Original Article

Preventive Effect of Ultraviolet Photofunctionalization on Peri-implant Biofilm Formation: An *In vivo* Randomized Study

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Abstract

Background: Peri-implant biofilm formation due to local bacterial colonization is one of the important factors for the instability of temporary anchorage devices (TADs). **Aim:** The aim of this study was to quantify and compare the colonization of *Streptococcus sanguinis* on ultraviolet (UV) treated and untreated titanium TADs. **Materials and Methods:** This prospective, *in vivo* study included 20 subjects requiring orthodontic treatment with first premolar extraction, followed by retraction of the anterior teeth with absolute anchorage using TADs. TADs were placed interdentally, in the keratinized tissue between the upper second premolar and the first molar on the buccal side, at the mucogingival junction. It was a split-mouth study where one side of TAD was UV-treated for 15 min, and the other side was kept untreated as a control. TADs were removed after 6 months for *S. sanguinis* quantification on both sides and were compared for biofilm reduction. **Statistical Analysis:** Statistical software was used to perform unpaired *t*-tests for the individual samples as well as for comparing total UV-treated and untreated samples. P <0.05 was considered significant. **Results:** The mean bacterial count (per ml) was found to be 2.2×10^6 copy numbers and 8.9×10^6 copy numbers in the UV group and untreated group, respectively. The total count of bacteria was found to be less in the UV-treated group compared to the untreated group. **Conclusions:** The study concludes that UV photofunctionalization results in a significant reduction of *S. sanguinis* colony on TADs with reduced chances of failure due to inflammation.

Keywords: *Biofilm, Streptococcus sanguinis, temporary anchorage devices, ultraviolet photofunctionalization*

Introduction

Anchorage loss has always been a concern in orthodontics. Various options have been tried to prevent or limit anchorage loss such as Nance palatal button,[1] transpalatal arch,[2] headgears,[3] inter-arch elastics,[4] and banding of the second molars.[5] However, these appliances resulted in two major disadvantages: (i) they could not provide full control over anchorage loss and (ii) the effectiveness of these appliances relied on patient compliance. With the advent of temporary anchorage devices (TADs) in the form of miniscrews, mini-implant, or mini-plates, the patient compliance factor was eliminated as well as absolute control over anchorage loss was achieved.[6] TADs immediately gained popularity because of their cost effectiveness, minimal invasiveness, and ease of placement and removal.

The success of a TAD is mainly dependent on its stability over the bone, and factors affecting the stability of TAD are miniscrew geometry (i.e. shape, length, and diameter), bone density, insertion angle and depth, insertion site, immediate or delayed loading, and inflammation around miniscrews.[6,7] Miyawaki *et al.* reported that a 30% failure rate was due to inflammation around TAD.[8] Therefore, maintaining uninflamed tissue around TAD is important because the healthy tissue acts as a biological barrier against bacterial colonization. Usually, microbial colonization first starts with aggregation of primary colonizers, such as *Streptococcus sanguinis*.[9] In previous studies, *S. sanguinis* has been detected at a very high frequency of 83.3% in peri-implant sulcus and 29.2% at titanium implant surface.[10] It has been documented in many previous studies that *S. Sanguinis* is associated with a biofilm formation on implants and leads to subsequent instability of the miniscrew.[11-13]

A technique of "ultraviolet (UV) photofunctionalization" had been effectively

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used for the prevention of biofilm formation on titanium implant surfaces in many previous studies.[11,13-15]

Therefore, we suppose that the technique of UV photofunctionalization can prevent bacterial colonization around implant or TAD, but all previous studies were *in vitro* and need confirmation of the same effectivity by *in vivo* study. Thus, we planned to conduct the first *in vivo* study "to evaluate the effectivity of UV photofunctionalization on quantification of bacterial growth over titanium surface." The aim of this study was to quantify and compare *S. sanguinis* on UV-treated and untreated orthodontic titanium miniscrews in an *in vivo* setup.

Materials and Methods

This was a single-center, split-mouth randomized controlled trial, and no changes were made to the trial design after commencement. The clinical trial was approved by the Institutional Ethics Committee (EC/NEW/INST/2019/329). The study was conducted on participants who reported for orthodontic treatment in a tertiary care center and teaching hospital.

