Ultraviolet A and Ultraviolet C Light-induced Effect on Titanium Implant Surface

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ABSTRACT
Photofunctionalization of titanium implants results in increasing hydrophilicity without altering the surface topography. Limited research has been done to observe the surface changes following UV irradiation and none have been performed on sandblasted acid-etched (SLA) titanium implants. Thus, this interventional experimental study aimed at assessing the changes in pore diameter on titanium dental implants surfaces following UV irradiation with varying wavelengths through field emission scanning electron microscopy analysis (FESEM). A total of nine fixtures were acquired at random and distributed into three groups. Group A was the control group receiving no radiation, while fixtures in group B were exposed to ultraviolet A irradiation (UVA) and group C to ultraviolet C irradiation (UVC) respectively. Alterations or changes in pore diameter were analyzed on implant surfaces through FESEM and a comparison was made among three groups. Significant changes in pore diameter were identified in all three groups. Moreover, the mean pore diameters on the surfaces of UVC treated implants were significantly larger compared to UVA and control group. In conclusion, UVC irradiation has the ability to increase the pore diameter.

Keywords: Field Emission Scanning Electron Microscopy; Photofunctionalization; Sandblasted acid etched; Ultraviolet A irradiation; Ultraviolet C irradiation.

Efecto inducido por Luz Ultravioleta A y Ultravioleta C en Superficies de Implantes de Titanio

RESUMEN
La fotofuncionalización de implantes de titanio permite aumentar hidrofilicidad sin modificar su topografía superficial. La investigación se ha limitado a observar cambios superficiales luego de irradiación UV y no existen avances para implantes de titanio con arenado y grabado ácido. Así, este estudio experimental de intervención se centró en evaluar los cambios en el diámetro de poro de superficies de implantes dentales de titanio expuestas a irradiación UV con diferentes longitudes de onda, a través del análisis por Microscopía Electrónica de Barrido de Emisión de Campo (FESEM). Un total de nueve piezas de implantes aleatorias se utilizaron como muestra y se distribuyeron en tres grupos. El grupo A fue el grupo de control sin radiación, las piezas del grupo B se expusieron a radiación ultravioleta A (UVA), y las del grupo C a radiación ultravioleta C (UVC), respectivamente. Las alteraciones o cambios en el diámetro de poro de las superficies de los implantes, fueron analizadas por FESEM, y se compararon entre los tres grupos. Se identificaron cambios significativos en el diámetro de poro para cada grupo. En efecto, la media de los diámetros de poro de las superficies de los implantes tratados con UVC, fue considerablemente superior, en comparación con los tratados por UVA y el grupo de control. En conclusión, la radiación UVC es capaz de incrementar el diámetro del poro.

Palabras claves: Microscopía Electrónica de Barrido de Emisión de Campo, Fotofuncionalización, Arenado y Grabado ácido, Irradiación ultravioleta A, Irradiación ultravioleta C.

INTRODUCTION

The implant fixture surface designs perform a vital role in an efficient biological response leading to successful osseointegration between implant and bone. Although most of the commercial implants are made up of pure titanium, but they vary in their designs, surface topography and manufacturing techniques [1]. The physical characteristics of implant fixtures imply a

macroscopic in addition to microscopic and nanometric features [2]. The main goal of inculcating these characteristics is to enhance the surface area, clear up surface contaminants and prevent corrosion in order to decrease the chances of implant failure [3].

The modified implant surface provides greater surface roughness to promote the attachment of osteoblasts, proliferation of osteoblastic cells, and protein absorption, needed to promote bone formation to enhance bone-to-implant contact (BIC) [4-7]. The previous literature inclines towards the fact that an increase in roughness provides more irregularities resulting in more surface area to encourage three-dimensional bone development around implants [8-10]. The smallest particle size provides an appropriate environment for protein absorption, cellular spread, attachment and proliferation resultantly upregulates to higher surface energy [11]. This will permit a stronger mechanical connection between the new developing bone and the implant fixture. Thus, secondary implant stability will increase owing to more BIC resulting in optimal osseointegration [12]. In the past few decades, researchers have developed various implants by modifying surface characteristics like roughness with altering geometry in the whole body of the implant fixture. Surface alterations of different types of implants have been done to attain this feature [13].

Sandblasted acid-etched (SLA) implants are currently the most popular commercial implants because the titanium implant fixtures are firstly blasted by particles to promote abrasion on the surface of implants resulting in macro-roughness. Next, they are subjected to acid-etching to create microroughness on titanium surfaces. In brief, the SLA method results in a blend of macro-pits and micro-pits on the implant surfaces [14].

