium-zirconium disks, and laser for the zirconia disks.

DISCUSSION

This study evaluated the effect on surface roughness and cell attachment following the use of instruments

out number of strokes by disk type.

used for implant maintenance and for the surgical treatment of peri-implantitis or implant exposure during a stage-two procedure on disks made of titanium, titanium-zirconium, and zirconia.

This study showed statistically significant differences in surface roughness following instrumentation of titanium and titanium-zirconium disks with plastic curettes, titanium curettes, rotary titanium brush, and diode laser compared with control, while no significant changes in surface roughness were found for zirconia disks instrumented with the same instruments.

For titanium disks, instrumentation with the titanium curette and the rotary titanium brush yielded the greatest mean surface roughness, while the plastic curette created a surface less rough than the control.The findings for the plastic curette are in general agreement with previous studies.^{9,10,14,19-24} Although not evaluated by profilometry, Hallmon et al found the plastic scaler produced minor alterations of titanium abutment cylinders.20 Other studies found little to no surface alterations after instrumenting titanium cylinders, abutments, or disks with plastic curettes.^{10,11,14,19,24,25} Similar to the present study, Homiak et al found the instrumentation of a machined titanium abutment with a plastic curette created a somewhat smoother surface than the untreated abutment surface.²¹

Although not tested in the present study, a titanium alloy curette was shown to create a significantly rougher surface than other instruments, including stainless steel.²⁵ In contrast to the present study, Mengel et al found a titanium curette left only slight working traces on machined titanium abutments.¹⁴ A device was used by Mengel et al¹⁴ that restricted the instrumentation force applied to the abutments to 0.2 N, which could have led to lighter forces being applied compared with the present study, and hence, less surface alteration of the abutment by the instrument. The present study sought to simulate how the instrument would perform on various implant and abutment surfaces under human hand application, as was reported by Duarte et al.¹⁰ In a subsequent study by Mengel et $al₁²³$ heavy pressure (4 N) with a titanium curette left moderate to severe traces on machined titanium abutments and led to increased roughness depth compared with light pressure (0.4 N), which coincides with the findings of the present study.

In the present study, the titanium rotary brush was found to create a surface roughness similar to that of a titanium curette on a titanium disk. This is in contrast to a study that found no significant change in surface roughness following instrumentation of a machined titanium disk with a rotary titanium brush.²⁶ Irrigation was used in the study by Park et al,²⁶ which probably cleared debris that could scratch the implant or abutment surface during instrumentation. Additionally, a longer treatment time (60 seconds) was used in the present study for the rotary titanium brush on the titanium disks. The diode laser tip left the surface of the titanium disks slightly rougher than the control disks. This finding is in contrast to a study by Castro et al,¹⁸ who found no visible surface alterations between diode laser-treated and nontreated smooth surface titanium implants viewed under SEM.

For titanium-zirconium disks, instrumentation with the titanium curette created the greatest surface roughness, while the plastic curette increased the surface roughness slightly compared with the control group. The surface alterations following instrumentation of the titanium-zirconium disks were similar to those of the titanium disks, with the exception of the plastic curette.

For zirconia disks, no significant differences were found in surface roughness following instrumentation with any instrument. No other studies were found that evaluated the effect of instrumentation of titanium-zirconium implant surfaces or zirconia abutment surfaces with implant maintenance instruments or a diode laser.

This study also evaluated the effect of repeated instrumentation of an abutment or implant surface with a titanium and plastic curette to simulate implant maintenance occurring on a repetitive basis.This study found no statistically significant increases in surface roughness with increased number of instrument strokes when evaluating 20 strokes versus 100 strokes with each instrument tested. This finding is in agreement with a study comparing 25 and 50 strokes, which found no cumulative effects on surface alterations of

titanium abutment cylinders with increasing number of instrument strokes, with the exception of the plastic-tipped sonic attachment,²⁰ which was not evaluated in the present study.

Similarly, Rapley et al found no differences in the surface alteration of titanium implant abutments between shorter and longer instrumentation times, except that a rotary cup with flour of pumice created a smoother surface with increased treatment time.²⁴ Homiak et al found a smoother titanium implant abutment surface following 30 strokes of a plastic curette compared with three strokes with the instrument.²¹ Such a difference between fewer and greater instrument strokes with the plastic curette was not observed in the present study, possibly due to the increased number of strokes evaluated in this study. As such, the effect of a very small number of strokes was not captured in the present study.

The effect of various instruments on cell attachment as evaluated in this study showed the greatest cell counts on titanium disks following instrumentation with plastic curettes, a finding in agreement with Dmytryk et al.¹¹ Instrumentation with a titanium curette resulted in significantly fewerattached fibroblasts compared with the plastic curette for the zirconia surface, while fibroblast attachment to the titaniumzirconium surface was not significantly affected by instrumentation with a titanium curette. It appears that there is a trend indicating a clinician could safely instrument a titanium-zirconium surface with a titanium curette without reducing the subsequent fibroblast attachment to the surface. It is interesting to note that instrumentation with plastic and titanium curettes on titanium disks and plastic curettes on zirconia disks resulted in increased fibroblast attachment compared with control disks. In contrast, Dmytryk et al showed impaired fibroblast attachment on titanium surfaces following instrumentation with a titanium-alloy curette compared with untreated control surfaces.11 This difference could be explained by the use of a harder instrument by Dmytryk et al¹¹ (titanium-alloy curette) compared with the titanium curette used in the present study. In contrast to the present study, Augthun et al found significantly reduced mouse fibroblast cell counts on plasma-sprayed, smooth titanium screw, and hydroxyapatite-coated implants treated with plastic scalers.27

A limitation of this study is that the instruments were tested on titanium, titanium-zirconium, and zirconia disks rather than implant or abutment fixtures. This study evaluated the effect of instrumentation on surface roughness and gingival fibroblast attachment to the implant surface in vitro without consideration of subsequent plaque retention. Further research is needed to evaluate the efficacy of each instrument on in vivo removal of contaminants of the implant surface and the effect of instrumentation on subsequent plaque retention.

CONCLUSIONS

Within the limitations of this study, repeated instrumentation, as would be expected by multiple recall appointments, did not result in cumulative changes in surface roughness of implant materials made of titanium grade 2, titanium-zirconium, or zirconia. Within the confines of this study, instrumentation with plastic implant curettes on titanium grade 2 and zirconia surfaces appeared to be more favorable than titanium implant curettes in terms of gingival fibroblast attachment on these surfaces.

ACKNOWLEDGMENTS

This research was partially supported by a research grant from Straumann USA (IIS 15-12). The authors thank Dr Toshiki Takamizawa of Creighton University School of Dentistry for performing profilometer and SEM scans of the disks; Dr Laura Harris-Vieyra and John Kum of Creighton University School of Dentistry for performing masked, independent cell counts in this study; Dr Terry Wilwerding of Creighton University School of Dentistry for the design and fabrication of specimen holders for use in the profilometer; Dr Wayne Barkmeier of Creighton University School of Dentistry for his expertise of the use of the profilometer and analyzing software; Dr Andrew Baruth, and Ryan Gnabasik of Creighton University Department of Physics for the atomic force microscopic specimen analysis. The authors reported no conflicts of interest related to this study.

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