Twenty subjects (10 males and 10 females) were allocated for the study requiring routine orthodontic treatment with first premolar extraction. The inclusion criteria were as follows: adult participants (age: 18–30 years) requiring routine fixed orthodontic treatment with extraction of both the upper first premolars (Class I bimaxillary protrusion or Class II div 1 malocclusion) and requiring absolute anchorage for retraction, good oral hygiene status (plaque index score of 0–1), and adequate inter-radicular space between the upper premolar-molar for miniscrew insertion. Any participant with poor oral hygiene (plaque index >1), age above 30 years, smoking habit, or not requiring absolute anchorage were excluded from the study. The participants were informed about the procedure, and an informed consent was taken. Each participant was assigned a code, and a suffix "L" and "R" was used to represent the left and right sides of the upper arch, respectively.

Fixed orthodontic mechanotherapy was started for the included participants following extraction of the maxillary first premolars. MBT 0.022 Prescription (3M Unitek™ Gemini Metal Brackets) was used. The leveling and alignment were done in the upper arch till 0.019" × 0.025" NiTi after which space closure was started on 0.019" × 0.025" stainless steel using miniscrews as anchorage. A force of 150 g was used for retraction using NiTi coil springs.

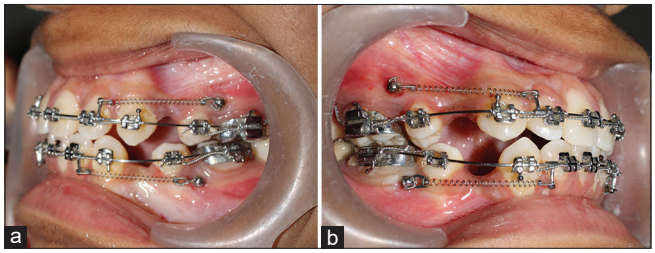

Figure 1: Miniscrew in the inter-radicular keratinized area between the upper second premolar and first molar at the mucogingival junction; (a) Left side; (b) Right side

Randomization and blinding

The participants selected for the clinical trial were randomly allocated the sides for UV-treated and untreated using a lottery method, and the results were kept in a sealed opaque envelope that was opened chairside before the treatment.

The participants were not informed about the side selected for the placement of UV-treated and untreated miniscrews. The laboratory personnel and the statistician were also blinded. All the procedures were performed by a single operator who was not blinded.

Intervention

The miniscrews (AbsoAnchor, Dentos, Korea; diameter – 1.4 mm, length – 7 mm) were inserted interdentally, in the keratinized tissue between the upper second premolar and first molar at the mucogingival junction [Figure 1]. The miniscrews were inserted in a sterile environment under local anesthesia (infiltration). The placement of miniscrews was carried out by a single operator. The study design was a split-mouth study, where one side miniscrew was UV-treated in a calibrated UV chamber [Figure 2] used in a previous study by Rampurawala *et al*.[16] for 15 min and the other was inserted without any prior modification. The UV chamber with UV-A (power-15 W, wavelength-350 ± 20 nm, intensity-0.1 mW/cm^2) and UV-C light (power-15 W, wavelength-250 ± 20 nm, intensity-2.0 mW/cm^2) was used. The miniscrews were retrieved after 6 months and were sent to the laboratory in an Eppendorf tube containing 1 ml sterile phosphate-buffered saline solution for quantification of *S. sanguinis* using real-time qPCR. Liquid nitrogen was used as a transporting media to ensure the viability of bacterial DNA. The copy number was used as a standard unit for bacterial quantification.

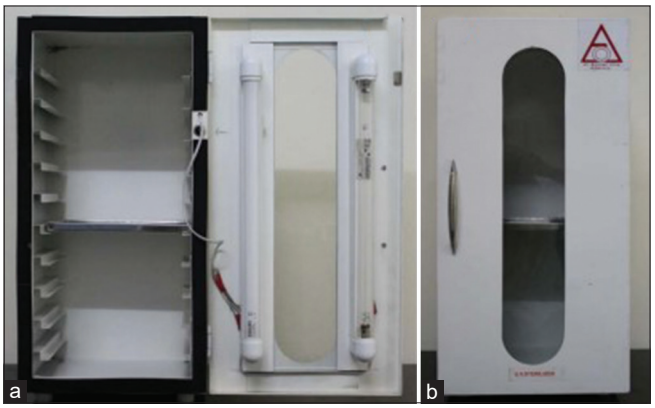

Figure 2: A calibrated ultraviolet chamber. (a-open; b-closed)

Statistical analysis

Statistical software (SPSS version 21, SPSS Inc.) was used to perform all statistical analyses. An unpaired *t*-test was carried out for the individual samples as well as for comparing total UV-treated and untreated samples. $P < 0.05$ was considered significant.

