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QUANTITATIVE EVALUATION OF BIOFILM FORMATION ON IMPLANT SURFACE AFTER THREE DIFFERENT MECHANICAL DEBRIDEMENT METHODS: AN IN VITRO MICROBIOLOGICAL STUDY

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ABSTRACT

Like teeth, the dental implant is surrounded by soft and hard tissues. These tissues are also prone to infection and are called peri-implant diseases. The peri-implant diseases resemble periodontal diseases that they are both initiated by plaque formation. The plaque control on the implant surface is thus necessitated for control of the biofilm formation and resultant adverse effects. The present study aims to evaluate and compare the difference in biofilm formation on implant surface after different mechanical debridement methods. Of total 20 implants, 5 implants underwent instrumentation using stainless steel scaler (Hu Friedy 204S Stainless steel sickle scaler), 5 implants underwent instrumentation using plastic scaler (Hu Friedy IMPL 204S IMPLACARE II, 5 implants underwent instrumentation using titanium scaler (Hu Friedy IMPL 204SDT Titanium Scaler) and 5 implants were left uninstrumented. The implant surfaces were irrigated using sterile normal saline in a syringe to clear off all the scrapings if generated and then visualized under the stereomicroscope at 40x magnification to evaluate for surface changes. Four organisms were used for the *in vitro* biofilm formation in the study - *Streptococcus mitis, Streptococcus oralis*, *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. At the end of incubation period of 4 weeks, the quantification of the bacteria was carried out in terms of optical density and colony forming units. The findings of the present study show that in spite of different degrees of alterations caused by different instruments, there is no statistically significant difference in the bacterial colonization with different treatment modalities. The study suggests that instrumentation types are safe and non-damaging and can be implemented as needed for mechanical debridement.

INTRODUCTION

Modern dentistry aims to restore the normal form, function, esthetics and patient comfort. A plethora of methods has emerged over the years for the management of tooth loss. The options for the replacement of the teeth depend on the cause of their loss and their strategic position in the oral cavity. The introduction of implants has changed the paradigm for the replacement of missing natural teeth.

Like teeth, the dental implant is surrounded by soft and hard tissues. These tissues are also prone to infection and are called peri-implant diseases. Where peri- implant mucositis is related to infection of peri-implant soft tissues, the involvement of the bone is called peri-implantitis. These are caused by biofilm formation.

Mechanical plaque control in addition to local and/or systemic antibiotics or antiseptics remains the mainstay of peri-implant disease therapy. However, such mechanical means might lead to surface alterations and the release of ions and by- products that might alter the tissue biocompatibility of the surface. Careful evaluation of such effects must be evaluated before initiating the treatment.

Various studies have evaluated the effect of mechanical instrumentation on titanium discs and smooth implant

surfaces. Some other studies have also evaluated the formation and nature of microbial colonization on the implant surface. Lacunae still exist whether the biofilm formation on implant surface will differ due to different mechanical debridement methods.

The present study aims to evaluate and compare the difference in biofilm formation on implant surface after different mechanical debridement methods.

MATERIALS AND METHODS

The current study was an in vitro study and therefore needed no approval by the institutional ethical committee.

Mold formation and mounting of implants

An acrylic block of 2cm x 2cm x 2cm was prepared and used as a template to prepare a mold using soft putty impression material (Denstply Aquasil soft putty impression material). Implants (Bioline spiral cone hydroxyapatite coated implants, 5.00 mm x 10.00 mm) were mounted in the mold in cold-cure acrylic resin and assigned to four groups and three instrumentation techniques: (a) Stainless steel scaler, (b) Plastic scaler, (c) Titanium scaler and (d) no treatment group. The implants underwent instrumentation and were later subjected to microbial colonization.

Instrumentation of implants

Implants were grouped depending on the type of instrument used – 5 implants were included in one group. Each group underwent instrumentation using stainless steel scaler (Hu Friedy 204S Stainless steel sickle scaler), plastic scaler (Hu Friedy IMPL 204S IMPLACARE II, titanium scaler (Hu Friedy IMPL 204SDT Titanium Scaler) respectively and 5 implants were left uninstrumented. Each instrument was used with 20 root planning strokes in apico-coronal direction.

Stereomicroscopy of implants

The implant surfaces were irrigated using sterile normal saline in a syringe to clear off all the scrapings if generated before visualization under the stereomicroscope at 40x magnification to evaluate for surface changes.

