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RESTORATION OF EXPIRED DENTAL IMPLANTS BY ULTRAVIOLET C PHOTOFUNCTIONALIZATION: AN IN VITRO STUDY

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ABSTRACT

Objective: This study examines the effect of ultraviolet C (UVC) irradiation on the wettability and the potential bacterial surface contamination of expired dental implants.

Materials and Methods: Twenty expired titanium implants in intact packaging were tested. The examined implants were exposed to UVC light of 254 nm wavelength and 8-watts power at two intervals for 15 minutes each. Bacterial culture was performed for all implants in three events; immediately after unpacking, after experimental contamination with freshly collected saliva, and after the first UVC exposure. As an indicator of surface wettability, Static-contact angle (S-CA) measurement was done for all implants three times; Pre-radiation and after the first and second UVC exposures.

Results: All tested implants showed hydrophobic surface characters before exposure to UVC, and superhydrophilic surfaces were obtained following the second UVC exposure. All sealed expired implants didn't show any bacterial growth, and all saliva contaminated implants showed no bacterial growth after the first UVC exposure. There was no statistical correlation between implants' aging periods and the expired implant statistic contact angles.

Conclusion: The used UVC source showed the ability to restore the expired implants to a superhydrophilic status and eliminate the bacterial contamination on the implant surfaces. Intact packaging protects outdated implants from bacterial contamination.

KEYWORDS: Expired dental implant, Ultraviolet C photofunctionalization, Contact angle, Hydrophilicity, Implant surface contamination.

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INTRODUCTION

Titanium dental implants are commonly used in dental rehabilitation. The passing of the expiration date of the titanium implants despite frequent usage is a challenge to be investigated. Expired titanium dental implant carries two crucial issues: the biological aging of the titanium oxide (TiO_2) surface layer[1] and the potential loss of sterilization of the packaged implant.

Biological aging of the titanium implants is a time-dependent degradation of the titanium surface owning to TiO_2 surface layer hydrocarbon contamination during storage under ambient conditions.[1] Hydrocarbon contamination progressively decreases the surface wettability of the implant[2,3] and reduces protein adsorption on the surface of the implants[4]. Both, in turn, reduce osteoblasts' migration, attachment, spread, and proliferation on the implant surface, leading to incomplete osseointegration.[5,6] Several studies indicated that clinically and experimentally used titanium implants show hydrocarbons surface contamination[7,8] resulting in 45 to 65% bone-titanium contact[9,10]. Elimination of titanium surface hydrocarbon contaminations regaining superhydrophilic surface improves the bone to titanium contact percent and hence, the process of osseointegration.[11-13] On the other hand, potential bacterial surface contamination of dental implants following expiration could affect the implantation success rate. Bacterial contamination increases the risk of mucositis or periimplantitis.[14-16] Addressing these two issues is essential to restoring the expired implants to the ideal working status.

UVC photofunctionalization can reverse the aging process, regain surface hydrophilicity [17,18] and sterilize the implant surfaces[19-23]. However, the previous studies examined the effect of photofunctionalization on titanium only after short aging periods (4 to 8 weeks)[6,24-27] or on non-expired implants[28]. To the best of the authors' knowledge, no previous study examined UVC photofunctionalization effects on expired dental titanium implants. Hydrocarbon contamination of the TiO_2 surface layer starts after four weeks of manufacturing.[1] This could reveal the pronounced effect of the prolonged aging periods of the expired implants, which is the working period, four years in most of the used titanium implants[1], plus the time following the expiration date.

The present study was conducted to test the capability of UVC photofunctionalization to restore surface hydrophilicity and eliminate potential bacterial contamination of the expired implants. Moreover, the study tested the ability of intact packaging to protect the outdated implants from bacterial contamination and the correlation between expired implant aging periods and the S-CA measurements.

