ABSTRACT: Zirconia has become an excellent choice of dental implants because of its excellent mechanical strength, aesthetic, and biocompatibility. Although some studies have shown ultraviolet (UV) irradiation is effective to photofunctionalize dental zirconia that can improve osteoblastic function, the scattered information has not identified the most effective exposure time and wavelength of UV. Herein, this study has investigated the effects of UV irradiation on zirconia after UV-A (365 nm) or UV-C (243 nm) photofunctionalization for different times (15 min, 3 and 24 h). After irradiation, the zirconia surface was analyzed by color spectrophotometry, scanned electron microscopy (SEM), energy-dispersive X-ray spectrometry, water contact angle (WCA) with goniometer, and X-ray diffraction. Osteoblastic (MC3T3-E1) cells were cultured on zirconia discs and evaluated with a CCK-8 test kit for cell proliferation (3 h and 1 day) and with alkaline phosphatase (ALP) activity (14 days). Significant color change ($\Delta E$) was observed by irradiating with UV-C for 15 min (1.99), 3 h (1.92), and 24 h (3.35), whereas only minute changes were observed with UV-A (respectively, $\Delta E$: 0.18, 0.14, 0.57). No surface textural changes were observed nor a monoclinic phase was detected on both the UV-A and UV-C irradiated samples. UV-C significantly decreased the C/Zr ratios and WCA, with irradiating for 24 h presenting the lowest values, and it was the only condition to give significantly higher ALP activity at 14 days ($p < 0.05$) and CCK-8 values for 1 day culture ($p < 0.05$). It is concluded that UV-C (but not UV-A) irradiation can significantly change the aesthetic in color, and only prolonged 24 h UV-C irradiation can enhance MC3T3-E1 cell adhesion on zirconia by photofunctionalization.

INTRODUCTION

Zirconia is a crystalline oxide form of zirconium which can present in three phases: (1) monoclinic phase, (2) tetragonal phase, and (3) cubic phase. The tetragonal phase is stable between the temperatures of 1170–2370 °C with suitable mechanical properties that are desirable for biomedical use.\textsuperscript{1,2} In order to obtain tetragonal zirconia polycrystals (TZP) at room temperature, commonly 3 mol % yttria is added\textsuperscript{3} in dental zirconia and these TZP have shown outstanding performance in terms of mechanics, biocompatibility, and aesthetics.\textsuperscript{4,5} Because of its qualities of excellent mechanical performance, strength, and fracture resistance,\textsuperscript{6} TZP may be a potential alternative to titanium (Ti) as an implant material. The osseointegration capability and durability of TZP implants has been reported to be similar to that of Ti implants.\textsuperscript{7,8}

Initial attachment, proliferation, and differentiation of osteoblasts at the implant–bone interface play an important role in the early stages of osseointegration.\textsuperscript{6} Surface characteristics are very essential in the early stages of osseointegration, such that the process of protein adsorption, initial attachment, proliferation, and differentiation of osteoblasts would be influenced at the implant–bone interface.\textsuperscript{9–11} Various in vitro studies reported that adsorption characteristics of cell attachment can be affected by surface wettability. High surface...
wettability is generally recognized to promote more cell attachment than low surface wettability.\textsuperscript{12–14} In fact, a smaller static water contact angle (WCA) indicates higher wettability, whereas a previous study\textsuperscript{15} has shown that a smaller WCA would yield higher cell attachment of human gingival fibroblasts on different dental implant abutment materials. The effect has not been diminished; even the average-arithmetic roughness ($R_a$) was less than 0.2 $\mu$m.\textsuperscript{16} This said, static WCA is an important parameter to estimate cell adhesion on materials.