Revival of microorganisms

Four organisms were used for the *invitro* biofilm formation in the study - *Streptococcus mitis, Streptococcus oralis, Porphyromonas gingivalis* and *Fusobacterium nucleatum*. Standard strains of the organisms wereprocured from the ATCC and revived in thioglycolate broth and incubated at 37^{o}C. The growth of organisms was checked by an increase in turbidity. The organisms were then subcultured on blood agar and incubated at 37^{o}C. The growth pattern was checked and the morphology was evaluated by gram staining. These individual organisms were transferred to thioglycolate broth. Thin suspensionof the organism was prepared for each organism. An equal quantity of each organism was transferred in a different tube. A suspension of mixed culture was made for developing the biofilm.

Biofilm formation on the implant surface

Implants were sterilized in an autoclave at 121^{o} C for 15 minutes under pressure of 15 lbs and transferred individually to labeled Eppendorf tubes according to the groups. Thioglycolate broth was added to each Eppendorf tube till the neck of the implant was covered in the broth. This thioglycolate broth was used to enhance the growth of facultative and anaerobic organisms. This was sterilized by autoclaving at 121^{o} C for 15 minutes under pressure 15 lbs. This ensured complete sterilization which was checked by incubation at 37^{o}C.

For the biofilm formation, 10 microliters of the prepared suspension were added to each tube containing implant. This was incubated anaerobically for 4 weeks. The media was changed twice weekly to replenish the organisms.

Quantification of the biofilm bacteria

At the end of incubation period of 4 weeks, the bacteria was quantified. Swabs of the biofilm were collected from the upper 1/3rd threads of each implant fixture and transferred to a different Eppendorf vial individually and shaken. This suspension was allowed to settle for 30 minutes before further quantification.

Evaluation of the optical density

The suspension was transferred to sterile microtitre plate and optical density was evaluated at 590 nanometres in ELISA reader (BIORAD-i Mark).

Evaluation of colony forming units

Nichrome resistance wire loop was charged with prepared suspension and a loopful of the specimen was transferred using streak culture onto well-dried blood agar plates containing vitamin K and haemoglobin to enhance facultative and anerobic organisms. These blood agar plates were incubated for 48-72 hours at 37^{o}C anaerobically. At end of incubation time, colony count was done to get the colony forming units.

These values of the optical density and colony forming units were used for intergroup and intragroup comparison.

Statistical analysis

Statistical analysis was carried out by one way ANOVA f-test and Tukey's post hoc test which is a modification of the student t-test.

RESULTS

The present study evaluated and compared biofilm formation on implant surface after three different mechanical debridement methods quantitatively. Alterations on the implant surface after instrumentation were also evaluated under a stereomicroscope.

Analysis of instrumented implant surface

The stereomicroscopic images of each instrument group have been illustrated in the figures 1 to 4. Upon first glance, stereomicroscope imaging revealed modifications on the implant fixture surface. Alterations of the surface differed between each instrumentation group and from the control (uninstrumented) implant surface.

Stainless steel scaler group showed evident changes on the surface of the implant. The titanium scaler group lead to surface changes lesser than the stainless steel scaler group but more than plastic tip scalers. The plastic tip scaler group did not show significant changes when compared to the control (uninstrumented) group.

The control group exhibited grainy appearance owing to the surface treatment of the implant. Plastic scaler yielded seemingly unchanged areas when instrumented. However, the titanium and the stainless steel group seem to create a wiped appearance, later showing more apparent changes than the former.

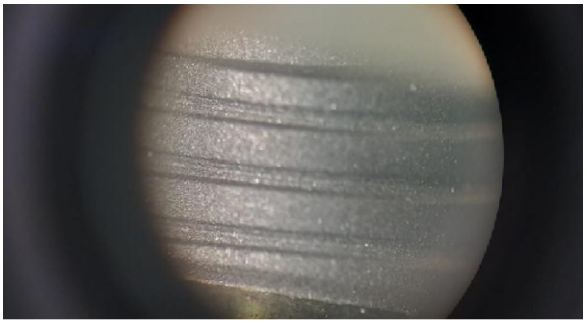

Figure 1: Stereomicroscopic Image of Control Group.

