

## The effect of a decontamination protocol on contaminated titanium dental implant surfaces with different surface topography in edentulous patients

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### ABSTRACT

**Objectives:** To investigate if it is possible to achieve complete decontamination of dental implant surfaces with different surface characteristics.

**Materials and methods:** Twelve implant pieces with an Osseotite<sup>®</sup> surface and 12 implant pieces with a Ti-Unite<sup>®</sup> surface were attached on to the complete lower dentures of six patients and were allowed to accumulate plaque for 30 days. When retrieved, the implant decontamination protocol used, involved both mechanical (PeriBrush<sup>™</sup>) and chemical (3% H<sub>2</sub>O<sub>2</sub>) decontamination. The number of colony forming units per millilitre was determined and the dominant micro-organisms in selected samples was identified by 16s rRNA gene amplicon sequencing. The effect of the titanium brush on the implant surface was examined by SEM.

**Results:** Complete decontamination was achieved in five out of 24 implants (four Osseotite<sup>®</sup> and one Ti-Unite<sup>®</sup>). The mean CFU/ml detected after decontamination were 464.48 for Osseotite<sup>®</sup> and 729.09 for Ti-Unite<sup>®</sup> implants. On the surface of the implants in which complete decontamination was not achieved, all of the predominant bacteria identified were streptococci except for one which was identified as micrococcus. SEM images revealed that the surface features of the decontaminated implants were not significantly altered.

**Conclusions:** Mechanical decontamination using a titanium brush supplemented with chemical treatment for one minute (3% H<sub>2</sub>O<sub>2</sub>) can achieve complete decontamination of implant surfaces in edentulous patients.

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### Introduction

The similarity of the subgingival microbiota found in peri-implant lesions to the subgingival flora at natural teeth, indicates that natural teeth may act as a reservoir for colonization of pathogens in partially edentulous patients [1–4]. It has been suggested that in edentulous patients no such reservoirs are present as periodontal pathogens are eliminated following the extraction of teeth [5]. In a recent study though, clearance of all teeth did not result in eradication of all pathogenic species when PCR technology was applied [6]. As residual periodontal pockets are not present to harbour periodontal pathogens in edentulous patients, saliva, tonsils or crypts, may act as the source of bacteria instead [7]. This is supported by cross-sectional studies showing the presence of periodontal pathogens in the oral cavity of denture-wearing edentulous patients [8–10]. Additionally, periodontal pathogens such as *A. aggregatibacter* and *P. gingivalis* were present in samples taken from the surface of denture teeth [11]. Overall though, there is a higher number of pathogenic microorganisms in partially edentulous patients than fully edentulous patients [12].

Many implant supported prostheses in fully edentulous patients are bulky and difficult to keep clean. Additionally,

they may be placed in patients with limited manual dexterity and a suppressed immune system due to age or/and medications. This may result, in the presence of multiple implants with significant bone loss and exposed threads, covered by calcified plaque under a fixed or removable restoration.

The majority of dental implants used today have a medium rough surface structure which has been developed to increase the bone to implant contact. However, this 'roughness' may complicate the management of peri-implant infections. The surface roughness and the chemical composition of the implant surface can have an impact on plaque accumulation and as a result increase the difficulty in reducing the bacterial load to a level necessary for resolution of the peri-implant inflammation [13].

The elimination of the biofilm from the implant surface can be difficult. It has been demonstrated that the biofilm formation is partially controlled by an inter-bacterial communication mechanism dependent on bacterial population density. This mechanism is called *quorum* sensing [14,15]. When faced with a peri-implant infection, the primary objective is to eliminate the pathogenic microflora or at least to alter the microbiota to such a degree that the

resident microbiota at the implant surface is compatible with the host. By doing so, the host's immune system is given a better chance to effectively eliminate putative pathogens.

To decontaminate an implant surface, pure titanium instruments including titanium brushes are recommended [16,17]. Titanium brushes are used due to their good performance in removing plaque and for being gentle to the implant surface [16,18,19]. Following mechanical decontamination, chemical decontamination of the implant surface should be performed. Chlorhexidine, citric acid, hydrochloric acid, tetracycline, chloramines, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) or sodium chloride are some of the substances that have been used. However, no single agent has proven to be superior [20]. Three percent H<sub>2</sub>O<sub>2</sub> has been recommended as it is considered safe to use even during surgical therapy [21,22].

The surface topography of implant surfaces differs between implants from different manufacturers. A number of animal studies have demonstrated that in implants with a certain surface structure, peri-implantitis may progress even after treatment. This indicates that this surface may be more difficult to decontaminate [23–26].

