ORIGINAL ARTICLE

Effect of sandblasted large grit acid etching and ultraviolet-c photofunctionalization of titanium on osteoblast cell viability: an in vitro study

Andreas Tjandra[1]
Kwartarini Murdiastuti[2]
Ahmad Kusumaatmaja[3]
Heni Susilowati[4]

[1]Doctoral Study Program of Dental Sciences, Faculty of Dentistry, Gadjah Mada University, Yogyakarta
[2]Department of Periodontics, Faculty of Dentistry, Gadjah Mada University, Yogyakarta
[3]Department of Physics, Faculty of Mathematics and Natural Sciences, Gadjah Mada University, Yogyakarta
[4]Department of Oral Biology, Faculty of Dentistry, Gadjah Mada University, Yogyakarta

Correspondence:
kmurdiastuti@ugm.ac.id

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ABSTRACT

Introduction: Sandblasted Large Grit Acid Etching (SLA) is a widely used titanium implant surface modification technique that enhances osseointegration. Ultraviolet C (UV-C) photofunctionalization is a method that modifies the physicochemical properties of titanium surfaces by removing hydrocarbon contaminants and increasing hydrophilicity. However, the combined effects of these modification techniques on MC3T3-E1 osteoblast cell viability require further investigation. Therefore, this study aimed to analyze the effect of SLA treatment and its interaction with UV-C irradiation on MC3T3-E1 osteoblast cell viability on titanium disks. **Methods:** This *in vitro* experimental study used titanium disks (10 mm in diameter and 1 mm in thickness), which were divided into SLA-treated and non-SLA groups. Each group was further subdivided according to UV-C irradiation (90 W, 254 nm) into four subgroups: non-UV (control), 10 minutes, 30 minutes, and 48 hours (n = 4 per subgroup). MC3T3-E1 osteoblasts (1.2×10^4 cells/well) were seeded onto disk surfaces and cultured for 24 hours. Cell viability was assessed using the MTT assay. Data normality was evaluated using the Shapiro-Wilk test, homogeneity using the Brown-Forsythe test, and differences between groups using two-way ANOVA, with statistical significance set at $p < 0.05$. **Results:** Two-way ANOVA revealed that UV irradiation had no significant effect on cell viability (F=2.388; p=0.093). Similarly, SLA treatment showed no significant effect (F= 1.679; p = 0.207), and no significant interaction was observed between SLA treatment and UV-C irradiation (F=0.041; p=0.988). The highest mean cell viability was observed in the non-SLA group exposed to UV-C irradiation for 10 minutes (88.53 ± 25.22), whereas the lowest mean cell viability was found in the SLA group without UV-C irradiation (45.55 ± 16.29). **Conclusion:** Neither UV-C irradiation nor SLA treatment significantly affected MC3T3-E1 osteoblast cell viability, and no significant interaction was observed between the two treatments.

KEYWORDS

Acid etching, dental, ultraviolet rays, osteoblasts, cell survival, titanium

INTRODUCTION

Tooth loss represents a significant oral health problem that negatively affects quality of life. According to the 2019 Global Burden of Disease (GBD) report, more than 44.5% of the global population is affected by oral disorders, including tooth loss, with low- and middle-income countries bearing a disproportionate share of the burden.[1] Titanium dental implants have become the preferred treatment modality for tooth replacement because of their superior biocompatibility, bioinertness, and corrosion resistance.[2]

The success of dental implants is largely determined by the quality of osseointegration, defined as the direct bond between the implant surface and the

alveolar bone. This process involves a series of biological events, beginning with protein adsorption, followed by osteoblast adhesion and proliferation on the implant surface. One of the major challenges in implantology is titanium aging, a phenomenon in which the surface of a titanium implant gradually becomes hydrophobic due to the progressive deposition of hydrocarbons from the atmosphere after manufacturing. This alteration can reduce the bioactivity of the implant surface.[3]

To address these issues, two titanium surface modification strategies have been developed: (1) topographic modification using the Sandblasted Large Grit Acid Etching (SLA) technique and (2) physicochemical modification using ultraviolet (UV) photofunctionalization. The SLA technique produces micro-rough surfaces through a sandblasting process with alumina (Al_2O_3) particles followed by immersion in a mixed acid solution (H_2SO_4 and HCl), creating a topography that has been shown to increase surface roughness, implant-bone contact area, and osteoblastic differentiation in vitro compared to smooth surfaces.[4] Implants with SLA surfaces reportedly have a 97–98% success rate over a 10-year period.[5]

Photofunctionalization, which involves ultraviolet (UV) irradiation within a wavelength range of 100–400 nm, is recognized as an effective method for enhancing surface properties without altering the surface topography.[6] Compared to untreated titanium surfaces, UV photofunctionalization has been shown to achieve nearly 100% bone-to-implant contact and approximately threefold greater mechanical anchorage strength in animal models.[7]

UV-C photofunctionalization (254 nm wavelength) operates through a photolysis mechanism that decomposes hydrocarbon compounds on the surface of titanium dioxide (TiO_2), thereby altering the surface properties from hydrophobic to superhydrophilic and shifting the surface charge from electronegative to electropositive. Studies conducted on murine pre-osteoblasts (MC3T3 cell line) have demonstrated that UV-C irradiation of titanium surfaces enhances protein adsorption, adhesion, and proliferation, and stimulates osteoblastic differentiation of osteoblast cells.[3, 8, 9] Furthermore, Huang et al. (2022) demonstrated that UV-C exposure for 1/6 hour, 1/2 hour, and 1 hour on a fixture implant significantly increased Bone-Implant Contact (BIC) and bone volume in a beagle animal model.[10]

MC3T3-E1 cells were selected as the in vitro model due to their advantages, including unlimited numbers, homogeneous characteristics, and their ability to differentiate phenotypically from pre-osteoblasts to mature osteoblasts, closely resembling the conditions of human cells.[11] The MC3T3-E1 cells used in this study represent a relevant and validated in vitro model for the study of osteoblast cell viability.

