Titanium Particles within Mucosa during Non-Surgical Implant Debridement

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Titanium Particles within Mucosa during Non-Surgical Implant Debridement

by

Holvin Louie, DDS

A Thesis Submitted to the
Faculty of Graduate Studies
in Candidacy for the Degree of
Master of Science (MS) in Periodontics

Loma Linda, California
June 2022
Each person whose signature appears below certifies that this thesis in his/her opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.

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Abstract of the Thesis

Titanium Particles within Mucosa during Non-Surgical Implant Debridement

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Holvin Louie, DDS

Master of Science, Advanced Education in Periodontics
Loma Lind University, June 2022
Dr. Yoon Jeong Kim, Chairperson

Titanium implants affected by peri-implantitis lesions are often treated non-surgically with various instruments, which may produce titanium particles that can become lodged within the peri-implant mucosa. Thus, a foreign body reaction may ensue. The aim is to assess the embedment of titanium particles into soft tissues from titanium implants via a stainless-steel ultrasonic scaler (SSU) versus a titanium hand scaler (TH). Sand-blasted, large grit, acid-etched surface-treated titanium implants were implanted into pig legs after creating peri-implantitis defects. Each implant was subjected to only one instrument type. Sequential strokes were conducted followed by thorough irrigation with sterile water. Soft tissue curettage was not conducted. The surrounding soft tissue was then removed, prepared, and analyzed with a hemocytometer under a light microscope to ultimately determine the mean total number of metallic particles present in each group. Soft tissue samples were also analyzed with scanning electron microscopy (SEM) and elemental analysis (EDS). The mean titanium particle count based on the hemocytometer squares as well as the mean total number of titanium particles based on the samples in each group were significantly higher when scaled with the SSU as compared with the TH. SEM and EDS subsequently confirmed the presence of titanium
within the soft tissue. Photomicrographs of the scaled implant surfaces depicted altered surface topography. Within the limitations of this study, the SSU and TH were both able to produce and embed titanium particles within the adjacent peri-implant mucosa. However, the SSU produced significantly more embedded titanium particles than the TH.

*Keywords:* dental implants, titanium particles, non-surgical implant debridement, stainless-steel ultrasonic scaler, titanium hand scaler
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Introduction and Literature Review

Titanium implants afflicted by peri-implantitis may be treated with various types of instruments, such as ultrasonic and hand scalers, which may inadvertently produce foreign particles. For instance, metallic particles were detected when new sandblasted, large grit, acid-etched (SLA) coated implants were subjected to ultrasonic scaling\(^1\). These foreign entities were further analyzed via scanning electron microscopy (SEM) and subsequently confirmed to be titanium via elemental analysis (EDS)\(^1\). Such fragments may become subsequently lodged within the peri-implant mucosa if they are not or cannot be thoroughly flushed out of the treated sites. Foreign bodies inside the peri-implant soft tissues may initiate and ensue a foreign body reaction as described by Albrektsson et al\(^2-5\). Regardless of whether biofilm or calculus are effectively removed from the implant threads, the healing outcome may still be compromised by the titanium particles embedded within the mucosa. This is supported by the finding that peri-implant diseases typically initiate in the coronal soft tissues and subsequently advance apically, thereby potentially resulting in bone loss\(^6\).

Peri-implant diseases, which include both peri-implant mucositis and peri-implantitis, are classically believed to be pathological diseases primarily related to bacterial infection. The bacteria causing peri-implant diseases are typically composed of mixed anaerobes\(^7\). The composition of such bacterial flora may be rather similar to that of chronic periodontitis, which is predominately Gram-negative\(^7\). However, despite these similarities, the disease progression in terms of marginal bone loss around the natural tooth versus the foreign implant cannot be considered identical. Evidence suggests that
inflammatory peri-implant lesions are typically larger and progress more rapidly than periodontitis lesions.\textsuperscript{6,8,9}

There is evidence that titanium particles are associated with localized inflammation around titanium implants in humans. Such titanium particles and ions may become widely dispersed throughout the body via the bloodstream.\textsuperscript{10} Additionally, a systematic review discusses the term “metallosis”, which refers to the frequently noted occurrence of inflammation associated with metallic particles around failing medical titanium implants.\textsuperscript{11} Thus, the medical literature has documented the production and release of such titanium particles and their relationship to implant failure.