The null hypothesis for the study herein was that it is not possible to achieve complete decontamination of two different, previously contaminated implant surfaces using a certain decontamination protocol. Additionally, there was an attempt to observe changes on the surface topography of the dental implants following the use of the titanium brush.

## Materials and methods

Ethical approval was obtained from the Joint Hospital's Research Ethics Committee (JREC) in Dublin.

The participants were recruited from the implant assessment clinics or the removable prosthodontics assessment clinics.

### Inclusion criteria

- Patients with a complete lower denture and in the waiting list for a new prosthesis.
- Patients able to give consent to participate in this study and sign a consent form approved by the Research Ethics Committee of Saint James's Hospital.
- Patients able to attend all required appointments.

### Exclusion criteria

- Patients unable to provide a written consent.
- Patients who did not have dentures with buccal flanges of adequate width and height.
- Antibiotic therapy within the preceding 6 months or in the 30 days following the commencement of the study.

## Implant preparation

Ten hybrid Osseotite<sup>®</sup> dental implants (dual acid etched, BIOMET 3i<sup>™</sup>) with an internal connection and 10 Ti-Unite<sup>®</sup> dental implants with an external connection (anodized dental implants, Nobel Biocare<sup>™</sup>) were prepared for the study.

An Isomet<sup>®</sup> (Buehler, Lake Bluff, IL) slow speed saw was used to cut the implants into two pieces. Four Inch, 15HC Isomet<sup>®</sup> wafering blade (Buehler, Lake Bluff, IL) was sharpened with cutting speed 10 prior to each implant. The implants were cut horizontally leaving 5 mm of the rough surface intact in addition to the head of the implant. In the Osseotite<sup>®</sup> implants, the coronal part of the body of the implant which had a smooth surface, was considered part of the head of the implant. Sterile water was used throughout the cutting procedure as a lubricant to prevent over heating of the implants and the cutting disk. The position of the connection apparatus was changed to place the implant vertically and the implants were cut longitudinally to the head of implants, producing two pieces from each implant. A total of 20 implant pieces from each type were prepared. Following preparation of the implants, they were autoclaved for future use.

Four Osseotite<sup>®</sup> and four Ti-Unite<sup>®</sup> implant pieces were used for training in order to identify the most secure way of attaching the implant pieces on to the dentures. Two Osseotite<sup>®</sup> and two Ti-Unite<sup>®</sup> implant pieces were kept, to be used as a control for the scanning electron microscope (SEM) evaluation. From the remaining 14 Ti-Unite<sup>®</sup> and 14 Osseotite<sup>®</sup> implant pieces, 12 Osseotite<sup>®</sup> and 12 TiUnite<sup>®</sup> were used for the clinical part of the study. The remaining four pieces were not used.

A cavity was created on both sides of the dentures with a straight hand piece and a bullet shaped bur. The cavity was designed to accommodate two implant pieces on each side with the head of the first piece facing the second implant's head for better adaptation. The well was filled with cold cure acrylic resin and the implants were placed on top of the soft acrylic (Figure 1).

Care was taken not to contaminate the rough surface of the implant with the acrylic pro-base polymer or the liquid monomer. The implants were submerged enough so that they would not protrude from the denture and irritate the



Figure 1. Implants used for training to identify the best fit.



**Figure 2.** Contaminated implants in participant no. 2.



**Figure 3.** Contaminated implants in participant no. 6.

patient's buccal mucosa. Any excess acrylic near the margin of the implants or the well was removed with a size 11 scalpel blade. A minute amount of super glue was used between the heads of the implants to ensure the implants were secured in place. Patients were then asked not to dip the denture in a disinfectant for 30 days. They were allowed to brush their denture using just water but not the specimens or the area around them. The implants were left in function for one month, allowing plaque to accumulate and become calcified. The patients were instructed to use the dentures as they would normally.

Following 30 days of plaque accumulation, implants were retrieved from the prosthesis (Figures 2 and 3). A small groove was created between their heads. The heads of the implants were undermined and slight pressure with an amalgam excavator was applied until the implants were detached from the surface of the denture and each piece was placed into a separate sterile gallipot.

### **Decontamination of implants**

The implant decontamination protocol involved both mechanical and chemical decontamination. Titanium brushes (PeriBrush™, Tigran™ Technologies AB, Malmö, Sweden) were used to mechanically disrupt the plaque and calculus

from the implant surfaces. The bristles of the PeriBrush™ are made of pure titanium, which is softer than titanium alloy, in order to minimize the risk of extensive damage of the implant surface. The brush was fixed on a W&H (Bürmoos, Austria) 20:1 hand-piece and the speed was set to 600 rpm according to the manufacturer's recommendation.