Results

A total of 20 participants were included in the study (10 males, 10 females, mean age of 23 ± 4.2 years). Hence, out of 20 participants, 9 received UV-treated miniscrew on the left side, and the remaining 11 participants received it on the right side. Table 1 shows the participants' code along with the sides where UV-treated and untreated miniscrews were inserted.

Real-time polymerase chain reaction

The results of real-time quantitative polymerase chain reaction (qPCR) analysis were obtained from the machine (StepOne™ Plus) and are presented in Table 2. The total number of copies of DNA (per ml) and the standard deviations were tabulated as per the treated side and untreated side.

The total sum of bacterial count (per ml) in the UV group was found to be 44,611,894.78 copy numbers, whereas in the untreated group, it was found to be 177,212,475.06

Table 1: Participant's data with their codes, ultraviolet-treated and untreated sides

Participant number	Participant code	UV-treated side	Untreated side	Participant number	Participant code	UV-treated side	Untreated side
1	AL/AR	Left	Right	11	KL/KR	Left	Right
2	BL/BR	Left	Right	12	LL/LR	Left	Right
3	CL/CR	Left	Right	13	NL/NR	Right	Left
4	DL/DR	Left	Right	14	OL/OR	Left	Right
5	EL/ER	Right	Left	15	PL/PR	Right	Left
6	FL/FR	Right	Left	16	QL/QR	Right	Left
7	GL/GR	Left	Right	17	RL/RR	Left	Right
8	HL/HR	Right	Left	18	SL/SR	Right	Left
9	IL/IR	Left	Right	19	TL/TR	Right	Left
10	JL/JR	Right	Left	20	UL/UR	Left	Right

Suffix "L" was used in the participant's code for their left side and suffix "R" was used for the right side. UV: Ultraviolet

Table 2: Total copy numbers of bacteria (per mL) in ultraviolet-treated and untreated samples along with their standard deviations

Participant code	UV-treated (copy number/mL)	UV-SD	Participant code	Untreated (copy number/mL)	Untreated SD
AL	648,046.13	62,531.13	AR	532,817.25	141,626.46
BL	1,009,859.50	42,184.97	BR	655,990.88	74,885.21
CL	6,005,312.00	117,199.41	CR	50,026,808.00	5,861,131.5
DL	564,711.38	103,091.70	DR	7,924,622.00	933,184.75
ER	3,044,850.06	88,979.05	EL	23,494,292.00	3,756,974.25
FR	9,577,500.00	3,997,927.25	FL	4,407,417.50	1,038,770.81
GL	4,740,766.00	435,062.65	GR	1,454,541.25	14,627.65
HR	53,542.20	8047.70	HL	602,214.50	120,901.73
IL	60,211.40	8455.43	IR	1,304,862.13	34,204.70
JR	902,568.06	43,055.91	JL	15,430,352.00	3,434,610
KL	1,895,769.00	530,779.37	KR	3,247,673.00	129,690.80
LL	1,327,370.00	164,811.46	LR	491,200.50	46,202.60
NR	3,529,526.50	251,943.39	NL	52,917,720.00	4,427,303
OL	12,459.58	2569.27	OR	615,576.31	38,771.19
PR	500,390.62	3337.11	PL	701,311.65	2311.32
QR	85,783.53	23,067.21	QL	117,552.09	25,110.66
RL	4,861,926	234,971.58	RR	5,109,344	108,632.76
SR	1,014,076	65,504.83	SL	1,128,705	108,633.11
TR	4,698,211	201,843.64	TL	6,911,201	209,781.09
UL	79,015.82	3991.82	UR	138,274	2892.61
Mean	2,230,594.74	319,467.74	Mean	8,860,623.75	1,025,512.31
Total	44,611,894.78	6,389,354.88	Total	177,212,475.06	20,510,246.2

SD: Standard deviation; UV: Ultraviolet

copy numbers. The mean bacterial count (per ml) in the UV group was 2,230,594.74 copy numbers, and in untreated, it was found to be 8,860,623.75 copy numbers. The total count of bacteria was less in the UV-treated group.

The results for the unpaired *t*-test applied to individual participants are tabulated in Table 3. $P < 0.05$ was considered significant.

The highlighted values [Table 3] show the UV-treated samples with their *P*-values and significance. Out of 20 samples, 11 samples (3, 4, 5, 9, 10, 13, 14, 15, 17, 19, and 20) showed a significant bacterial reduction in UV-treated samples compared to the untreated samples. Out of the remaining nine samples, only two samples (2 and 12) showed a statistically increased bacterial count in UV-treated samples than the untreated sample.