To date, there is no standardized treatment regimen for peri-implant diseases since the most effective therapy has yet to be conclusively identified.\textsuperscript{12} When treating peri-implant mucositis and peri-implantitis, titanium implants are often debrided and scaled with conventional ultrasonic and hand scalers, as well as various adjuncts. The ideal material of choice for the instrument still appears to be a subject of controversy with regards to efficient biofilm removal and implant surface alteration. However, the stainless-steel ultrasonic scaler (SSU) has been used in recent literature. For example, in a prospective in-vivo human study published in 2020, the non-surgical therapy regimen for peri-implantitis involved a steel alloy ultrasonic scaler, glycine air powder, and metronidazole, along with supportive maintenance.\textsuperscript{13} The authors believe that the relevance of surface alteration is surpassed by the potential to effectively disrupt the biofilm around the implant threads.\textsuperscript{13} Similarly, other recent in-vivo human studies have utilized ultrasonic scalers with metal tips in their treatment protocol.\textsuperscript{14-16} Hence, some clinicians still advocate the use of the SSU in treating peri-implant diseases due to its
effectiveness in removing biofilm, calculus, and even cement, despite its potential to increase implant surface roughness. Further clarification may be necessary regarding the application of the SSU in peri-implant diseases and the potential consequence of increased production and subsequent embedment of foreign titanium particles within the peri-implant mucosa. This holds clinical relevance since practitioners may also inadvertently use the SSU around both natural teeth and implants.

In contrast, an ultrasonic scaler tip with a plastic insert may cause minimal implant surface alteration. However, according to an in-vitro study, plastic inserts tend to exhibit more of a polishing action due to its motion-dampening effect and may even leave behind plastic deposits on the implant surface. Other authors have also noted plastic-like remnants after debridement, which may not be easily removed between implant threads. Such plastic debris may interfere with the healing or biocompatibility of the implant surface. If there is peri-implant mucosa present, then such plastic debris may even become lodged within the soft tissue. The bulkiness of the plastic insert may also hinder the operator from effectively maneuvering the tip in the submucosal environment. Moreover, the plastic insert may simply rub or burnish the calculus instead of effectively removing it from between the implant threads. Another in-vitro study analyzing the effectiveness of various mechanical instruments determined that the ultrasonic scaler with a metal tip was the most effective in removing residual artificial calculus, whereas the ultrasonic scaler with a plastic tip failed to remove any calculus deposits.

Various instruments have been shown to alter the surface topography of the implant as well as produce and release titanium particles into the surrounding environment, as discussed by Harrel et al. However, the titanium hand scaler (TH) may
induce less surface alteration than the SSU due to material compatibility\(^2\). In addition, Albrektsson et al elucidated the concept of a “foreign body reaction” that refers to a foreign entity embedded in hard or soft tissue that can elicit an inflammatory response\(^2\). Essentially, if there is a continued exacerbated host response towards this foreign body, then marginal bone loss around the implant will inevitably occur. Foreign body reactions following implantation of medical devices, prostheses, or biomaterials have been well documented\(^2\). Such a foreign body reaction often involves a modulated interaction between macrophages and foreign body giant cells\(^2\). Titanium ions in higher concentrations can also negatively affect the viabilities and differentiation capabilities of osteoblasts and osteoclasts\(^5\).

Wilson et al, who conducted peri-implantitis human biopsies, stated that titanium particles are foreign bodies that can ultimately lead to a subacute and chronic inflammation primarily dominated by plasma cells\(^6\). Interestingly, the particles were often noted several millimeters into the adjacent soft tissue via light microscopy (LM). SEM and EDS subsequently confirmed the presence of titanium. This foreign material also appeared to be long-standing, based on the localized inflammatory response. A systematic review also detailed findings of titanium particles within epithelial cells, connective tissue, macrophages, and bone around implants affected by peri-implantitis\(^2\). If left unaddressed, such chronic inflammation stemming from these foreign bodies may initiate and/or further exacerbate peri-implant diseases via loss of connective tissue attachment and surrounding bone.