Ultraviolet (UV) radiation wavelength (100–400 nm) is classified as UV-A (320–400 nm), UVB (290–320 nm), and UV-C (100–290 nm) according to the dermal biological actions of UV radiation.\textsuperscript{16} Photochemical modification (a.k.a. photofunctionalization) by UV irradiation was reported for zirconia implant materials. Machined zirconia surfaces after UV photofunctionalization would increase surface hydrophilicity and enhance the initial attachment of osteoblast cell, and this process was sometimes claimed as “bioactivation”.\textsuperscript{15,17} Hence, UV-induced bioactivation might represent a promising method to improve surface chemistry of zirconia-based implant materials.\textsuperscript{18}

Previous studies demonstrated that UV photofunctionalization at the mixed-wavelength of 360 and 250 nm for as little as 15 min can accelerate healing and increase bone-to-implant contact,\textsuperscript{19} although the shortest irradiation time may be as short as 10 min.\textsuperscript{20} Some other studies set the time of UV light irradiation to be 2 h at the intensity of 19 mW cm\textsuperscript{-2},\textsuperscript{21,22} and even mixing two UV wavelengths at 0.05 mW cm\textsuperscript{-2} in intensity (360 nm in wavelength) and 2 mW cm\textsuperscript{-2} in intensity (250 nm in wavelength) to photofunctionalize the zirconia surface.\textsuperscript{23} At present, most of the investigations concerning the impact of photofunctionalization on wettability and osseointegration of zirconia have achieved satisfying results. However, UV lights can change the aesthetics of zirconia, such that light yellow color was observed on zirconia surfaces after UV-C irradiation.\textsuperscript{24} Furthermore, no study has been conducted to correlate the most effective exposure time (such as a prolonged exposure) and wavelength of UV light. Therefore, studies are needed to solve these problems and identify the most effective approach for zirconia osseointegration.\textsuperscript{16}

This study had two objectives: the first was to analyze the surface characteristic of zirconia after dynamic UV-A and UV-C irradiation. The second was to compare the dynamic osteoblast attachment and proliferation behavior on the zirconia surface. The hypothesis of the study was that the surface characteristic of zirconia will not be changed after dynamic UV-A and UV-C irradiation. Furthermore, the osteoblast attachment and proliferation behavior on the zirconia surface will be the same with different UV treatments. We expect to discover the proper UV irradiation wavelength and time, thus achieving relevant possibilities for zirconia implants for improved osseointegration.

\section*{MATERIALS AND METHODS}

\subsection*{Zirconia Disks Preparation.} Zirconia disks from Wieland (Zenostar T0, Ivoclar Vivadent, Jagst, Germany) were used in this study. The composition of the Zenostar T0 was $\text{ZrO}_2$, $\text{HfO}_2$, $\text{Y}_2\text{O}_3$, $\text{Al}_2\text{O}_3$, and other oxides. The percentage of $\text{Y}_2\text{O}_3$ was between 4.5 and 6 wt % (2.6–3.5 mol %). The zirconia blocks were cut into square-shaped specimen ($\sim 9.0 \times 9.0 \times 1.0 \text{ mm}^3$) using a precision saw (IsoMet 5000, Buehler, Lake Bluff, Illinois, USA) with a diamond blade. The samples were polished with 1000-grit SiC abrasive paper before sintering according to the manufacturer instructions. Then, all fully-sintered zirconia specimens were ultrasonically cleansed in 70% ethanol solution and de-ionized water for 15 min and dried in clean ambient air. The polished specimens without any further modification were used as control.

\textbf{UV Photofunctionalization.} The zirconia samples were randomly divided into three study groups and treated with one of the following surface modification protocols (Table 1). UV-

| irradiation | UV irradiation time | groups |
|------------|--------------------|-------|
| no (control) | NA | control |
| UV-A | 15 min | UV-A 15 min |
| | 3 h | UV-A 3 h |
| | 24 h | UV-A 24 h |
| UV-C | 15 min | UV-C 15 min |
| | 3 h | UV-C 3 h |
| | 24 h | UV-C 24 h |

A irradiation (UV fluorocence cabinet CL 150, SPECTROLINE, Westbury, New York, USA) was 365 nm in wavelength and 550 $\mu$W cm\textsuperscript{-2} in intensity, whereas UV-C irradiation (UV fluorescence cabinet CL 150, SPECTROLINE, Westbury, New York, USA) was 243 nm in wavelength and 490 $\mu$W cm\textsuperscript{-2} in intensity. After UV treatment, the zirconia samples were immediately subjected to the following experimental evaluations.