MATERIALS AND METHODS

Sample size determination

A pilot study was held to determine the sample size and the UVC light source specifications. Eighteen expired titanium implants with intact packaging were divided into three groups and exposed to three different UVC light sources at two sessions for 15 minutes each. Group-I source consisted of a commercial sterilizer box (FBFL UV Sterilizer box, Zhejiang SORBO Technology Co., Ltd, China) that supplies UVC light with a wavelength of 185 nm and a power of 0.5-watts. Group-II source was A UVC lamp (LAVAED UV Lamp, China) that emits rays with 254 nm wavelength and 8-watts power. Group-III source: UVC lamp (Coospider CTUV-36, Aopu Lighting (JinYun) Co. Ltd, China) with a wavelength of 254 nm and power of 36-watts. The three examined UVC sources were equipped with a built-in 15-minute timer.

S-CA measurements were done on three events: before exposure, following the first and second UVC exposures. Bacterial culture was done for the three groups on three events; immediately after implants unpacking, after experimental contamination of the

implants with freshly collected unstimulated saliva, and following the first UVC exposure.

All tested implants showed hydrophobic surface characters before exposure to UVC sessions. The three groups' implants showed superhydrophilic characters after the second UVC exposure. However, only UVC sources-II and -III showed an effective antibacterial effect. According to the findings of this pilot study, the UVC source II was chosen, and the sample size was determined. The online sample size calculator computed the minimal sample size at 0.8 power and 0.05 alpha level.[29] The minimal sample was 12 implants and was increased to twenty implants.

Study design

The current study was conducted on twenty expired titanium implants (Anthogyr, Axiom, France) subjected to bacterial culture in a class II safety cabinet on three events. UVC light exposure was done at two intervals for 15 minutes each. S-CA measurement was used to indicate the hydrophilicity of the implant surface before and after each UVC exposure.

Bacterial culture

Implants' packages of the tested implants were examined for any damage or apparent leakage. Intact packages were marked to indicate the implant's number, and each implant's manufacture and expiration dates were recorded. Twenty blood agar plates were labeled following implants' numbers, and each was marked into three sectors with a permanent marker. Twenty 3D printed implant-holders were designed to fit the implant connection site and enable implants manipulation without touch (Fig 1C-l). The bacteriological culture was made on three events. The first was immediately after opening the implant sealed package (sector I). The second was after dipping the apical third of the implant, carried with the implant-holder, in freshly collected unstimulated saliva to experimentally contaminate the implant surface (sector II). The third was done after the first UVC exposure interval (sector III). The apical third of each implant was pressed from three surface sites against the designated blood agar plate in the corresponding marked sector of the plate. Pressing the implant from three surface sites represented almost the entire examined surface.

S-CA measurements

Measurement of S-CA was done on three incidents: before UVC exposure and after each UVC exposure.

1. Equipment:

Four specially designed 3D printed implant-holders-carrying trays were made with six insertion sites to accommodate six implant-holders each. (Fig 1C-m) The carrier sites were indexed with implants' numbers. A specially designed 3D printed implant-photographing tray was made to accommodate three implant-holders-carrying trays to take photos for S-CA measurement. (Fig 1A-g, Fig 1C)

A specially built camera mount setup was designed to confirm a constant distance between the camera lens and the implants with a perpendicular orientation of the lens to the photographed implant during photographing of each tested implant. This camera mount consisted of a horizontal tripod arm (Fotoconic, DCFANBOY Co., Ltd, China) (Fig 1A-a) attached to a steady tripod (2 in 1 camera tripod, Neewer, China). A four-way horizontal macro slider (Pro 4-way Macro focus slider, Neewer, China) (Fig 1A-c) was attached on top of 360˚ rotating tray (DSLR-KIT 360˚, Neewer, China) (Fig 1A-b) at one side of the horizontal bar, allowing for incremental camera position adjustment in the X (right/left) and Z (anterior/posterior) axes (Fig 1B-j, i). On top of the horizontal slider, a vertical macro slider (DSLR-KIT 360˚, Neewer, China) (Fig 1B-k) was attached to allow incremental camera position adjustment in the Y-axis (up/down). A DSLR camera (Canon 70D, Canon INC., Japan) was attached to the vertical slider (Fig 1A-d). The camera was equipped with a fixed zoom macro-lens (Macro 100mm L lens, Canon

Fig. (1) Section A: a. Horizontal tripod arm, b. 360° rotating tray c. Four-way horizontal macro slider, d. DSLR Camera, e. Remote shutter release, f. Tripod head, g. Implant-photographing tray, h. the three implant-holder trays with 18 implant holders carrying 18 expired implants; Section B: i. Z-axis anterior/posterior slider, j. X-axis right/left slider, k. Y-axis vertical slider; section C: l. implant holder, m, implant-holder tray.