The combined mean values of UV-treated sides were also compared with untreated sides. An unpaired *t*-test was used for comparison, and the results are tabulated in Table 4 ($P = 0.013$).

The comparison of the combined mean of all UV-treated samples and the untreated samples showed a highly

Table 3: Bacterial concentration (counts per ml) in individual sample and the respective *P* values assessed by real-time polymerase chain reaction

Participant number	Participant code	Bacterial concentration (copy number/ml)	SD	*P*	Significance
1	AL$^{\perp}$	6,48,046.13	62,531.13	0.2067	ns
	AR	5,32,817.25	1,41,626.46		
2	BL$^{\perp}$	10,09,859.50	42,184.97	0.0283	*
	BR	6,55,990.88	74,885.21		
3	CL$^{\perp}$	60,05,312.00	1,17,199.41	0.0088	**
	CR	5,00,26,808.00	58,61,131.50		
4	DL$^{\perp}$	5,64,711.38	1,03,091.70	0.0080	**
	DR	79,24,622.00	9,33,184.75		
5	EL	2,34,94,292.00	37,56,974.25	0.0137	*
	ER$^{\perp}$	10,44,850.06	88,979.04		
6	FL	44,07,417.50	10,38,770.81	0.2187	ns
	FR$^{\perp}$	95,77,500.00	39,97,927.25		
7	GL$^{\perp}$	47,40,766.00	4,35,062.65	0.4476	ns
	GR	14,54,541.25	14,627.65		
8	HL	6,02,214.50	1,20,901.73	0.328	ns
	HR$^{\perp}$	53,542.20	8,047.70		
9	IL$^{\perp}$	60,211.40	8,455.43	0.0004	***
	IR	13,04,862.13	34,204.70		
10	JL	1,54,30,352.00	34,34,610.00	0.0268	*
	JR$^{\perp}$	9,02,568.06	43,055.91		
11	KL$^{\perp}$	18,95,769.00	5,30,779.37	0.0729	ns
	KR	32,47,673.00	1,29,690.80		
12	LL$^{\perp}$	13,27,370.00	1,64,811.46	0.0203	*
	LR	4,91,200.50	46,202.60		
13	NL	5,29,17,720.00	44,27,303.00	0.004	**
	NR$^{\perp}$	35,29,526.50	2,51,943.39		
14	OL$^{\perp}$	12,459.58	2,569.27	0.0021	**
	OR	6,15,576.31	38,771.19		
15	PL	7,01,311.65	2311.32	0.0002	***
	PR$^{\perp}$	5,00,390	3337.11		
16	QL	1,17,552.09	234971.58	0.8	ns
	QR$^{\perp}$	85,783.530	23067.21		
17	RL$^{\perp}$	48,61,926	234971.58	0.006	**
	RR	51,09,344	108632.76		
18	SL	11,28,705	108633.11	0.32	ns
	SR$^{\perp}$	10,14,076	65504.83		
19	TL	69,11,201	209781.09	0.008	**
	TR$^{\perp}$	46,98,211	201843.64		
20	UL$^{\perp}$	79,015.82	3991.82	0.0034	**
	UR	1,38,274	2892.61		

*$P<0.05$; **$P<0.01$; ***$P<0.001$. $^{\perp}$signifies UV treated samples. Ns: Nonsignificant; SD: Standard deviation

Table 4: Combined comparison of ultraviolet-treated and untreated samples

UV-treated side (mean copy number±SD)	Untreated side (mean copy number±SD)	*P*	Significance
2,230,594.74±319,467.74	8,860,623.75±1,025,512.31	0.013	*Statistically significant

SD: Standard deviation; UV: Ultraviolet. *$P < 0.05$ – Statistically significant

statistically significant reduction in bacterial count by UV treatment. A total of 62.5% reduction was observed in the UV group compared to the untreated group [Table 4].

Discussion

This novel *in vivo* study on the effect of UV photofunctionalization showed that this technique is significantly effective in the prevention of bacterial colonization over the implant surface. Thus, this is indirect evidence that UV photofunctionalization can be an effective method for implant stability. The results were in accordance with the other *in vitro* studies where a significant reduction in bacterial count was seen.[11,13-15] We found a total 62.5% reduction in *S. sanguinis* count on UV-treated miniscrews compared to untreated miniscrews ($P = 0.01$).