SLA coated titanium implants demonstrate excellent biological response [13], greater propagation of cellular osteoblasts, and bone growth owing to the existence of both types of pits on the implant fixture [15]. These SLA coated titanium implants are believed to be significantly better, due to more BIC [2]. Nevertheless, commercially available titanium implants, irrespective of their surface characteristics, are extremely reactive, and ultimately deteriorate over a period of time due to absorption of hydrocarbons from the adjoining environment [16]. The increased carbon content on implant surfaces eventually reduces the cellular attachment and delays the process of osseointegration [17]. The surface hydrocarbons content can be decreased using ultraviolet (UV) exposure of implants also termed as photofunctionalization [18]. Ultraviolet (UV) light-induced irradiation of titanium dental implants has achieved significant consideration as a means to enhance the biological activity and osteoconduction of implant fixtures [19] because it re-establishes their wettability, decreasing surface hydrocarbons and increasing surface electrostatic potential [20].

Ultraviolet radiations are further classified as UV - A ($320 < \lambda < 400$ nm), UV - B ($290 < \lambda < 320$ nm) and UV - C ($10 < \lambda < 290$ nm) [21,22], with UVC radiation having the highest intensity. To our knowledge, very few in vitro studies have been done to compare the effects of UVA and UVC irradiation in improving the hydrophilicity on various surface modifications like zirconia, PEEK, grit blasted acid-etched, anatase coating and MAO coated titanium implants [23]. However, different surface modifications showed varying responses in increasing the hydrophilicity and reducing surface hydrocarbons, without causing any changes in surface topography [24,25]. But there is a general concept that UVC radiation shows promising results due to the generation of more hydrophilicity and greater surface energy, thus, leading towards a fact that UVC is better than UVA irradiation [23-27]. Furthermore, in-vitro studies conducted on different commercial titanium dental implants irradiated specifically with ultraviolet-C (UVC) concluded that the implants turned super hydrophilic without causing any changes or compromising the surface topography of the implants [28-30]. But there is no concrete evidence as to which wavelength of ultraviolet radiation was used and

how super-hydrophilicity was achieved without altering the surface topography of the implants. Therefore, this interventional study aimed to assess the changes in pore diameter on the surface of SLA coated titanium implants with varying ultraviolet wavelengths, using field emission scanning electron microscopy analysis (FESEM).

MATERIALS AND METHOD

An interventional experimental study was performed at SEM Laboratory, School of Health Sciences, Health Campus, Universiti Sains Malaysia. The study protocol was approved by Human Research Ethics Committee USM (USM/JEPem/17060290). A total of nine implants (Dio UFII HSA implants, Haeundae-gu, Korea) were obtained for field emission scanning electron microscopy (FESEM) analysis. Purposive (non-probability sampling) was used for this study. The samples were randomly selected and assigned into group A (control group), group B (UVA irradiated implants) and group C (UVC irradiated implants) through a random method (drawing cards). All implants had similar dimensions, 4.0 mm in diameter and 10 mm in length.

Ultraviolet Irradiation

Implants placed in group A were not irradiated and thus were kept as control. However, groups B and C samples were UV-irradiated for 10 min by placing them in a UVACUBE 100 (Honle, Germany) [32]. Group B implants were irradiated with UVA (382 nm, 25 mW/cm2) and group C with UVC radiations (260 nm, 15 mW/cm2). All implants were removed from their plastic/glass casings before placing in the UVACUBE 100 to ensure proper UV exposure, as it is proven that UVA can pass through glass and clouds and alter the chemical bonds, whereas UVC cannot [31].

Scanning electron microscopy analysis

The implants were removed from the chamber after fifteen minutes of exposure to radiation and then placed on a sample holder. The implants were then placed in a sputter coater SCD 050 (Bal-Tec, Liechtenstein) machine with gold coating was deposited on the surface of implant to voltage of 60mA for 120s, during which time a layer to achieve clearer images.

The sputter coated implants were placed in FESEM machine for analysis under 10,000 × magnifications, using an accelerating voltage of 10 kV and 30μm aperture. The images were evaluated by using software (XT microscope control) in a computer system incorporated to the FESEM machine for comparison of surface topography.

FESEM image analysis

Only the coronal third parts of the implants were visualized. Fiji is Just ImageJ software (an image processing package which facilitates scientific image analysis) was utilized to calibrate and analyze mean difference of 20 major independent pores on individual implant fixtures. The scale of 5μm was set on the software corresponding to the requirement of the study. Changes in pore diameters were compared among the three groups, based on previous studies [32].

Error analysis for FESEM

Repeat determinants were performed for FESEM to assess the reproducibility and reliability of previous readings. The pore diameters among the three groups were repetitively verified by other examiners at the same time to validate or confirm the authenticity of the readings.