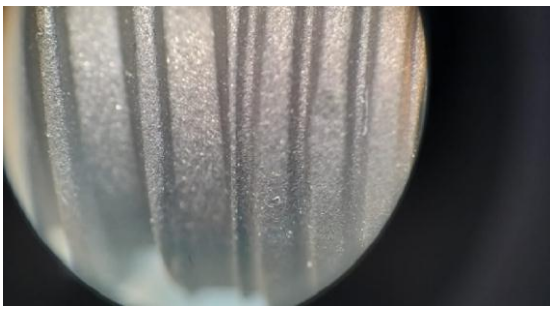

Figure 2: Stereomicroscopic Image of Plastic scaler Group.

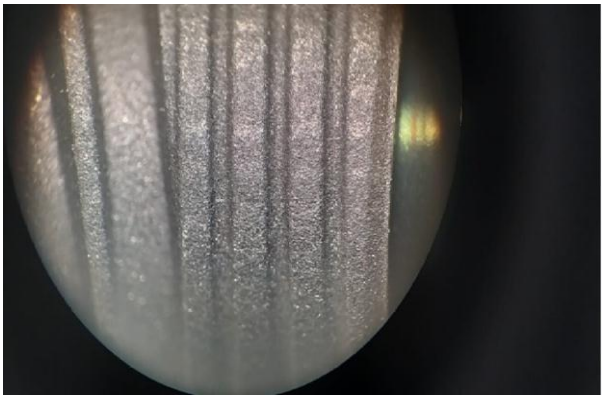

Figure 3: Stereomicroscopic Image of Titanium Scaler Group.

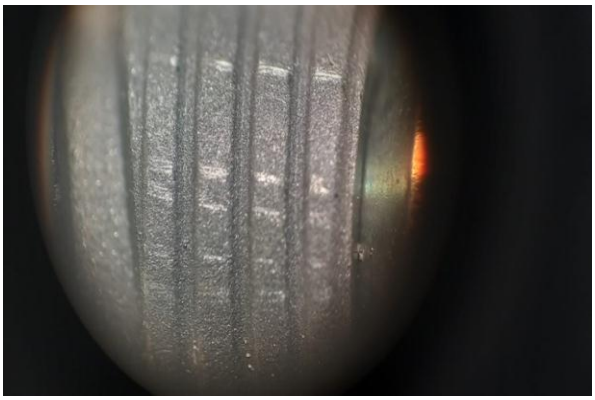

Figure 4: Stereomicroscopic Image of stainless steel scaler Group.

Quantitative analysis of biofilm formation

Optical density

The optical densites for the instrumentation groups have been depicted in the table 1.

Table 1: Optical density.

	OPTICAL DENSITY					
	1	**2**	**3**	**4**	**5**	**Mean**
Control	0.061	0.062	0.083	0.074	0.07	0.07
Titanium scaler	0.101	0.065	0.078	0.071	0.073	0.0776
Plastic scaler	0.067	0.069	0.075	0.08	0.081	0.0744
Stainless steel scaler	0.053	0.07	0.07	0.075	0.079	0.0694

Colony Forming units

The optical densities for the instrumentation groups have been depicted in the table 2.

Table 2: Colony forming units.

	COLONY FORMING UNITS					
	1	**2**	**3**	**4**	**5**	**Mean**
Control	80	110	180	190	180	148
Titanium scaler	150	60	95	75	72	90.4
Plastic scaler	50	60	100	110	110	86
Stainless steel scaler	62	89	110	130	115	101.2

The blood agar plates culture of the instrumentation groups have been depicted in figures 5 to 8.

Figure 5: Blood Agar Plate after Incubation for Control Group.

Figure 6: Blood Agar Plate after Incubation for Plastic Scaler Group.

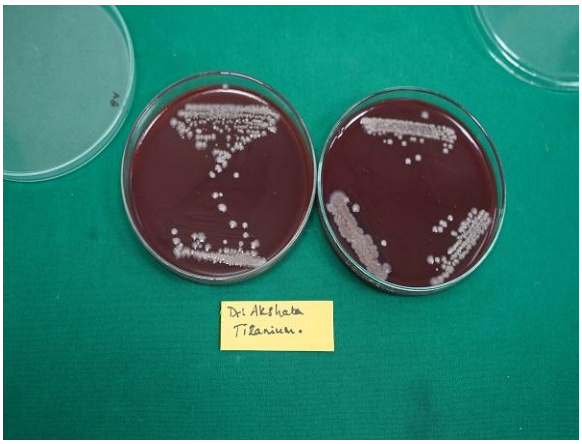

Figure 7: Blood Agar Plate after Incubation for Titanium Scaler Group.