UV photofunctionalization is a phenomenon of modification of titanium surfaces occurring after UV treatment, including the alteration of physiochemical properties and the enhancement of biologic capabilities.[17] The physiochemical properties are affected by three key mechanisms: (i) generation of superhydrophilicity, (ii) carbon reduction, and (iii) electrostatic conversion of surface charge from negative to positive.[16-20] The ability of UV photofunctionalization to convert the natural hydrophobic surface of titanium to a superhydrophilic surface has been shown to reduce the attachment of hydrophobic bacteria like *S. sanguinis* that finally results in the reduction of bacterial colonization.[20] Overall, the result of this physiochemical alteration generates a photocatalytic effect on the titanium surface which is responsible for killing a wide spectrum of bacteria.[19]

The UV light lies in the wavelength spectrum ranging from 10 to 400 nm, and they are classified as UV-A (320–400 nm), UV-B (290–320 nm), and UV-C (10–290 nm) as per the dermal biological actions of the UV radiation.[21] Both UV-A and UV-C irradiation have been shown to reduce biofilm formation. A study by Jain *et al.*, in 2018, demonstrated a 50% killing efficacy shown by UV-C irradiation and a 20% killing efficiency of UV-A irradiation.[11] In this study, also the calibrated UV chamber contained both UV-A (power-15 W, wavelength-350 ± 20 nm, and intensity-0.1 mW/cm^2) and UV-C (power-15 W, wavelength-250 ± 20 nm, intensity-2.0 mW/cm^2) lights as per the range used in biological investigations.[18] We found similar results in the current study where bacterial reduction was seen after UV-A and UV-C treatment.

The selected duration of UV treatment of titanium surface varies considerably among different studies, ranging from a few minutes to several hours.[22] A recent review by Chang mentioned that most of the studies had good effectivity with a treatment duration of 15 min;[11,22] therefore, in the current study, we had fixed the duration of UV treatment for 15 min.

S. sanguinis was selected for this study due to two main reasons; (i) it is a primary colonizer and initiator of biofilm formation[20] and (ii) it has an affinity toward hydrophobic titanium surface because of its similar hydrophobic membrane characteristics.[20,23] Therefore, we decided to assess the reduction in biofilm indirectly by assessing *S. sanguinis* count on the UV-treated and comparing it with untreated titanium miniscrews. Other *in-vitro* studies have also assessed similar properties like hydrophobic membrane characteristics and role in wound infection in diverse oral microbial community[13] and wound bacteria such as Staphylococcus aureus, Streptococcus pyogenes.[14]

Based on these findings, it can be concluded that UV photofunctionalization reduces the *S. sanguinis* count on titanium miniscrews by photocatalytic activity that maintains its potency even in a dynamic oral cavity. We also hypothesize that other hydrophobic bacteria such as *S. pyogenes* and *Staphylococcus aureus* will also have the same effect of UV photofunctionalization.

Conclusions

The following conclusions can be drawn from the present *in vivo* study that chairside UV treatment (UV-A and UV-C light) can significantly reduce the biofilm attachment on orthodontic titanium miniscrew. A rapid method of UV treatment for 15 min duration is sufficient in day-to-day clinical practice, and it can reduce the chances of miniscrew failure caused by inflammation around miniscrews. Therefore, UV photofunctionalization can increase the longevity of miniscrews in the oral cavity and can prevent the participants from the trouble of repeated administration of miniscrews. Future directions from the current study are: (i) finding to be confirmed from other large-scale *in vivo* studies; (ii) effect on other bacteria such as *S. pyogenes* and *S. aureus* should also be studied; and (iii) protocol for UV photofunctionalization should be made universally applicable with multicentric studies.

Limitations and risks

One observation of our study was that two of our participants had risen bacterial count on UV-treated miniscrews. Both the participants (participants number 2 and 12) had their UV-treated implant on the left side, and

they mentioned that due to their habitual better cleaning on the right side of the mouth more than the left, both of these participants were left-handed. This could be one of the reasons for finding less bacterial attachment on the right side, which was the untreated side.[24] This observation has to be critically analyzed in future *in vivo* studies.

The biofilm reduction was indirectly assessed by quantifying only one species of bacteria. Therefore, it would be more accurate to assess a wide spectrum of bacteria in future studies. More human studies with a larger sample size and multicentric studies are required to confirm the efficacy of UV-treated titanium on bacterial reduction.

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Conflicts of interest

There are no conflicts of interest.

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