Statistical analysis

SPSS version 20.0 (SPSS, Inc., Chicago, IL, USA) was employed for the entry of data and its analysis. The mean difference of pore diameter was compared among three groups control, UVA and UVC determined by Kruskal Wallis Test. p-value < 0.05 was considered statistically significant. For pairwise comparison Hodges- Lehman estimate was used as post hoc analysis.

RESULTS

Paired t-test was applied to compare the repeated measures. Cronbach’s Alpha was employed to check

whether the repeated values are coherent with preceding interpretations. The pore diameters were calculated by the principal investigator through Fiji's ImageJ software. The pore diameters were again evaluated by the other investigator and the second readings were also noted. Thus, inter-examiner reliability was assessed, and no significant results were drawn (Table 1). The pore diameters of both UV treated groups were significantly larger than control group, meaning that UVA and UVC irradiation resulted in significant increase in pore diameter in comparison to the control group samples ($p < 0.05$; Table 1). Nevertheless, UVC irradiated implants showed noticeable increase in pore diameter (3.196 µm + 0.168) in contrast to UVA group (2.327 µm + 0.159) as shown in (Table 2).

The group-wise comparisons Hodges-Lehman estimate was used as post hoc analysis that revealed all three groups had significant differences from each other (Table 3). The UVA and UVC had significantly larger pore diameters when compared to the control group ($p < 0.05$). Furthermore, UVC irradiated implants had significantly larger pore diameters as compared to UVA irradiated ones ($p < 0.05$; Table 3).

The FESEM images of SLA coated titanium implants of each group are shown in figure 1 taken under 10,000X magnification.

Table 1. Repeatability measures for FESEM for two readings and reliability on basis of Cronbach alpha.

Measures of FESEM	**Values compared**	**Difference**			**Comparison**			**Reliability 0.640**
		Mean	**SD***	**SE***	***t****	**df***	***p*-value**	**Alpha**
Control	*Reading 1 – Reading 2*	-0.00588	0.01736	0.00224	-2.625	59	0.011	1.00
UVA	*Reading 1 – Reading 2*	-0.01637	0.10009	0.01292	-1.267	59	0.210	0.998
UVC	*Reading 1 – Reading 2*	-0.01663	0.06996	0.00903	-1.842	59	0.071	0.999

***SD:** Standard deviation, **SE:** Standard error, **df:** degrees of freedom, ***t*:** test statistics value for paired *t* test.

Table 2. Comparison of pore diameter among three groups by using Kruskal Wallis Test.

Groups	**N**	**Mean**	**Standard Deviation**	**Standard Error of Mean**	**X^2statistics (df)**	***p* value**
Control	3	1.3482	0.68	0.087	2	< 0.01*
UVA	3	2.3271	1.24	0.160		
UVC	3	3.1962	1.30	0.168		

Table 3. Group-wise comparison among three groups using Hodges - Lehman estimate for post hoc analysis.

Sample 1 – Sample 2	**Test Statistic**	**Standard Error(I-J)**	**Standardized Test Statistic**	***p* value**	**Adjusted *p* value**
Control- UVA	-42.6	9.513	-4.478	< 0.001	< 0.001*
Control- UVC	-76.1	9.513	-8.000	< 0.001	< 0.001*
UVA- UVC	-33.5	9.153	-3.521	< 0.001	0.001*