INC., Japan). A remote shutter release (WTR-2 transmitter, Shenzhen Aodelan technology Co., Ltd, China) (Fig 1A-e) was used to trigger the camera without shaking the mount. Lens focusing was kept at automatic mode, and exposure was adjusted according to the ambient light. On the other side of the horizontal bar, the implant-photographing tray (Fig 1A-g) was attached to a tripod-head (Fig 1A-f), allowing for horizontal orientation of the tray carrier.

2. Photo acquisition

Three implant-holders-carrying trays holding eighteen implants were set on the implant-photographing tray (Fig 1C). The photographing tray level was adjusted to a horizontal level parallel to the floor by the tripod-head leveling bubble water. Meanwhile, the camera was kept horizontally to the floor according to its electronic leveling system. The camera position was adjusted with the X-axis and the Y-axis sliders so that the camera field of view was centered at implant number one of the

first implant-holders-carrying tray. The field of view was tweaked to include the specific implant and its number mark in the middle third of the photo by moving the Z-axis slider anteriorly and posteriorly. The Z-axis slider was fixed after adjusting the field of view to standardize the photographing distance. A drop of five microliters [30] of normal saline was trickled on each implant using a micropipette with a sterile tip. The saline drop was dripped on the implants away from the apical area contaminated with saliva. Autofocusing was done, and the photo was taken. After photo acquisition, the Y-axis slider knob lowered the camera to the next implant for photographing till the end of the first implant-holders-carrying tray. Then the X-axis slider knob moved the camera to the next implant-holders-carrying tray. After taking photos of the 18 implants, the remaining two implants carried on the fourth implant-holders-carrying tray were placed on the photographing tray for photo acquisition with the same fixed distance.

3. Measuring the S-CA

The obtained photos were analyzed by ImageJ software (ImageJ 1.53e, National Institutes of Health, USA) utilizing the Low Band Axis-symmetric Drop Shape Analysis module (LB-ADSA). Each image was renamed following the relevant implant number and then cropped to view the drop with the implant surface and imported to the software. The image type was adjusted to 8 bits. LB-ADSA module was initiated, and the green reference arc was modified to fit the drop. The vertical position of the green arc was adjusted using the slider (Y0) till its summit was aligned with the top of the drop arc. The slider (X0) was then used to adjust the horizontal position till the center of the green arc coincided with the center of the drop arc. The green arc height and width were adjusted using sliders h and b, respectively. (Fig 2a) The measured angle was recorded in the datasheet. S-CAs lower than 90˚ indicate a hydrophilic surface, while those approaching 0˚ indicate superhydrophilic surface characteristics. On the other hand, S-CAs above 90˚ indicate hydrophobic surfaces, and those above 150˚ indicate superhydrophobic surfaces.[30]

UVC exposure

The used UVC source was a UVC lamp with its base (LAVAED UV Lamp, China), emitting rays with 254 nm wavelength and 8-watts power equipped with a built-in 15-minute timer. Specially designed box (34X16X12.5 cm^3) made of compressed black foam board fully lined with mirrors. The lamp base was fixed on the box cover. (Fig 3A-a) This box was lined with mirrors to allow maximum implant surface exposure. The four implant-holders-carrying trays were inserted into the box and exposed twice for 15 minutes each. (Fig 3B)