The PeriBrush™ was placed parallel to the implant surface and the decontamination process took place under copious irrigation with sterile saline. Only minimal pressure was applied to avoid damaging the titanium bristles and reduce their cleaning effect. The implant debridement with the brush was stopped only if no plaque or calculus could be seen with the naked eye. One PeriBrush™ was used for each patient (one brush to decontaminate four implant pieces). Each time, the pieces were decontaminated in no particular order and the time for the decontamination process was calculated using a stopwatch.

Hydrogen peroxide 3% was used as the chemical agent in this study. A small amount was placed in a gallipot and a cotton pellet was dipped into the freshly prepared H<sub>2</sub>O<sub>2</sub> and the surface of the implant was gently swabbed for one minute. The implants were then rinsed with sterile saline for 1 min and each implant piece was placed in a labelled Eppendorf tube and was sent for the microbiological analysis.

### **Microbiological analysis**

Twelve hours before decontamination, blood agar (BA) for microbiological culturing was made. Under sterile conditions, 500 ml of Tryptic Soy Agar supplemented with 5 g/l yeast extract, 0.50 g/l L-cysteine hydrochloride, 5.0 µg/ml of Hemin, 1.0 µg/ml menadione and 5% horse blood (Oxoid™) were prepared. The plates were stored in a fridge and 30 minutes prior to use they were dried under a sterile hood.

One millilitre of Brain Heart Infusion broth was added to the Eppendorf tube containing the implant samples and a vortex mixer was used for one minute to agitate the implant and generate a homogenous suspension of bacteria. From this, 100 µl was taken and added to 0.9 ml (900 µl) of Brain Heart Infusion broth to make a 10-fold dilution. On the previously prepared agar plates, 100 µl of the sample and its dilution were then plated in duplicate. As the implants were not

covered by a peri-implant soft tissue pocket, the vast majority of the bacteria expected to be found would be aerobic, microaerophilic and facultative anaerobes. As a result the plates were incubated at 37°C under aerobic conditions for 72 hours.

Following the incubation period, the plates were removed and assessed for bacterial growth. For each plate, a photographic record was made using the colour camera on a colony counter (Flash & Go™) and were stored electronically for a manual count of colonies. Bacterial densities were calculated and expressed in CFUs. The dominant colony type from selected samples (i.e. that representing at least 60% of the colony morphologies present) was purified by subculture on BA until pure cultures were obtained.

Each of the previously isolated dominant bacteria species were grown on agar plates for three days at 37°C under appropriate conditions to provide pure colonies. Several of the colonies were sampled using a sterile metal loop and were transferred into Brain Heart Infusion Broth and incubated at 37°C overnight to generate a dense culture.

Following this, the broth was centrifuged in sterile Eppendorf tubes at maximum speed (20,817×g) for one minute. A solid pellet was obtained and bacterial DNA was prepared as described by Mawhinney et al. [27].

In order to identify the dominant colony type to the species level, we utilized the most accurate method currently available for this purpose; DNA sequence analysis of the 16S rRNA gene. Following DNA extraction from each dominant bacteria sample, the DNA was prepared for amplification of 16S rRNA genes. The 27F – forward primer (5'-AGAGTTTGATCCTGGCTCAG) and the 1492R reverse primer – (5'-GGTACCTTTTACGACTT) sourced from Sigma-Aldrich® were selected for the reaction. For amplification, 1 µl of DNA of each sample was added to 49 µl of the reaction mixture to give 50 µl. The amplification process was completed as described by Mawhinney et al. [27].

The DNA samples, in addition to a sample of the primers, were forwarded to an external laboratory (Source BioScience, Nottingham, UK) for sequencing. When sequencing data were obtained, they were submitted to an online database (HOMD, [www.homd.org](http://www.homd.org)) for identification using BLAST. Organisms were identified to the species level where a sequence match  $\geq 98\%$  could be identified in the database.

### Evaluation of implant surfaces by SEM

A SEM was used to observe the effect of the PeriBrush™ on the implant surfaces caused by the mechanical action of the brush.

Two pristine Osseotite® and two pristine TiUnite® were used as controls and were examined under the SEM. They were further compared to the decontaminated and autoclaved samples. Fifteen samples from four randomly chosen participants were used for this comparison. A total of 19 implants (nine TiUnite® and 10 Osseotite®) were used for the SEM evaluation by examining the middle area of each implant (2 × 2 mm).