\textbf{Surface Characteristics of Zirconia Surface. Color Change.} The colors of zirconia surfaces before and after UV treatment in different groups were assessed by a color spectrophotometer (NR10QC, 3 nh, Shenzhen, China) at three different positions on three independent samples. The color change value ($\Delta E$) $L^*a^*b^*$ was calculated according to the following formula

$$
\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}
$$

where $L^*$ stands for lightness, $a^*$ for green-red ($-a^* = green; +a^* = red$), $b^*$ for blue-yellow ($-b^* = blue; +b^* = yellow$).

\textbf{Scanning Electron Microscopy and Energy Dispersive X-ray Spectrometry.} A scanning electron microscope (SU1510, HITACHI, Ibaraki, Japan) was used to observe the surface morphology of zirconia surfaces before and after UV irradiation. The samples were gold sputtered for 45 s before observation and three images were randomly taken for each sample. The magnification was at 1000X. Three scanning electron microscopy (SEM) images were obtained from three independent samples, and that were further accessed by two independent viewers. For elemental compositions, on the three zirconia specimen for each group, carbon and zirconium on the zirconia surface were analyzed by energy dispersive X-ray spectrometry (EDX) three times in different areas.

\textbf{Water Contact Angle.} Static contact angle was determined using the sessile drop method (optical contact angle and interface tension meter, SL200KB, KINO Industry, Boston, Massachusetts USA). Ultrapure type 1 water was used as probe liquid. The syringe needle was positioned 5 mm above the zirconia surface and a drop of the test liquid (1 $\mu$L) was dispensed. Photographs were taken after droplets impacted on the zirconia surface and were in equilibrium situation for 20 s, using the built-in software (CAST3.0; KINO, Norcross, GA,
USA). Three zirconia specimens of each group were tested, and the contact angles for each specimen were measured and calculated from three drops of the liquid droplets. Each test was repeated three times.

X-Ray Diffraction Analysis. X-ray diffraction (XRD) (Rigaku SmartLab, Tokyo, Japan) examination was used to analyze the changes of surface crystalline structure on three specimens for each group. The scanning was carried out within the $2\theta$ range between 20 and 80° at a speed of 4°/min with a voltage of 45 kV and a current of 200 mA. The result was analyzed by the software Jade 6.5 (Materials Data, Inc., Livermore, California, USA).

Biological Study. Osteoblastic Cell Culture. Murine pre-osteoblastic MC3T3-E1 cells (ATCC, Manassas, Virginia, USA) in passage number of P20 to P50 were grown in alpha-modified Eagle’s medium ($\alpha$-MEM) (Gibco, Carlsbad, California, USA) supplemented with 10% (v/v) fetal bovine serum (Hyclone, Logan, Utah, USA) and 1% (v/v) penicillin/streptomycin (Invitrogen, Carlsbad, California, USA). The cells were cultured at 37 °C with 5% CO$_2$ in the humidified incubator. At 80% confluence, MC3T3-E1 cells were trypsinized and seeded onto zirconia samples of the seven independent test groups (shown in Table 1) for respective time-points of 3 h ($n$ = 3) and 1 day ($n$ = 3) at a density of $8 \times 10^4$ cells/well in a 24-well plate.

Cell Counting Kit-8 (CCK-8) Test. Initial attachment of cells was evaluated by measuring the quantity of the cells attached to zirconia substrates for each group after 3 h and 1 day incubation. These quantifications were performed using Cell Counting Kit-8 (CCK-8, Model: CK04, Dojindo Molecular Technologies, Tokyo, Japan). In brief, the original cell culture medium in each well was discarded at certain time points, 300 μL of CCK-8 solution was then added, immersing the zirconia samples, and incubating with the samples for 3 h. Absorbance of the supernatant was measured at 450 nm by a SpectraMax M2 microplate reader (Molecular Devices, Sunnyvale, California, USA). There were three replicates for each group.