Hence, the failed implants may be the main source of these titanium particles, but the mechanism by which such particles are dislodged from the implant surface remains
elusive. There are several hypotheses as to how such titanium particles become embedded inside the peri-implant mucosa, which include but are not limited to friction during implant placement, surface corrosion, and implant stress and wear during mechanical debridement\textsuperscript{28-30}. Evidently, increased titanium surface roughness after usage of various ultrasonic and hand scalers has been demonstrated in the literature\textsuperscript{31,32}. Therefore, the clinician’s attempt to treat peri-implant diseases may inadvertently cause the shedding and trapping of these titanium particles within the adjacent mucosa, which ironically may contribute to the progression of such peri-implant diseases.

To date, the production and embedment of titanium particles within the peri-implant soft tissue due to ultrasonic and hand scaler usage have not been investigated. Hence, the aim was to quantify the titanium particles that are shed from an implant surface and subsequently entrapped within the adjacent mucosa after using conventional ultrasonic and hand scalers. The null hypothesis is that there is no difference in the total number of titanium particles embedded in the soft tissues between the SSU and the TH. It is hypothesized that the SSU will produce and embed a greater total number of titanium particles within the peri-implant mucosa than the TH.
Materials and Methods

In this in-vitro study, the production and subsequent embedment of titanium particles into the peri-implant mucosa were assessed. SLA-surface-treated titanium (Grade 5 Alloy) implants (Pulsar®, Sydent, Israel) with an internal hex configuration were inserted within prepared osteotomy sites in soft tissue-bone models and subsequently scaled. The pig leg section, which was directly above the pig’s foot or trotter, was utilized for the models. The two instrument groups tested were the SSU (Cavitron FSI Slimline Focused Spray Ultrasonic Insert - 30K FSI-SLI 10S, Dentsply Sirona) as the test group and the TH (Titanium Implant Scaler Langer ½ - IMPLG1/2T, Hu-Friedy) as the active control group.

A total of 35 implants were placed in 35 soft tissue-bone models: 16 implants in the test group, 16 implants in the active control group, and 3 in the passive control group. In the test group and the active control group, 2 specimens each were reserved for SEM/EDS analysis only after instrument scaling. Each model was subjected to only one treatment type with either the SSU or the TH.

The attached soft tissue of the model was first measured and determined to be 4 mm thick. It was then circumferentially removed using a 6 mm diameter mucosa punch. Only the tissue directly coronal to the site of interest was removed without flap elevation. A 3.8 mm in diameter twist drill was used to create an 8 mm deep implant osteotomy site. Subsequently, a reamer drill that is 6 mm in diameter (DASK - Dentium Advanced Sinus Kit) was used to create a standardized crater-like simulated bony defect. Therefore, the simulated bony defect was 6 mm in diameter and 2 mm in depth. The total height of 6 mm, which was measured from the coronal aspect of the soft tissue to the apical portion.
of the defect, was created to simulate an early peri-implantitis lesion which was then subjected to initial non-surgical therapy.

The implant (4.2 mm diameter x 8 mm height) was thus carefully placed until primary stability was achieved. Thus, the implant protruded two millimeters coronal to the bone due to the prepared bony defect. A healing abutment (4.5 mm diameter x 5 mm height) was then placed, which mimicked removal of the restoration and protected the underlying implant prior to non-surgical debridement. The goal was to immobilize the implant when subsequently subjected to scaling strokes by the ultrasonic or hand scalers.

Three additional non-scaled implants were reserved as the passive control group with intact soft tissue and bone. For the passive control specimens, the non-scaled implant surface was subjected to only syringe irrigation with sterile water, followed by SEM preparation and analysis of the soft tissue.