Figure 8: Blood Agar Plate after Incubation for Stainless steel scaler group.

DISCUSSION

Implant dentistry is one of the fastest growing areas of dentistry to replace missing natural teeth.[1] Peri-implant diseases resemble gingivitis and periodontitis as they are caused due to presence of biofilm.[1] The prevalence, aetiology and treatment of peri-implant diseases are topics of research in modern dentistry. Peri-implant diseases or infections can either be defined as peri-implant mucositis or peri- implantitis.[2] Peri-implant mucositis has been defined as an inflammatory lesion of the mucosa surrounding an endosseous implant without loss of supporting peri-implant bone.[3] Peri-implantitis is a pathological condition occurring in tissues around dental implants, characterized by inflammation in the peri-implant mucosa and progressive loss of supporting bone.[4]

A systematic review and meta-analysis reported the prevelance of patients with peri implantitis to be 18.8% and that of implants with peri-implantitis to be 9.6%.[5] Another review reported the prevalence of peri-implantitis to range from 1-47% with an estimated weighted mean prevalence of 22%.[6] Implants affected by peri-implantitis ranged from 0-3.4% during the first 5 years of implant function. However, 10.7-47.2% of implants were seen to be affected by peri-implantitis after an observation period of 10 years.[7] Frequency of patients with peri-implantitis was seen to decrease to 14.3% after enrolment in supportive maintenance programmes.[5] Therefore, a minimum implant recall interval of 5 to 6 months for a significant positive impact on the incidence of peri-implantitis.[8]

Preventive maintenance therapy around implants plays a crucial role in maintenance of peri-implant clinical

stability and homeostasis of microbial condition.[9] There is a need of recommendations for the clinicians to safely and effectively carry out mechanical debridement of soft and hard deposits to arrest the disease progression and recurrence around the implants.[1] Alteration of the implant surface should be avoided to increase the life of the implants after mechanical debridement.[1] Stainless steel tends to alter the implant surface. Other materials like titanium, plastic curettes, carbon-fiber curettes, Teflon coated curettes or polyether- etherketone-coated ultrasonic tips have been used to overcome the drawbacks ofconventional stainless steel curettes.[10] The focus of the present study was to evaluate and compare the difference in the biofilm formation after different mechanical instrumentation modalities that may be carried out for treatment of peri-implant diseases or as a part of supportive therapy when implant threads are exposed in the oral cavity.

Periodontopathic microorganisms colonize implants in health and disease.[11] Peri-implant health is associated with gram positive cocci and non- motile bacilli. Increase in presence of cocci, motile bacilli and spirochete is seen in peri-implant mucosistis. Whereas transition to peri-implantitis shows an increase in gram-negative, motile, and anaerobic species.[12] The primary colonizers of the peri- implant sites are the *Streptococcus sp*..[11] *Fusobacterium nucleatum* has been associated with periodontal and peri-implant diseases as its opportunistic characteristic serves a bridging bacteria between the early and the late colonizers.[13] *Porphyromonas gingivalis* has been the most frequently found red complex organism at peri-implantitis sites.[11] It has also been associated more strongly with peri-implantitis than peri-implant mucositis.[14] *P.gingivalis* tends to induce more bone loss at sites with peri-implantitis and periodontitis.[15] Therefore, the present study included *S.mitis*, *S.oralis*, *F.nucleatum* and *P.gingivalis* for *in vitro* formation of the biofilm.