Alkaline Phosphatase Activity. MC3T3-E1 cells were first seeded in 24-well plates at a density of $8 \times 10^4$ cells/well in standard medium (the same medium as described in the Osteoblastic Cell Culture section). For osteogenic differentiation, an osteogenic medium that contains 50 μg/mL L-ascorbic acid (Sigma-Aldrich, St. Louis, Missouri, USA) and 10 mmol/L β-glycerophosphate (Sigma-Aldrich) supplemented in $\alpha$-MEM was used. For each group, three zirconia specimens were used. One zirconia specimen was put into each well and cultured for 14 days. The medium was changed every 3 days. At day 14, the cells were lysed by Triton X-100 (Anaspec Inc., Fremont, California, USA). Then, the cell differentiation was determined by measuring the level of Alkaline Phosphatase (ALP) activity using the ALP Assay Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The solution would show a yellow-colored product, and the absorbance was measured at 405 nm by a SpectraMax M2 microplate reader. ALP activity was normalized to the protein concentration of cellular lysates.

Statistical Analyses. The data were analyzed by Statistical Package for Social Science (SPSS, Version 23, IBM, Armonk, New York, USA). The statistical analysis was performed using one-way analysis of variance (ANOVA) at a significance level ($\alpha$) of 5%, that is, $p < 0.05$ was considered to be statistically significant.

Figure 1. Zirconia samples irradiated with UV-A or UV-C with respect to different times (15 min, 3 and 24 h). NB: UV-C irradiation caused color changes in the samples. SEM images of surface morphology and EDX analysis.

Figure 2. Results of SEM examination (original magnification 1000×) on zirconia surfaces. There is no observable surface texture change.
**RESULTS**

**Color Change.** The color change values ($\Delta E$) $L^*a^*b^*$ after UV-A irradiation for 15 min, 3 and 24 h were, respectively, 0.18, 0.14, and 0.57. The color change which was not visually observable was detected on UV-A irradiation. The values of $\Delta E$ after UV-C irradiation for 15 min, 3 h, 24 h were, respectively, 1.99, 1.92, and 3.35, such that the color changes from white to light yellow were visually observable (Figure 1).

Figure 2 shows the SEM images of the zirconia surface before and after UV irradiation in different groups. All tested zirconia specimens have similar surface morphology in all groups after different UV treatments, such that small pits and shallow grooves from the polishing procedures could be seen. EDX results (Table 2) show that the C/Zr ratio decreased significantly after UV-C irradiation at all times. [Different superscript letters indicate Significant differences ($p < 0.05$)]

![Figure 3. Results of XRD analysis on zirconia surface after UV-A and UV-C irradiations for different time (T represents tetragonal phase zirconia)](Image)

Table 2. Values of the C/Zr Ratio of the Zirconia Samples in Different Groups by EDX

| groups     | C/Zr ratio (average ± SD) | WCA (average ± SD) |
|------------|---------------------------|---------------------|
| control    | 0.45 ± 0.09$^a$          | 70.7 ± 0.3$^c$      |
| UV-C 15 min| 0.33 ± 0.06$^b$          | 49.6 ± 0.3$^d$      |
| UV-C 3 h   | 0.35 ± 0.03$^b$          | 52.8 ± 3.6$^e$      |
| UV-C 24 h  | 0.37 ± 0.05$^b$          | 17.3 ± 1.1$^f$      |
| UV-A 15 min| 0.45 ± 0.04$^g$          | 54.4 ± 2.4$^g$      |
| UV-A 3 h   | 0.45 ± 0.13$^h$          | 60.1 ± 2.6$^i$      |
| UV-A 24 h  | 0.45 ± 0.10$^h$          | 58.3 ± 2.7$^i$      |

$^a$C/Zr ratio decreased significantly after UV-C irradiation at all times. Significantly different among groups by EDX.