The SSU or TH was positioned parallel to the implant surface. Each instrument was utilized an equal number of times, achieving an apico-coronal distance of 2 mm and a total of 30 scaling strokes along the implant surface in the bony defect. The instrument tip also always remained submucosal when scaling such that the instrument did not protrude from the soft tissue. Copious medical-grade sterile water was used with the ultrasonic scaler that was set at high power. Additionally, a high-volume suction was positioned adjacent to the implant. Each treated site was thoroughly flushed out with sterile water using an irrigation syringe. Soft tissue curettage was not conducted. After 30 scaling strokes, each hand scaler was carefully sharpened with a sharpening stone via 30 subsequent strokes.
The soft tissue (3 mm in thickness) directly adjacent to the scaled implant site was then marked and removed from the bone section in a half circumference using a scalpel blade and hand instruments. In preparation for hemocytometer analysis, each soft tissue sample was carefully debrided with a stainless-steel curette (Gracey 5/6 Curette, Hu-Friedy) until no dark entities within the sample were visually detected. By comparison, pristine soft tissue adjacent to non-instrumented implants in the passive control group did not display such foreign material. The collected specimen on the curette was subsequently irrigated off the instrument with sterile water into a plastic tube, which was then centrifuged at 7000 rpm for 5 minutes, so that any metallic particles collected were separated at the bottom of the tube. The supernatant was carefully removed without disturbing the metallic particles. Afterward, each sample was completely evaporated in a desiccator. Each tube was then rehydrated with 0.05 mL of sterile water and gently shaken to evenly disperse any metallic particles within the solution. The rehydration steps thus allowed standardization of the sample volumes.

The total number of metallic particles in each liquid sample could then be assessed using a hemocytometer under a light microscope (EVOS XL Core by Advanced Microscopy Group, Bothell, Washington, USA) at 40x magnification. A 0.01 mL sample was pipetted onto the hemocytometer and counted. Metallic particles were identified as dark or black entities when visualized under a microscope (Figures 1 and 2). The four corner squares in the hemocytometer were then counted, followed by the calculation of the average titanium particle count per square. Since the volume of the hemocytometer counting area (Improved Neubauer by AO Spencer, USA) and the volume of the water in the rehydrated samples were known, the total number of particles in each sample could
be estimated. More specifically, the total number in each sample = average count per square x volume of squares on the hemocytometer x initial volume of sample. The mean total number of titanium particles based on all samples in each group was then calculated.

Two soft tissue samples each from the test group and active control group were first sputter-coated with a thick layer of gold and then qualitatively analyzed with SEM (Quanta 250 FEG by Thermo Fisher Scientific Inc., Madison, Wisconsin, USA) at 300x magnification, which produced descriptive images that are indicative of embedded titanium particles. Additional details regarding the SEM setup for the soft tissue samples include high-voltage (HV) ranging from 10-20 kV, working distance (WD) ranging from 7.8-12.4 mm, horizontal field width (HFW) at 691 microns, Everhart-Thornley Detectors (ETDs), and secondary electron (SE) mode.

Following implant removal with reverse torque, the implants were visually evaluated with SEM for any surface alterations after instrumentation. In addition, photomicrographs of the scaled implant surfaces were taken at 40x magnification. Additional details regarding the SEM setup for the implants include HV ranging from 10-20 kV, WD ranging from 9.6-19.9 mm, HFW at ranging from 4.14-5.18 mm, ETDs, and SE mode.

EDS (Pathfinder 1.4 X-ray Microanalysis by Thermo Fisher Scientific Inc., Madison, Wisconsin, USA) was further conducted in five regions where titanium was likely to be present based on the radiodensity. In addition to confirming the presence of titanium, EDS was utilized to determine the relative proportion of such metallic particles to other organic and non-organic compounds in various sample regions.
For the statistical analysis, the alpha level was set at 0.05 whereas the power value was set at 0.85. The paired-samples t-test was used to determine if there is a difference in the mean total number of titanium particles.
Results

As previously described, out of an initial 35 implants, 3 were allocated to the passive control group, whereas 2 implants from both the test group and active control group (which totals to 4 implants) were subjected to only SEM/EDS analysis after instrumentation. Therefore, a total of 28 samples with 14 samples in each group were quantitatively assessed based on hemocytometer analysis. Examples of foreign particles visibly detected in the hemocytometer under a light microscope are shown in Figures 1 and 2, which correspond to the test group and active control group, respectively.