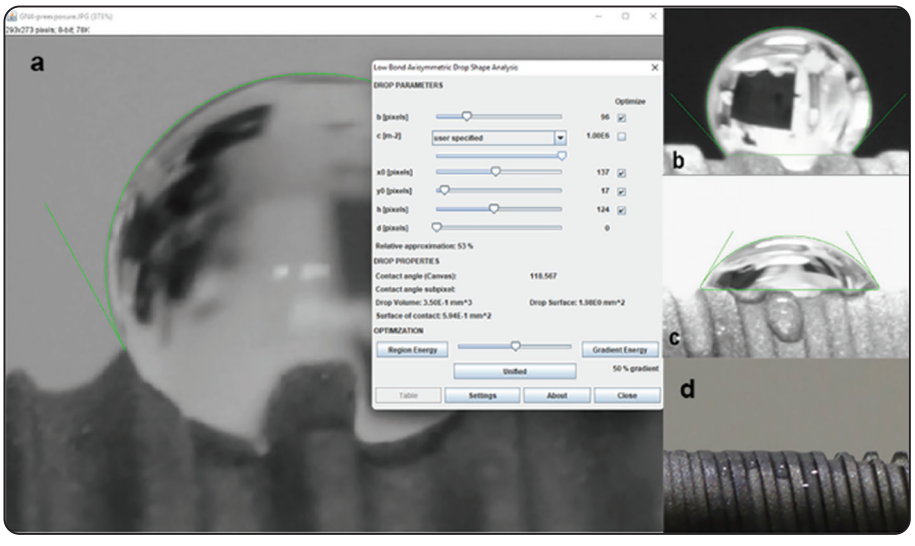

Fig. (2) a. Pre-radiation S-CA measurement (Implant No 15); b, c, and d. Pre-radiation, 1st-Radiation, and 2nd-Radiation-CAs, respectively (Implant No 2).

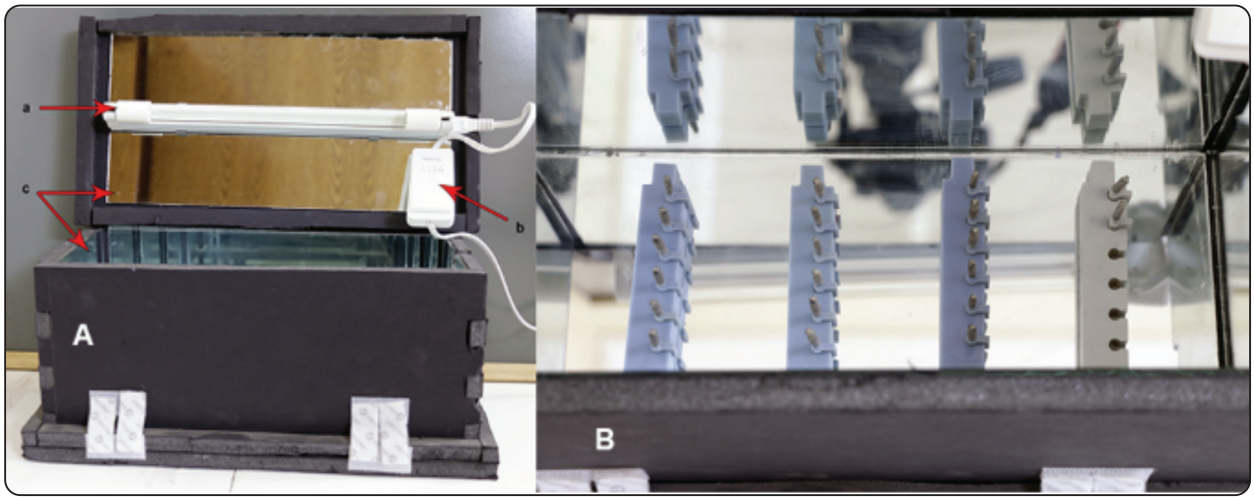

Fig. (3) UVC source: Section A: a. LAVAED UV Lamp attached to the specially designed box cover, b. UVC source built-in timer, c. mirror lining; Section B: the four implant-holders carrying trays with the tested 20 expired implants inside the mirror-lined UVC source-box.

Statistical analysis

Numeric data were presented as mean ± SD using Statistical Package for Social Science (SPSS) software (ver. 25). Shapiro-wilk test was utilized to examin the normality of pre-radiation and post-15 minutes S-CA data. Paired samples T-test was used to indicate a significant statistical difference between the means of S-CA measurements on the three events. Pearson's correlation test was used to examine the association of pre-radiation S-CA measurements to the length of aging. The p-value significance was set at $p<0.05$.