Implant surfaces were individually mounted on aluminium pin stubs using conductive carbon tabs. A Carl Zeiss Ultra

SEM (Zeiss, Oberkochen, Germany) was used to obtain images of the samples at three different magnifications. Micrographs were taken, using an acceleration voltage of 20 kV, at ×30, ×500 and ×2000 magnifications. Micrographs obtained were stored in a removable data storage device for future use.

### Data analysis

Descriptive statistics were used to analyse the results. The CFU/ml data were expressed by means and standard deviations.

### Results

A total of 49 patients were screened. Six patients were included in the study (five females and one male), allowing for a total of 24 implant pieces to be attached to their dentures. The mean age of the patients was 71 years. One patient was a smoker and two patients were on medication for high blood pressure.

Following 30-days of plaque accumulation, the adherent plaque was evident on to the attached implants. With the naked eye, the differences in the amount of plaque accumulated on the implants varied between patients but not between specimens within the same patient (Figures 3 and 4). One patient lost one of the Ti-Unite® implants during the study period and before the 'retrieval' day. As a result an overall of 12 Osseotite® and 11 Ti-Unite® implant pieces were available for decontamination and further analysis.

### Viable bacterial counts

Culturing of each implant sample was carried out in duplicate (neat, 1:10 and 1:100 dilutions) and the mean viable bacterial counts from each neat sample were calculated. The viable bacterial counts of each plate were measured and expressed in CFU/ml. When no viable bacteria were detected on the agar plate, a score of 0 was given for that plate. The TiUnite® II of participant 1, which was lost before retrieval, was recorded as N/A and was not included in the analysis (Table 1).

All the available Osseotite® and TiUnite® mean viable bacterial counts (from 1 to 12/11) along with the corresponding duration of the decontamination process for each implant piece are reported in Tables 2 and 3. An Osseotite® implant piece retrieved from participant no. 2, needed only 0:38:30 for 'complete' mechanical debridement. The maximum amount of time spent for mechanical debridement of an implant piece was again for an Osseotite® implant piece, retrieved from participant no. 4 (2:38:30 min) (Tables 2 and 3).

Summary of the data and the overall mean (SD), median and confidence interval is presented in Table 4.

### SEM micrographs

In the pristine Osseotite® samples, the peaks and valleys were well defined as were the open pores and craters in TiUnite® samples (Figures 4 and 5) However, in the

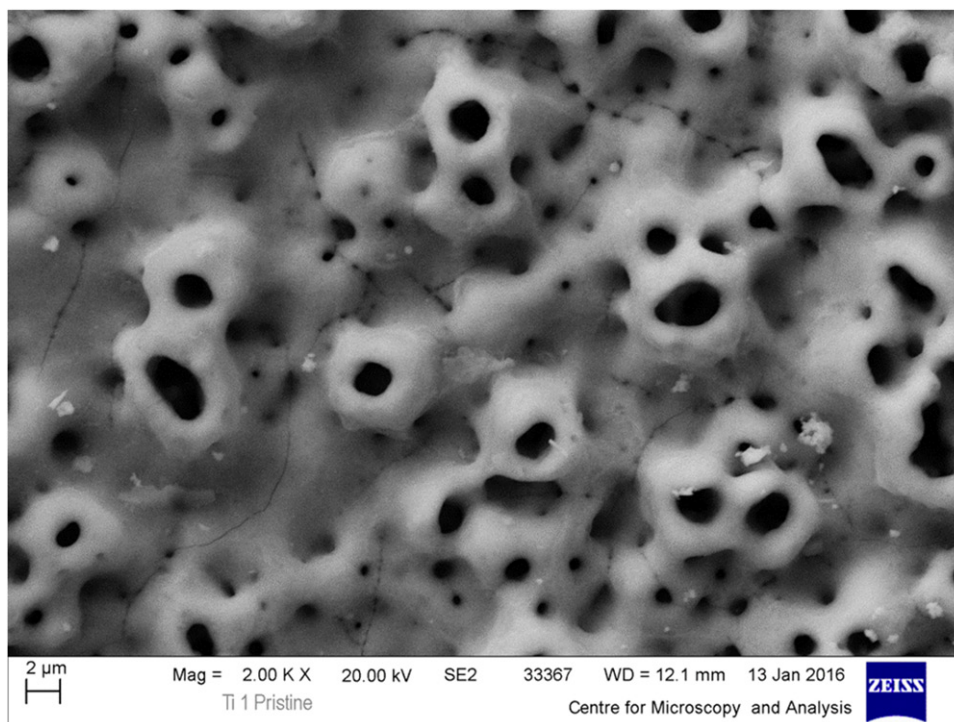


Figure 4. TiUnite® at ×2000 magnification.

Table 1. Mean colony forming unit/ml for each specimen.