The mean titanium particle count based on the hemocytometer squares was significantly higher when scaled with the SSU in the test group (6.54 ± 2.02) as compared with the TH in the active control group (3.87 ± 2.02) (p<0.001). Additionally, the mean total number of titanium particles based on the samples in each group was significantly higher when scaled with the SSU (3,271 ± 1,010) as compared with the TH (1,936 ± 1,009) (p<0.001) (Table 1). Hence, the SSU produced and embedded 69% more titanium particles than the TH.

Figures 3 and 4 depict SEM images of soft tissue samples obtained from the test group with the SSU and the active control group with the TH, respectively. The soft tissue specimens in the passive control group did not significantly display the presence of titanium based on SEM and EDS assessment, as shown in Figures 5 and 6. However, the samples in the test group and the active control group did display varying amounts of titanium, which was confirmed by EDS analysis, as displayed in Figures 7 and 8. Neither chromium nor nickel was detected in any of the samples.
Photomicrographs of the scaled implant surfaces depicted altered surface topography. Figures 9 and 10 portray SEM images of implants obtained from the test group with the SSU and the active control group with the TH, respectively. From a qualitative standpoint, the implant scaled by the SSU depicted more extensive visible damage compared to that scaled by the TH. There was no attempt to quantify the amount of damage to the roughened SLA implant surface due to the limited sample size in each SEM group.
Discussion

The results from the hemocytometer analyses evidently showed that both the SSU and TH produced and embedded detectable foreign particles within the peri-implant mucosa after instrumentation. However, the SSU generated approximately 69% more particles than the TH. By comparison, a different research model involving ultrasonic scalers with stainless-steel and titanium tips depicted a 45% difference in titanium particle production in favor of the SSU\textsuperscript{1}. Despite the differences in study design, the percentage difference appears to be within reason especially since an ultrasonic scaler was compared with a hand scaler in this research study.

Additionally, the ultrasonic insert used in the model had a slim diameter that facilitated submucosal insertion and instrumentation. Evidence suggests that a thin ultrasonic scaler tip and a high-power setting can induce the highest vibrations, which may be more effective at removing biofilm and calculus\textsuperscript{33}. Although it may be difficult to quantify the force used with the titanium hand scaler, the operator was careful to consistently utilize a steady fulcrum while conducting regular sequential strokes along the implant surface. Nevertheless, such findings may be extrapolated to the clinical setting in the sense that both the SSU and TH have the potential to not only cause visible damage to the implant surface but also entrap titanium particles within the peri-implant mucosa during non-surgical debridement. This extrapolation is supported by qualitative and quantitative assessment with SEM and EDS analysis, respectively.

In general, a rougher implant surface favors more biofilm retention\textsuperscript{34}. Moreover, a larger and more diverse bacterial population may hinder the overall healing efficacy of the peri-implant treatment and/or increase the susceptibility of future peri-implant disease.
development at that site\textsuperscript{7}. The embedded titanium particles in the surrounding soft tissues may also provoke a foreign body reaction, thus potentially further impeding a normal healing response. This dental finding may be rather comparable to metallosis, which can be associated with an inflammatory foreign body reaction\textsuperscript{11}. Ensuing macrophage migration may occur in the chronic presence of metallic debris. Phagocytosis of these foreign particles may lead to increased inflammation, which can then accelerate metal degradation and loosening, bone resorption, and production of granulation tissue\textsuperscript{11}.

Perhaps soft tissue curettage with a sharp curette should be considered as an adjunctive procedure after implant debridement to remove as many embedded foreign particles as possible within the peri-implant mucosa. In a 12-month clinical study, significant clinical improvement around implants afflicted with peri-implantitis was reported after nonsurgical therapy involving ultrasonic decontamination, soft tissue curettage, and submucosal air polishing\textsuperscript{15}.