RESULTS

Implants-expiration periods and titanium-aging period

The length of Implants-expiration periods of the tested implants ranged from 22 to 32 months with a mean of 26.35 ± 2.66 months. The titanium-aging period of the tested implants ranged from 84 to 94 months with a mean of 88.35 ± 2.66 months. (Table No 1)

S-CA measurements

All implants showed hydrophobic surface characters in the pre-radiation event with a mean value of 125.47 ± 8.57°. Following the 1st-Radiation, a statistically significant improvement in surface wettability of all implants was indicated with mean S-CAs of 53.20 ± 32.82° (P-Value < 0.001). Thirteen implants (65%) showed hydrophilic surfaces, four implants (20%) showed superhydrophilic surfaces (No 1, 6, 10, and 19), and three implants (15%) showed hydrophobic surfaces (No 4, 9, and 14). After the 2nd-Radiation, all tested implants showed superhydrophilic surface characteristics. (Table No 1) (Fig 2b, c, d)

Shapiro-wilk test indicated normality of the pre-radiation and 1st-Radiation-CA data with P-value of 0.377 and 0.052, respectively. Paired samples T-test indicated a statistically significant difference between mean S-CAs at pre-radiation, 1st-Radiation, and 2nd-Radiation events with P-Value < 0.001 in the three pairs. (Table No 2)

Bacterial culture and antimicrobial effect of UVC

All implants did not show any bacterial growth (Sector I), whereas the saliva contaminated implants showed bacterial growth (Sector II). After exposure to the UVC source for 15 minutes, all implants showed no bacterial growth (Sector III). (Fig 4)

TABLE (1) Implants-expiration periods, titanium-aging periods, and S-CA findings and means ± standard deviations of the studied implants' values.

Implant No	Implants-expiration periods (month)	Titanium-aging period (month)	S-CA °		
			Pre-radiation	1st-Radiation	2nd-Radiation
1	27	89	111.62*	0***	0***
2	27	89	132.21*	59.45**	0***
3	27	89	135.12*	67.98**	0***
4	27	89	128.31*	103.85*	0***
5	27	89	136.02*	60.02**	0***
6	30	92	124.12*	0***	0***
7	22	84	130.72*	52.55**	0***
8	24	86	121.61*	52.2**	0***
9	32	94	126.87*	93.5*	0***
10	27	89	112.87*	0***	0***
11	27	89	139.06*	28.59**	0***
12	25	87	120.47*	48.19**	0***
13	24	86	138.04*	75.32**	0***
14	24	86	135.2*	72.9**	0***
15	23	85	118.57*	71.55**	0***
16	27	89	124.34*	64.38**	0***
17	25	87	116.55*	104.69*	0***
18	25	87	116.77*	58.35**	0***
19	32	94	116.9*	0***	0***
20	25	87	124.08*	50.48**	0***
Mean ± SD	26.35 ± 2.66	88.35 ± 2.66	125.47 ± 8.57*	53.20 ± 32.82**	0 ± 0***

** Hydrophobic surface, ** Hydrophilic surface, and *** Superhydrophilic surface.*

TABLE (2) Paired samples T-test findings of the tested pairs.

Tested pair	T value	P-Value
Pre-radiation-CA and 1st-Radiation-CA	10.489	< 0.001
Pre-radiation-CA and 2nd-Radiation-CA	65.448	< 0.001
1st-Radiation-CA and 2nd-Radiation-CA	7.248	< 0.001

Association of pre-radiation-CAs and the length of titanium-aging periods

Pearson's correlation test indicated no significant association between titanium-aging periods and pre-radiation-CAs with P-Value= 0.657 and Person correlation score = -0.106.