Participant no.	CFU/ml			
	Osseotite® I	Osseotite® II	TiUnite® I	TiUnite® II
Participant 1	1500	1925	1350	N/A <sup>a</sup>
Participant 2	895	535	1300	580
Participant 3	0	0	120	20
Participant 4	295	360	140	290
Participant 5	10	0	0	20
Participant 6	0	55	1200	>3000

<sup>a</sup>TiUnite® II in patient no. 1 was not available (N/A).

Table 2. Mean CFU/ml for Osseotite® implants and duration of decontamination.

Osseotite®	Mean CFU/ml	Duration of decontamination
1	1500	0:58:18
2	1925	0:50:28
3	895	0:38:30
4	535	0:55:02
5	0	0:59:18
6	0	1:10:23
7	295	1:42:87
8	360	2:38:30
9	10	1:03:13
10	0	1:35:48
11	0	1:12:15
12	55	0:53:28

decontaminated implants, some of the surface features were altered. These surface alterations can be described as flat surfaces or partially disfigured topography probably due to the effect of surface debridement with the PeriBrush™ (Figures 6 and 7).

### Predominant colony forming species

Nine samples (four Osseotite® and five TiUnite®) were available for identification of the predominant colony forming

Table 3. Mean CFU/ml for TiUnite® implants and duration of decontamination.

TiUnite®	CFU/ml	Duration of decontamination
1	1350	1:34:62
2	N/A	N/A
3	1300	0:58:10
4	580	1:00:80
5	120	0:53:57
6	20	1:15:20
7	140	1:18:71
8	290	2:23:17
9	0	0:48:83
10	20	1:05:81
11	1200	1:25:00
12	3000	1:59:19

Table 4. Summary of data.

Parameter	Osseotite®	TiUnite®
Mean	464.58	729.09
Number of points	12	11
Standard deviation	651.75	925.23
Standard error	188.14	278.97
Minimum	0.000	0.000
Maximum	1925	<3000
Median	175	290
Lower 95% CI	50.478	107.55
Upper 95% CI	878.69	1350.6

species. The predominant bacteria identified were streptococci except in one case that was identified as micrococcus (*Rothia mucilaginosa*) (Table 5).

### Discussion

This proof of principle study aimed to test the null hypothesis that complete decontamination of previously contaminated implant surfaces is not possible when using a certain

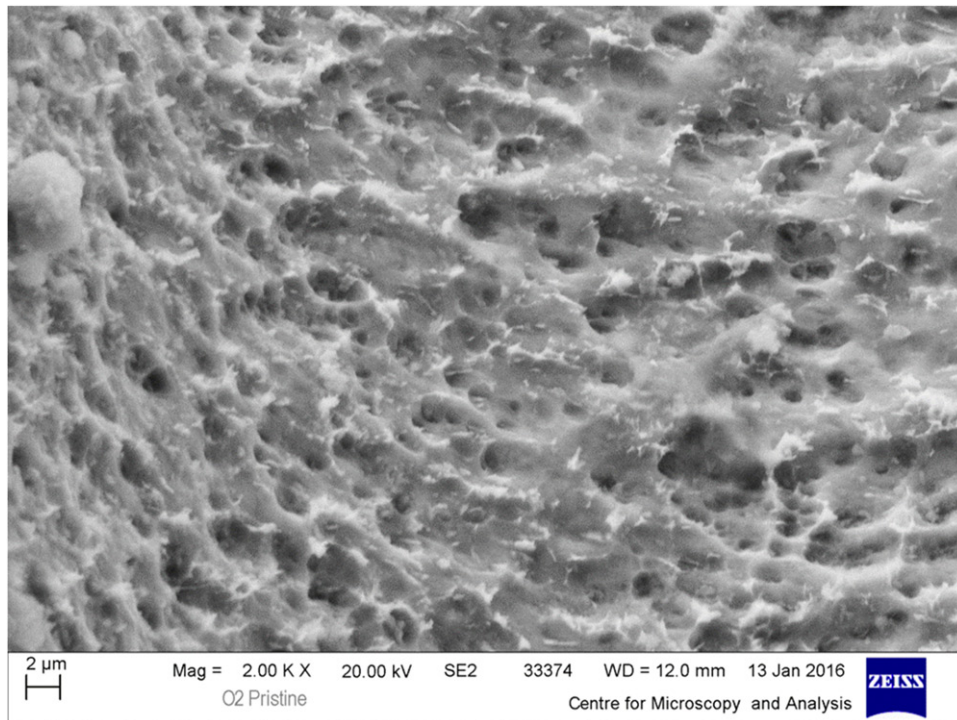


Figure 5. Osseotite<sup>®</sup> at  $\times 2000$  magnification.