Water Contact Angle. The WCA of the zirconia surface decreased significantly after 15 min (49.6°), 3 h (52.8°), and 24 h (17.3°) UV-C irradiation compared to the control group (70.7°) ($p < 0.05$). Upon UV-A irradiation, there was a slight decrease compared to the control group ($p < 0.05$); WCA values, respectively, were 54.4, 60.1, and 58.3° (Table 2).

**XRD Analysis.** In Figure 3, it is shown that only a tetragonal (T) phase structure could be detected on the zirconia surfaces of all groups. No monoclinic (M) phase was detected in the zirconia samples in all groups after UV-A and UV-C irradiation treatments.

**CCK-8 Test and ALP Activity.** The CCK-8 test demonstrated no significant difference on the proliferation of MC3T3-E1 cells on zirconia surfaces in different groups at the culture time of 3 h. However, after culturing for 1 day, a significantly higher MC3T3-E1 cell proliferation in Group UV-C 24 h was found, compared to the other six groups ($p < 0.05$) (Figure 4A). The ALP activity test after MC3T3-E1 cells cultured on zirconia surfaces for 14 days showed that significantly higher ALP activity was found in group UV-C 24 h compared to the control group ($p < 0.05$). However, no significant difference of ALP activity was found between all of the other groups after culturing for 14 days (Figure 4B).

**DISCUSSION**

This laboratory study investigated the effect of time-dependent dynamic UV irradiation process on the color, surface characteristics, and MC3T3-E1 osteoblast cell response of osteoblasts to zirconia-based surfaces.

One of the differences after UV-A and UV-C treatments observed was the color change values ($\Delta E$) $L^*a^*b^*$ detected in group UV-C and group UV-A, based on color assessment by the color spectrophotometer. The color change values ($\Delta E$) $L^*a^*b^*$ after UV-C irradiation were much larger than those after UV-A irradiation on the zirconia specimen for all time points. Clinically speaking, the discoloration may affect the aesthetic performance of zirconia. Thus, it should be taken into consideration if UV, in particular UV-C, irradiation is used on zirconia for the aesthetic aspects. CIE (Commission Internationale de l’Eclairage), the organization that creates international light and color standard, introduced the main color systems, color difference ($\Delta E$) concepts, and illumination patterns applied in color science. $\Delta E$ has been the standard assessment for total color difference between two objects; a greater value indicates larger color difference and, consequently, more perceptible difference to the human eye. The $\Delta E$ value that could be observed by the naked eye was reported to be approximately 1.74–1.80 color units by 50% perceptibility threshold. In another study, the mean $\Delta E$ that could be observed by the naked eye was 2.72 color units by 50% acceptable threshold. Thus, the color change by UV-A is not visually observable ($\Delta E = 0.18–0.57$), whereas that by UV-C, in particular to prolonged exposure, is easy to be seen ($\Delta E = 1.92–3.35$).

The color change can be explained by change of optical band gap ($E_g$). In this study, the dental white zirconia contains 3 mol % of yttria which has the band gap energy, $E_g$ of 4.60–4.99 eV. Upon UV irradiation, free electrons would be
generated in zirconia with a range of energies by absorption of the UV radiation energy, and subsequently become ionized.31 The photo-energy equation is as follows

\[ E_g = \frac{hc}{\lambda} \]  