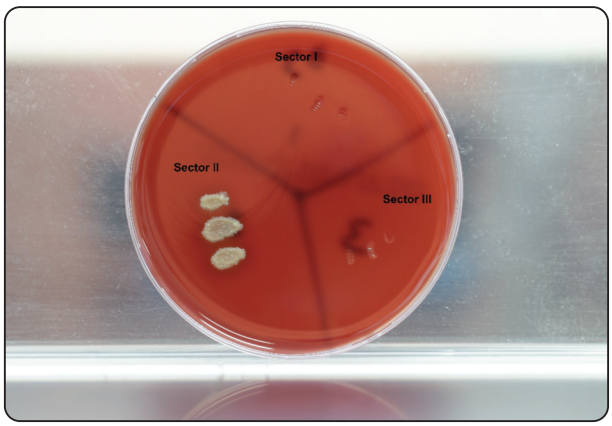

Fig. (1) Bacteriological culture: Sector I (pre-exposure culture) showed no bacterial growth; Sector II (after contamination with saliva) showed bacterial growth; Sector III (after first UVC exposure) showed no bacterial growth.

DISCUSSION

The increased need for dental implantation raised the issue of bypassing the expiratory date of commercially available titanium dental implants. Titanium is a biocompatible material with a nanoscale hydrophilic TiO_2 surface layer[31], which has a crucial role in the osseointegration of the titanium dental implants[2]. Titanium implants that passed their expiratory dates risk TiO_2 surface layer aging[2] besides potential bacterial surface contamination. The current study results indicated the hydrophobic surface characters of expired implants, which were significantly improved following the first UVC exposure and reached the superhydrophilic nature after the second UVC exposure. In addition, the utilized UVC source eliminated bacterial contamination of the experimentally saliva-contaminated implants after the first UVC exposure.

Several studies examined the effect of photofunctionalization on the wettability and surface characteristics of aged titanium implants with shorter titanium-aging periods of 4 to 8 weeks[5,6,17,26,32-34] compared to the current study utilizing implants with a mean titanium-aging period of 88.35±2.66 months from manufacturing. The TiO_2 surface layer aging process leads to progressive loss of titanium wettability[1], affecting the osseointegration[2-4,11-13]. Implant surface hydrophilicity improved the degree of osseointegration[12], implant fixation, and enhanced bone-implant contact[35]. The reduction of the aged titanium surface's wettability could be attributed to the inevitable progressive accumulation of surface hydrocarbon contaminants[1-3,36], which was indicated in one study to be 17.9% to 76.5% on 34 non-expired different implants[37]. However, the effect of wettability on surface protein adsorption showed diversity in the literature. Osteoblasts react with the surface adsorbed proteins not with the TiO_2 layer.[38] On one side, information indicated the importance of wettability regarding protein adsorption. Hydrophilicity directs protein adsorption, bonding strength, and tridimensional conformation.[2-4,36,39] On the other hand, other studies utilizing different chemistries[36] indicated no correlation between titanium surface wettability and surface protein adsorption[7,40]. Yet, increased hydrocarbons deposition reduces wettability[1] and degree of surface protein adsorption[40], and both affect osteoblastic activity[7,8,17,41]. The reduction of osteoblastic activity deteriorates the process of osseointegration[4] and affects long-term success [42].

The most common approach to reveal surface wettability is the S-CA measurements [30,43]. Several devices were utilized in S-CA measurements, such as CA Goniometer[44], automated CA measuring device[6,17], video-based CA system[34], simplified experimental setups using simple camera[45], or even smartphone[46]. The current CA measuring setup allowed for individualized photo acquisition of multiple implants (18 implants) at a fixed distance with minimal manipulation, reducing the risk of contamination. The designed implant-holder granted touchless manipulation of the implants during several bacterial culture sessions. Furthermore, the implant-holders-carrying tray allowed implants' photo acquisition and insertion into the UVC source box without touching, keeping enough spacing for complete UVC exposure of the entire surface of all implants. This setup permitted fine controlled incremental camera vertical movement (in the Y-axis) to take photos of each implant in the holders-carrying tray. Then, the X-axis slider allowed right to left camera movement to switch to the next holders-carrying tray. It also allowed for anterior and posterior movement of the camera in the Z-axis to achieve more zoomed images of the implant with the fixed zoom macro lens. The high-resolution camera with a fixed professional macro lens allowed high-quality image acquisition. S-CA measurement in the current study was done by applying the saline drop away from the saliva contaminated apical third to avoid measurements errors. It was indicated that saliva-contaminated titanium surfaces showed reduced wettability.[26]