From a clinical standpoint, care must be taken when conducting periodontal therapy, especially during prophylaxes or periodontal maintenances in patients with both natural teeth and implants. Practitioners may accidentally use the SSU around the implant threads, which if exposed, may create and leave behind embedded titanium particles in the adjacent mucosa. Thus, existing implants should be properly noted and documented, followed by careful implant debridement and soft tissue curettage, if indicated.

Moreover, nickel and chromium are known contact allergens that may cause delayed hypersensitivity. These two compounds may be commonly detected in stainless-steel alloys. However, neither chromium nor nickel was detected in any of the soft tissue
samples in this study. This finding suggests that the SSU did not undergo significant surface disintegration during usage.

This laboratory study was novel in its primary assessment of the production and embedment of titanium particles after non-surgical implant debridement with scalers. Other adjuncts, such as glycine powder air polishers, lasers, titanium brushes, and antimicrobials, were also considered since there is existing data in the literature supporting their usage. However, they appear to be less commonly used when compared with ultrasonic and hand instruments. Moreover, there is no consensus as to which therapeutic entity is the most effective. One strength noted from this investigation was that all specimens were standardized in terms of preparation and treatment by one operator. However, the operator was not blinded to the treatment type, which could be interpreted as a limitation. Additionally, the peri-implant mucosa in the soft tissue models was clearly not edematous or erythematous. This contrasts with the abnormal soft tissues that are typically observed when afflicted by peri-implant diseases. Caution is thus advised when interpreting these results and applying them in the clinical setting.

In addition, dark entities were occasionally visually noted around the marginal bone after soft tissue and implant removal, which may indicate titanium particles. Although this parameter was not assessed in this research study, this may be a topic of interest to pursue in future studies in terms of the impact of foreign particles on hard tissues around afflicted implants. Other interesting research variables to explore would be the influence of contaminated calculus intermingled with titanium particles within the inflamed peri-implant mucosa as a possible trigger of a foreign body reaction in conjunction with abnormal soft tissue. In consideration of these different variables,
additional research is needed to further examine the relationship between titanium particles and peri-implant diseases.
Conclusions

Both the SSU and TH caused visible damage to the SLA-layered titanium implants and embedded titanium particles within the surrounding soft tissue. However, the SSU shed more particles that subsequently became embedded in the peri-implant mucosa than the TH. Such foreign entities may trigger a foreign body reaction and play a role in peri-implant disease. Caution should be exercised even when utilizing the TH.
Table 1  Foreign particles detected within the hemocytometer and overall sample after instrumentation

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>SE</th>
</tr>
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<td>2.02</td>
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</tr>
<tr>
<td>Ti Particle Count (Hemocytometer) - Stainless-Steel Ultrasonic</td>
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<td>6.54*</td>
<td>6.55</td>
<td>2.02</td>
<td>0.540</td>
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<td>Total # of Ti Particles (Sample) - Titanium Hand Scaler</td>
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<td>1935.71*</td>
<td>2050.00</td>
<td>1009.27</td>
<td>269.739</td>
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<tr>
<td>Total # of Ti Particles (Sample) - Stainless-Steel Ultrasonic</td>
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<td>3271.43*</td>
<td>3275.00</td>
<td>1010.47</td>
<td>270.059</td>
</tr>
</tbody>
</table>

*p-value<0.001 with paired samples t-test
**Figure 1** A representative sample from the test group with the SSU where the foreign particles were visibly noted in the hemocytometer under a light microscope.
Figure 2  A representative sample from the active control group with the TH where the foreign particles were visibly noted in the hemocytometer under a light microscope
Figure 3  SEM image of a representative soft tissue sample from the test group with the SSU
Figure 4 SEM image of a representative soft tissue sample from the active control group with the TH
**Figure 5** SEM image of a representative soft tissue sample from the passive control group
Figure 6  EDS results of a representative soft tissue sample from the passive control group
Figure 7  EDS results of a representative soft tissue sample from the test group with the SSU
Figure 8  EDS results of a representative soft tissue sample from the active control group with the TH
Figure 9  SEM image of a representative implant from the test group with the SSU
**Figure 10**  SEM image of a representative implant from the active control group with the TH
References