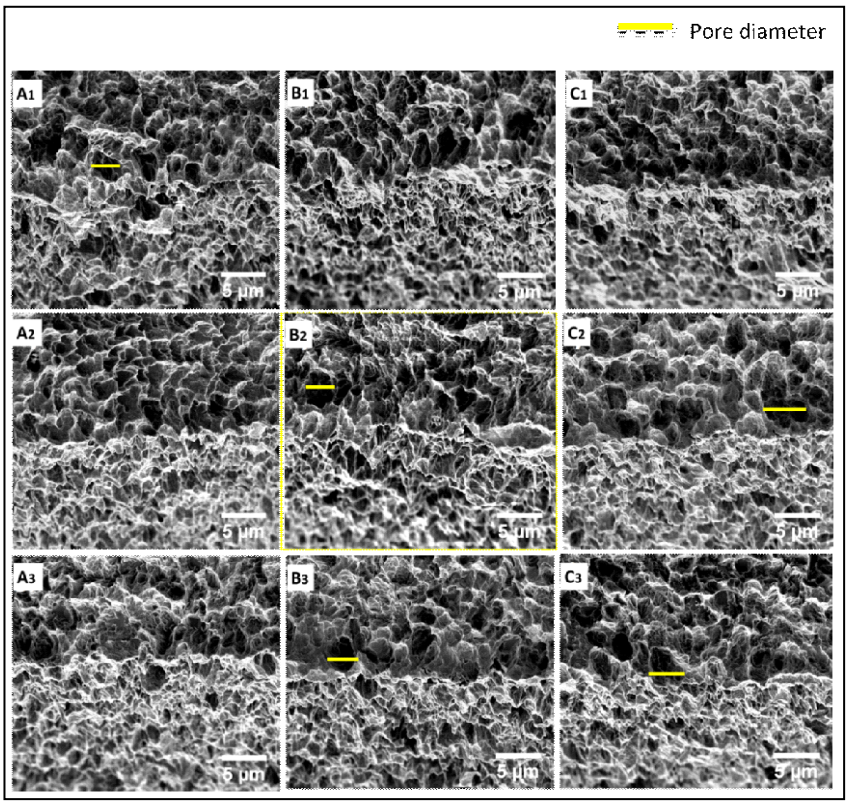

Fig. 1. FESEM of SLA coated titanium implants of three samples for each group; control group (A_1, A_2 and A_3); UVA group (B_1, B_2 and B_3) and UVC group (C_1, C_2 and C_3).

DISCUSSION

Traditionally, standard SLA coated implants were first sandblasted to attain macro roughness, followed up by acid-etching process in order to achieve microroughness [33-35]. Though, the Hybrid SLA coated titanium implants used in this study have both micro and macro-rough surfaces.

Micro-rough surface is observed in the coronal/upper third section of implant with roughness value (Ra) of 0.5 - 1.0 µm, whereas, both macro and micro roughness (ranging from 2.0 - 2.5 µm), can be seen in the lower third/body of the implant. So, for this study, the coronal third of the implant was chosen as the region of interest to evaluate changes in pore diameter after UV radiations because microporous texture at gingival tissue level is necessary to sustain soft tissue stability and aesthetically appealing results [36]. The incorporation and modifications of pits, grooves, and protrusions set the stage for biological responses at the bone-to-implant interface, leading to a rise in surface area and subsequently improving the osseointegration between bone and implant surfaces [7].

In this study, both UVA and UVC irradiations of implants resulted in substantial increase in pore sizes in contrast to non-UV irradiated group, thus showing a shift in physical condition of implant surfaces. On the contrary, majority of the previous studies have stated that only the physiochemical nature of the implant surfaces was enhanced following UV irradiation, without compromising the surface topography or altering the pore size of implants as shown in the FESEM and confocal laser scanning microscope (CLSM) [23,28,29,37]. Neither UVA nor UVC appeared to cause any surface changes even at the nano-scale on the Titanium surface [19,38]. Moreover, it was concluded that there was no change in surface topography of sandblasted acid-etched implants after UVC exposure [30]. Recently it has been reviewed

that no changes in surface roughness have ever been reported following UV treatment. Instead, it induced superhydrophilicity (0 angle) by lowering the concentration of hydrocarbons, which led to increase in proliferation, attachment, and differentiation of osteoblast [39]. These studies suggested that only the physiochemical changes due to UV radiation could enhance the biological activities on the titanium surfaces [20,37].

All the aforementioned findings contrast with our results in which UV radiation did cause changes in pore diameter. Furthermore, UVC irradiation resulted in greater increase in pore size as compared to UVA. Moreover, it has been stated earlier that UVC irradiation has the capacity to induce greater change in the electronic state on the implant surface, when compared to UVA, because it directly decomposes hydrocarbons through a process known as photolysis [40]. Consequently, this increased optimization caused by UVC irradiation might have imposed an increase in pore diameter.

This is a novel finding and the reason for these changes in pore sizes are yet unknown. However, several studies have established that increased surface roughness promotes more attachment and absorption of proteins on the implant surface to perform cellular osteoblastic function [23,41,42]. The outcomes of this research may lead to important scientific contributions for the achievement of desired results in implantology such as bone implant contact. In brief, an ideal surface with the appropriate roughness and mechanical properties might lead to improved osseointegration for successful dental implants.

CONCLUSIONS

Significant changes in pore diameter were identified in all three groups. Moreover, the implants treated with UVC irradiations demonstrated marked increase in pore diameter as compared to the UVA irradiated and the non-irradiated Implants. In conclusion, UVC irradiation has the ability to increase the pore diameter and this technique will ultimately provide more surface area for successful osseointegration. The differences in the results from previous studies may have been caused by the types of implants used, difference in experimental conditions, intensity, and wavelength of the UV generator. Further study is necessary to reveal this aspect. The surface topography can be evaluated through both FESEM and confocal laser scanning microscope (CLSM), to validate the results.

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