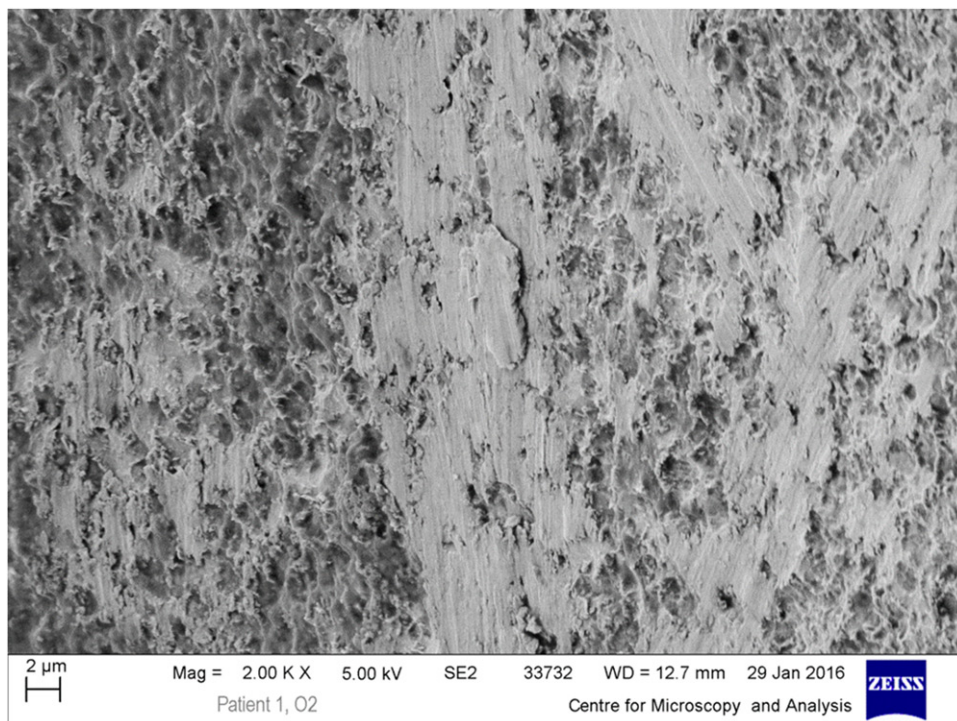


Figure 6. Decontaminated Osseotite<sup>®</sup> at magnification ( $\times 2000$ ).

decontamination protocol. Finally, it tried to identify topographical changes on the implant surfaces following decontamination. The null hypothesis was rejected and results suggest that complete decontamination can be achieved for both implant surfaces evaluated. Some changes of the surface topography were observed for both investigated surfaces.

Complete decontamination occurred in four out of 12 Osseotite<sup>®</sup> surfaces and one out of 11 TiUnite<sup>®</sup> surfaces. This

is not in agreement with other studies that evaluated the same decontamination protocol. In a study by Gustumhaugen et al., rotating titanium brush (TiBrush<sup>™</sup>) in combination with 3% H<sub>2</sub>O<sub>2</sub> was used to decontaminate titanium coins with three surface characteristics (SLA<sup>™</sup>, TiUnite<sup>™</sup> and OsseoSpeed<sup>™</sup>). Despite the fact that this protocol resulted in statistically significant bacterial biomass reduction, complete decontamination was not achieved [28].