The stereomicroscopic imaging showed maximum changes by stainless steel instruments followed by titanium instruments and the least by plastic tips when compared to control group. This is in accordance with previous studies where stainless steel and titanium curettes induced macroscopic modification of the implant surface and plastic instruments showed no changes.[16] A recent study, also showed similar results where metal instruments caused more damage to the titanium surface and plastic caused the least damage.[17]

The quantification of bacteria was first carried out by photospectrometry in terms of optical density. This is the most common method to measure number of cells in a suspension at wavelength of 600nm.[18] Optical density also has added advantages of being extremely fast, inexpensive, simple, relatively nondisruptive, high-throughput, and readily automated.[18]

The mean optical density by photospectrometry showed the least colonization by stainless steel scaler. This is comparable with a study where the fibroblast attachment was evaluated following different instrumentation techniques and titanium and stainless steel showed fewer cell attachment than control group, with stainless steel showing the least fibroblast attachment.[20] Adhered fibroblasts and the bacteria show same behaviour of attachment on the surface of implants.[19] This supports the findings of the present study which has evaluated the quantification of bacteria.[20]

Though plastic instruments cause least surface alterations on the implant surface, it tends to leave deposits and smear layer. This in turn, alters the biocompatibility of the titanium surface and cell attachment.[20] This supports the finding that the mean optical density for the plastic instrument is lesser than that for the titanium instrument.

Another study showed that titanium surfaces treated by metal curettes were less susceptible to colonization by *S.sanguinis* due to texture modification and presence of abrasive deposits.[21] In the present study, highest mean value of bacterial adhesion was shown by titanium instruments. This is in accordance with a study where the titanium instrument treated surfaces showed increased adhesion to *S.mitis* as these instruments tend to create deep scratches on the titanium surfaces leading to increased surface roughness and thereby increasing adhesion of the bacteria.[22]

A key shortcoming of OD measurements is that a direct measure of cell count is not actually provided. Except within a limited range, OD is not related to the cell count. Also, the OD levels are related to light scatter than absorbance and therefore are quite unreliable.[21] Therefore, alternative measure of cell count – colony forming units was used in the present study. Whereby, the CFU represents only the viable cells, the OD value gives numerical value of both dead and viable cells.[23] This makes the CFU values more reliable. The present study also shows some disparity in the cell counts by two different methods – OD and CFU owing to the aforementioned reasons.

The surface geometry is more favorable for cell attachment after stainless steel instrumentation.[20] However, stainless steel and titanium being dissimilar metals, an alteration in biologic properties of theimplant surface occurs following instrumentation.

Though plastic instruments cause least surface alterations on the implant surface, it tends to leave deposits and smear layer. This in turn, alters the biocompatibility of the titanium surface and cell attachment.[20] This explains the findings of the present study where control group showed maximum mean colony forming units compared to titanium group, plastic group and for stainless steel group.

Bacterial attachment and biofilm formation are

promoted by rough surfaces to a greater extent than smooth surfaces. This is due to increased contact area between the material and bacterial cells and the protection of bacteria from shear forces.[24,25] However, some studies have evaluated surface alterations after instrumentation and have shown that metal instruments cause more damage and macroscopic alterations than plastic instruments.[17,16]

Surface charge density on the implant surface is more determinant than the chemical composition of the implant surface.[20] The implant surface has an inherent layer of oxide which is slightly negatively charged.[24] Mechanical instrumentation of the implant surface alters this oxide layer and thereby alters the surface charge.[27] Bacterial attachment, adhesion and spreading depends on the surface charge and occurs more rapidly on a negatively charged surface.[28] The difference in alteration in the surface charge between the instrumented and uninstrumented surface supports the findings of the present study that the control group shows maximum mean colonization than any instrumented group.

An *in vitro* study observed that except for stainless steel, no significant differences were seen in the surface characteristics or bacterial colonization based on one-time instrumentation by other instrumentation techniques.[1] The findings of the present study show that in spite of different degrees of alterations caused by different instruments, there is no statistically significant difference in the bacterial colonization with different treatment modalities. We therefore suggest that all instrumentation types are safe and non-damaging and can be implemented as needed for mechanical debridement.

The present study does not use standardized force for instrumentation. The surface changes in this are evaluated under a stereomicroscope which fails to provide a significant insight into the surface changes after instrumentation. This study includes biofilm formation by only four organisms whereas the oral cavity presents with a plethora of organisms. Also the *in vitro* environment fails to replicate the complex oral conditions.

CONCLUSION

Within limitations of the present study, it can be concluded that,

1. Stainless steel instruments caused maximum alterations of the surface and plastic instruments caused the least alterations.
2. There is no difference in biofilm formation irrespective of the difference in the surface alterations.
3. There is no statistically significant difference in the microbial count irrespective of the instrument used.
4. Mechanical debridement of the implants can be carried out using commercially available instruments for maintenance.

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