(2)

where \( E_g \) is the energy for a given wave type \( \gamma \), \( \lambda \) is the wavelength, \( h \) is the Planck’s constant \((6.626 \times 10^{-34} \text{ J s})\), and \( c \) is the speed of light \((2.998 \times 10^8 \text{ m/s})\). In this study, \( \lambda \) of UV-A is 365 nm, that means \( E_{UV-A} \) is 3.397 eV; \( \lambda \) of UV-C is 243 nm, so the corresponding \( E_{UV-C} \) is 5.102 eV. The photon-energy of UV-C is higher than that of UV-A such that \( E_{UV-A} \) was lower than the \( E_g \) but \( E_{UV-C} \) was higher than \( E_g \). Thus, UV-C can excite valence electrons to conduction band, and charge transfer could happen from O\(^{-}\) to Zr\(^{4+}\).32 Accordingly, at the very proximal zirconia surface <0.5 nm, the oxides would be ionized and cleave the Zr–O bonds. Such a process can lead to: (1) creation of color centers by increasing the number of O deficiency that decreases the \( E_g \) after UV irradiation. Consequently, the white zirconia no longer absorbs UV only but also become yellowish and absorbs violet and/or blue colors. This may also be the underlying reason for the discoloration phenomenon of zirconia-containing resin composites after UV irradiation.33 It is also worth noticing that a sufficiently high number of O deficiency can significantly decrease the \( E_g \) and turn zirconia into black, that is absorb all visible lights;34 (2) together with the parallel reaction of charged oxygen species and the hydrolysis in the atmosphere, the number of –OH and hydrophilicity increase. Therefore, all UV-A and UV-C irradiated groups in this study have lower WCA than the control.

It is interesting to notice that the WCA on zirconia samples after UV irradiation for 15 min is significantly lower than that after UV irradiation for 3 h, in the treatment groups of both UV-A and UV-C. The exact reason remains unknown, but possibly because the UV-A and UV-C irradiations are both effective on chemical activity of oxygen, that is, oxygen becomes charged and makes this treatment induce oxidation on the zirconia surface. However, this effect could only be shown for the initial stage, and it would not give further improvement after 1 h.35 Furthermore, the lowest WCA was found in group UV-C 24 h of 17.3 ± 1.1°, which means a highly hydrophilic surface condition. The result was consistent with a previous study,36 which demonstrated obvious decrease in the contact angle on implant surface after 40 min UV-C irradiation.

As determined by SEM, the overall morphology of the zirconia surface did not seem to be affected by sterilization treatments used in this study. This result was consistent with the findings of previous studies,35,36 where similar zirconia surface morphology before UV irradiation and after UV irradiation treatment were observed. Unlike some destructive surface treatment methods,37–39 for example grit-blasting, hydrofluoric acid (HF) etching, and laser ablation, the UV treatment is a nondestructive surface treatment method that only alters the surface chemistry and does not induce morphological change.

EDX analysis showed that the C/Zr ratio decreased significantly after UV-C irradiation at all time points of 15 min, 3 and 24 h compared to the control group, but not for UV-A groups. Zirconia surface can easily adsorb any carbon species35 as compared to other dental ceramics. Study has shown that UV irradiation could induce the removal of adsorbed hydrocarbons and other carbonaceous species on titanium dioxide (TiO\(_2\)) by photo-oxidation and/or by the photo-induced production of surface oxygen vacancies resulting in a higher reactivity of the titanium surface, which then is susceptible for dissociative water adsorption.19 In this model, UV light should have high enough energy (i.e., larger than the band gap of TiO\(_2\), 3.2 eV) to create surface oxygen vacancies at bridging oxygen sites, resulting in the conversion of relevant Ti 4p sites to Ti 3p sites, which are favorable for dissociative water adsorption.40 As aforementioned, in this study, UV-C has higher photon energy than UV-A that excited the valence electrons to conduction. Therefore, UV-C irradiation can assist the direct photolysis of carbon contaminations on zirconia surface and increased hydrophilicity of the zirconia surface. It should be further stressed.

Figure 4. (A) OD\(_{450}\) value of the CCK-8 test in different groups of zirconia after culturing with MC3T3-E1 cells for 3 h and 1 d. (B) ALP activity in different groups of zirconia after culturing with MC3T3-E1 cells for 14 days. * denotes statistical significant (\( p < 0.05 \)) difference between different groups among the same time point.
that not all UV irradiation can induce this or can be representable as a method to improve surface chemistry of zirconia.