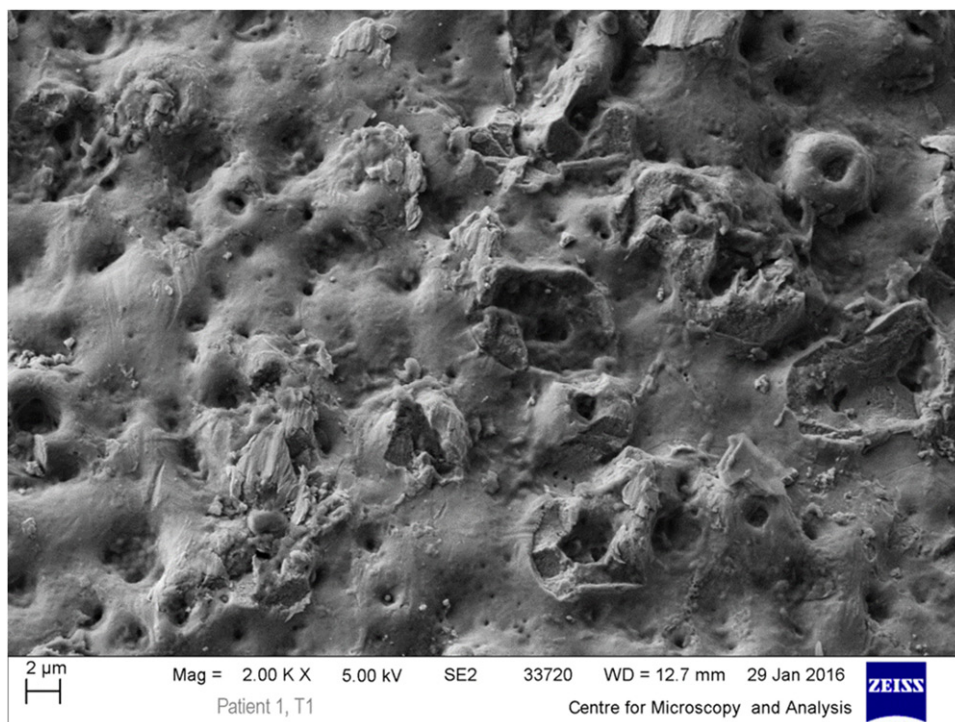


Figure 7. Decontaminated TiUnite® at magnification ( $\times 2000$ ).

Table 5. 16SrRNA sequencing.

Sample no.	Sample name	Bacterial species
1	P101	<i>Streptococcus vestibularis</i>
2	P102	<i>Streptococcus vestibularis</i>
3	P1T1	<i>Rothia mucilaginosa</i>
4	P2T1	<i>Streptococcus oralis</i>
5	P2T2	<i>Streptococcus vestibularis</i>
6	P201	<i>Streptococcus vestibularis</i>
7	P202	<i>Streptococcus salivarius</i>
8	P4T1	<i>Streptococcus salivarius</i>
9	P4T2	<i>Streptococcus vestibularis</i>

Many clinicians are faced with peri-implantitis related problems and are trying to tailor the treatment they provide on each patient's individual needs. Limited data are available in how to best handle implant surfaces colonized by microorganisms. In most of the existing studies the effectiveness of mechanical instruments on plaque removal was evaluated on flat titanium discs/coins [18,19,29]. In an *in vitro* study, six patients received acrylic resin splints for the upper jaw wherein four sandblasted and acid etched, flat titanium discs were integrated. The titanium discs were left in the mouth to collect plaque for 48 hours. In this particular study, the effectiveness of stainless steel curettes was compared to titanium brushes [18]. In other similar studies, discs were left only for 24 hours inside the oral cavity or were contaminated *in vitro* with certain bacterial species [28–31].

Flat titanium discs or sheets can be ideal for studies due to their shape and low cost but they are less challenging to decontaminate than a standard dental implant, especially if they have been contaminated with a soft pellicle and not mature biofilm. Additionally, despite trying to simulate the conditions of the oral environment, *in vitro* experiments do not reliably represent the conditions found in the oral cavity. The diversity of the microbiological profile, the presence of

saliva and shearing forces, the characteristics of individual patients and the host immune response are factors that may influence the result. There is available evidence demonstrating that initial bacterial adhesion is essential to determine the organization, diversity and strength of the biofilm [32,33]. Recently, successful efforts have been made to develop a peri-implantitis microcosm using a constant depth film fermenter but *in vivo* studies of adhesion and biofilm formation are still the gold standard for understanding the interaction between bacteria and implant surfaces [34].

In the study herein, the coronal part of two types of implants with different surface characteristics and geometry was used as this is the area of the implant body that gets exposed during the progression of peri-implantitis. Additionally the specimens were left into the oral environment for 30 days, allowing undisturbed initial adhesion and maturation of the biofilm, better replicating the clinical reality.

A small number of *in vivo* studies evaluating implant decontamination during surgical or non-surgical peri-implantitis treatment have been performed. In a study by de Wall et al., 30 patients with peri-implantitis were surgically treated. The treatment included bone recontouring, debridement and chemical decontamination of the exposed implant surfaces. Microbiological samples were taken during surgery (before and after implant decontamination) by rubbing a sterilized brush across the implant surface [35]. There is a good possibility that the brush used in this study or cotton pellets used for taking microbiological samples in similar studies [36,37] were not specifically designed to go between the threads of the implants or robust enough to rub off a significant amount of bacteria. In the study herein, specimen vortexing and

centrifuging, in addition to the mechanical debridement must have contributed on bacterial loosening and as a result, in to more accurate way of measuring the number of bacteria present.

Although it is still controversial as to what extent minor changes in roughness can affect biofilm formation, several studies have demonstrated that small differences in surface structure may not have an effect on the initial bacterial adhesion. Accordingly, with the 30 days experimental design employed in the present study, no major differences between the two surfaces were expected in their bacterial adhesion patterns [31,36,38,39].