In the current study, all tested expired implants showed hydrophobic surface characteristics before exposure to UVC light with mean S-CA measurements of 125.47 ± 8.57°. Several studies revealed different aged titanium S-CA less than 90°[5,17,26,27,34,47-49], indicating hydrophilic surfaces following the wettability categorization system utilized in the current study[30]. However, a study conducted by Gittens et al. in 2013[50] showed higher S-CA measurements of 131± 4° and 157±3°. This study applied autoclave sterilization at 121°C for 20 minutes during samples preparation before measuring the contact angles. Several studies demonstrated that autoclaving alters the surface characteristics of the titanium and renders the surface more hydrophobic.[20,22] The difference in S-CA measurements in the current and previous studies could be attributed to the prolonged aging period in the current study, indicating the progressive effect of titanium aging on the deterioration of the surface wettability characters of titanium implants. However, the present study did not show a statistical association amongst the length of the aging period and the pre-radiation S-CA.

UVC photofunctionalization restores the surface activity of the aged TiO_2 surface layer to levels similar or even higher than fresh surfaces[5], through direct hydrocarbon decomposition[6]. Sequentially, this leads to recovery of surface bioactivity of the aged titanium[51,52], 4-times faster bone-implant integration, and more than 98% bone-implant contact[7]. The current study showed a significant improvement of surface wettability following 15 and 30 minutes of UVC exposure. However, the complete transformation of all implants surfaces to superhydrophilic character occurred following the second exposure. This finding indicated the possibility of recovering surface superhydrophilicity of the expired titanium utilizing UVC photofunctionalization.

In this study, the tested implants kept in intact packaging showed no bacterial contamination, indicating the ability of undamaged packaging to protect the implant from bacterial contamination after expiration. A study done by Worthington in 2005[53] showed similar results. Worthington indicated that the intact implant package keeps the content sterile for years. However, Worthington's study used only one implant with six years expiratory period.

The antibacterial effect of the utilized UVC source was assessed by contaminating the apical third of the implants, followed by exposure to the UVC for 15 minutes. All tested implants did not show any bacterial growth following the exposure. UV exposure produces hydroxy-radicals at the titanium oxide surface, reacting with and diminishing the bacterial population.[23] Several studies indicated the effective antibacterial effect of UV light exposure.[20,21,23,54] However, the tested UV exposure showed variability regarding the used UV types, intensity, and exposure periods among litrature. A study utilized UVC light with 254nm wavelength for 90 mins.[20] Another study utilized 150 watt/set power and 360 to 450 nm wavelengths for 8 seconds on each side.[21] A third study used three different UV types for 10 min.[54] The current study utilized UVC light of 254 nm wavelength and 8-watts power for 15 minutes.

Several questions regarding expired implants still need to be investigated. Biological aging of the titanium implant is a time-dependent process.[1] This time-dependent process was indicated through the higher pre-radiation S-CAs of the aged expired implants revealed in the present study compared to previous studies. However, the current study did not significantly correlate the aging period with the pre-radiation S-CA measurements. This issue requires further investigation to determine the rate of deterioration of surface wettability of the titanium implants over time. Another point to be investigated is the proper UV exposure specifications that could be used to sterilize the potentially contaminated expired dental implants. The current study only examined the antibacterial effect of the utilized UVC light, and other studies tested the sterilization effect of UV with a wide variety of specifications.

CONCLUSION

According to the studied sample, the aging of titanium significantly affects the surface wettability of the expired titanium implants. The utilized UVC light recovered surface superhydrophilicity after 30 minutes of exposure and showed effective antibacterial influence after 15 minutes. Intact packaging prevents bacterial contamination of outdated titanium implants. UVC photofunctionalization is a practical, easy, and cheap method to regain the superhydrophilicity of the expired titanium implant and eliminate potential bacterial surface contamination if utilized for the proper period.

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