According to the XRD results, zirconia samples in our study only contained a tetragonal (t) phase structure in all of the groups. This result was consistent with a previous study which revealed that no evidence of zirconia phase transformation was observed in any zirconia. This result was consistent with our previous study,46 which showed that no evidence of tetragonal (t) to monoclinic (m) phase transformation of zirconia was detected in zirconia after being treated by UV irradiation for 30 min on each side of zirconia.46

The other aim of this study was to evaluate the effects of UV-A and UV-C irradiation for different times on MC3T3-E1 proliferation and differentiation ability on zirconia surface. UV light irradiation has been shown to promote initial osteogenic cell attachment, proliferation, and early bone apposition on titanium implant surfaces.42,43 In our current study, according to the CCK-8 test assessing the proliferation ability of MC3T3-E1 on zirconia surface, significantly higher MC3T3-E1 cells proliferation in group UV-C 24 h was shown compared to the other groups. Group UV-C 24 h was shown in this study to have the lowest WCA (i.e., highest hydrophilicity). Thus, this pivotal result is influential in protein/surface interactions after implant insertion, followed by subsequent cell/surface interactions. In fact, hydrophilic surfaces have been proposed to positively affect the biological reaction, such that it can increase fibronectin adsorption and osteoblastic cells adhesion and spreading during the initial stage of osseointegration.17 Therefore, this may be reason for the highest MC3T3-E1 cell proliferation in group UV-C 24 h on the zirconia surface after culturing for 1 day. Moreover, no significant difference on the proliferation of MC3T3-E1 cells on zirconia surfaces in different groups was observed at the culture time of 3 h. The underlying reason may be that the culture time was not long enough to observe the difference of MC3T3-E1 proliferation ability between different groups.

According to the ALP result, significant difference of ALP activity after culturing for 14 days was found between group UV-C 24 h and group control. The ALP activity of MC3T3-E1 improved significantly on the zirconia surface after UV-C irradiation for 24 h. Despite the exact reason remains unknown, UV-C indeed created the imbalanced oxygen stoichiometry on zirconia which triggered the electric field44,45 and sent out electrical signals46 that fostered electron and proton transports across biological membranes.47 On the osteoblast membranes, some channels such as gap junction channels which are responsible for different molecules (e.g., calcium, cyclic nucleotides, and inositol phosphates) exchange are sensitive to mechanical and electrical transductions.48,49 Various forms of titanium dioxide have shown to have different effects of the gap junction channels,50 and it is logical to think that zirconia might have similar electrical effects on the channels. Although inhibition of the channels would decrease ALP and osteocalcin levels,51 and vice versa,52 the exact proof on zirconia has not been found. Further study is necessary.

Previous studies applied different UV light irradiation time and wavelengths on implant surfaces, which make the results different when compared with each other and draw a clear conclusion. For example, it was stated that photofunctionalization for as little as 15 min could accelerate healing and increases bone-to-implant contact.19 Another study applied the exposure time of 40 min in total.17 Some other studies used the UV irradiation time of as long as 2 h,6,20−22 As such, there is no consensus about what are the most effective time exposure and UV wavelength values that could effectively produce the best surface for osseointegration. Nevertheless, in this study, the biological aspects of the zirconia surface on MC3T3-E1 proliferation and differentiation were shown to be improved by UV-C irradiation on dental zirconia for 24 h; however, this irradiation should be cautiously used because the color may change. Therefore, this could be useful for manufacturing implant body (screw part) but not the abutment or crown that has a high requirement of aesthetics.

The limitation of this study was that limited time points of UV irradiation were applied in the test. More comprehensive time points should be added in the future study. In addition, X-ray photoelectron spectroscopy (XPS) test may be applied to show chemical changes of zirconia induced by UV light in the future study.

## CONCLUSIONS

In conclusion, the color, surface free energy, and surface chemistry of zirconia changed after both UV-A and UV-C treatments. Prolonged 24-h UV-C photofunctionalization on zirconia can enhance MC3T3-E1 bioactivity but compromise the color aesthetic.

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