Although the study herein was conducted *in vivo* conditions, these experimental conditions represent mainly the oral environment in edentulous patients. Additionally, in the clinical situation, the composition of the plaque accumulated over the implant specimens might have been influenced by the capacity of the removable prosthesis to harbour certain types of bacteria inside the plaque biofilms. Another limitation of the present study is that it was conducted using a supra gingival biofilm and that the culturing technique employed favoured the aerobic microflora. However, by using DNA sequencing, we were able to accurately identify the predominant species in each sample. The results of the present study demonstrated that regardless of implant surface characteristics, Streptococci were the predominant species found on the implant surfaces following decontamination. These observations suggest that these bacterial species might adhere more efficiently to the treated titanium surfaces and be more resistant to shredding forces during decontamination. Overall though, a recent systematic review on the microbiologic profile associated with peri-implantitis in humans, concluded that it is the quantitative characteristics of the microflora cohabitants that represent the most important determinant of the disease and not its specific composition [40].

The results in the present study demonstrated great variability in that in some patients the decontamination protocol was very effective but in others rather ineffective. One could speculate that decontamination may be patient dependent rather than implant surface dependent as different microcosms with different bacterial characteristics develop in individual patients. Another reason for this variability could have been that both implants with similar surfaces were placed on the same side of the denture and this might have affected the accumulation of biofilm.

There is a general consensus that mechanical decontamination is necessary in order to achieve the best possible treatment outcome when treating peri-implant infections. The design of implants and the various surface modifications used to improve osseointegration though may eventually limit the effect of mechanical debridement and advocate the use of additional therapies. Therefore, the use of broad spectrum systemic antibiotics, in conjunction with surgical treatment has been recommended in aggressive cases [41]. A recent randomized clinical trial, studied the medium term effects of the adjunctive systemic and local

antimicrobial therapy in the surgical treatment of peri-implantitis. Results showed that although systemic antibiotics had a positive effect on the treatment success of implants with a modified surface, overall, these implants showed significantly lower odds for treatment success [42]. The majority of these implants (49%) had a TiUnite® surface, which according to earlier research from the same research centre might have had a negative effect on the success of the surgical treatment [25]. Additionally, the local use of chlorhexidine did not seem to have an overall effect on treatment outcomes and that is in agreement with an earlier study by the same research group [43]. The results from a study by Ungvari et al. which demonstrated that chlorhexidine gel negatively affects epithelial attachment on the implant surface following treatment could partly explain their findings [44]. In the present study, 3% H<sub>2</sub>O<sub>2</sub> was the adjunctive antimicrobial of choice due to its safety, low cost and proven antibacterial effect [20,45]. Additionally, it has been demonstrated that it slightly enhances human epithelial growth [44].

In a study by Charalampakis et al., it was reported that the amount of a four-day biofilm grown intra-orally on titanium discs was correlated with the amount of remaining plaque after decontamination [37]. This could be explained by the fact that all the discs were cleaned for 5 s each using three strokes with a cotton pellet soaked in either saline or chlorhexidine. In the clinical situation, when the implant surface is exposed, a clinician would stop decontaminating only when plaque can no longer be seen with the naked eye. Titanium brushes which were used in the present study, were effective in removing calcified deposits present on the implant surfaces but significantly more time had to be dedicated for mechanical debridement when these deposits were extensive.

In the study herein, since implant surfaces were not significantly altered following a maximum of ≈2.30 minutes of mechanical debridement, it might be beneficial to increase treatment time, especially for medium to rough contaminated implant surfaces. Additionally, it could also be beneficial to increase the time of H<sub>2</sub>O<sub>2</sub> application to 2 min for both surfaces.

Overall, a larger cohort of patients could have helped us identify or rule out any potential significant differences between these two different surfaces and also reach more meaningful conclusions regarding the possibility for complete decontamination of implant surfaces following this combination treatment.

## Conclusions

Within the limitations of the present study, it was demonstrated that mechanical decontamination using a titanium brush supplemented with chemical treatment for one minute (3% H<sub>2</sub>O<sub>2</sub>) can achieve complete decontamination of implant surfaces in edentulous patients. Alterations of the surface topography were observed for both investigated surfaces.

## Disclosure statement

Drs. Alotaibi, Moran, Grufferty and Polyzois declare no conflict of interest.

Dr Renvert reports grants and personal fees from Geistlich Pharma AG, Tigran Technologies and Te-Pe, personal fees from Straumann and Zimmer Biomet, and grants from OraPharma Inc. outside the submitted work.

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