MC3T3-E1 is a well-characterized subclone derived from the parental MC3T3 calvarial cell line, selected for its consistent and robust osteoblastic differentiation and mineralized matrix formation, which distinguishes it from other MC3T3 subclones with limited osteogenic potential; these properties make MC3T3-E1 one of the most widely used and reliable in vitro osteoblast models. This is in line with Izumiya et al., who reported that the MC3T3-E1 mouse calvaria-derived preosteoblast cell line has made a significant contribution to the study of osteoblasts' function in bone formation and remains one of the most commonly used cell lines for assayed cell viability under varying culture media conditions.[12] These cells possess the ability to differentiate phenotypically from pre-osteoblasts to mature osteoblasts and exhibit alkaline phosphatase (ALP) activity, which serves as a reliable osteogenic marker.[13] In addition, measurement of viability using the methyl thiazolyl tetrazolium (MTT) method is the gold standard for quantitative evaluation of cell viability or the number of metabolically active or living cells in a sample, in which tetrazolium salts are reduced to produce insoluble purple formazan crystals and quantified at a wavelength of 570 nm. The resulting absorbance value is directly proportional to the number of viable cells.[14]

Studies evaluating the combination of SLA and UV-C treatments with shorter irradiation time variations on titanium disks using MC3T3-E1 osteoblast cells as a model are still limited, particularly those directly comparing short-duration UV-C exposure (in the range of minutes) with prolonged exposure on the same SLA-treated surface; this gap in the literature constitutes the primary rationale. The novelty of this study lies in the evaluation of the interaction between UV-C irradiation durations of 10 minutes, 30 minutes, and 48 hours alongside a non-UV control on both SLA-treated and non-SLA titanium disks using MC3T3-E1 cells as an in vitro osteoblast model, providing a direct comparative assessment of short versus prolonged UV-C exposure in the context of osteoblast cell viability that may serve as a scientific basis for optimizing UV-C photofunctionalization protocols for dental implant surface modification. Therefore, this study aims to analyze the effect of SLA treatment and its interaction with UV-C irradiation on MC3T3-E1 osteoblast cell viability on titanium disks.

METHODS

This study employed an in vitro laboratory experimental design. A total of 32 titanium disk samples (10 mm in diameter, 1 mm in thickness) (Shandong Zehao Metal Materials Co. Ltd., Shandong, China) were prepared by polishing with sandpaper, followed by ultrasonic cleaning in acetone, 70% alcohol, and sterile distilled water for 15 minutes each. The samples were subsequently air-dried at room temperature for 1 hour. The titanium disks were divided into two surface types: SLA-treated and non-SLA. The SLA group was sandblasted with 50 µm alumina (Al_2O_3) particles, followed by acid etching in a mixture of H_2SO_4 (8 mL) and HCl (4 mL) for 30 minutes. After etching, the disks were rinsed with distilled water and dried in a vacuum autoclave at 134°C and under 2 bar pressure. In contrast, the non-SLA group did not receive any surface modification treatment. The sterilized titanium disks were then allowed to age in implant tubes and stored in a dark room for 30 days.

After the aging period, titanium disks from each group (SLA and non-SLA) were placed in a closed UV irradiation chamber and exposed to UV-C light (TUV 254 nm, Philips, Amsterdam, Netherlands) at an intensity of 5 mW/cm², 90 W power, and maximum wavelength of 254 nm for varying durations: 10 minutes, 30 minutes, and 48 hours. Based on the combination of surface treatment and irradiation duration, the samples were divided into eight experimental groups (n=4 biological replicates per group): Non-SLA Non-UV, Non-SLA UV 10 minutes, Non-SLA UV 30 minutes, Non-SLA UV 48 hours, SLA Non-UV, SLA UV 10 minutes, SLA UV 30 minutes, and SLA UV 48 hours.

The sample size was calculated according to the method by Ihwah et al. (2018), in which the number of treatment groups (t) multiplied by the number of replications per group (n-1) must be greater than or equal to 15. In this study, the factorial design consisted of eight treatment groups derived from the combination of four UV-C exposure durations and two surface modification types ($t = 8$). Substituting into the inequality, eight multiplied by $(n-1)$ must be greater than or equal to 15, which simplifies to $8n-8 \geq 15$ and $8n \geq 23$, yielding a minimum n of 2.875, rounded up to 3 replicates per group. The number of replicates was increased to n=4 per group to improve statistical reliability, resulting in a total of 32 titanium disk samples.[15]

For cell culture, MC3T3-E1 osteoblast cells were obtained from the European Collection of Authenticated Cell Cultures (ECACC) (catalogue number 99072810). The cells were cultured in α-Minimum Essential Medium (α-MEM 1x + GlutaMAX™, Gibco, Thermo Fisher Scientific Inc., MA, USA) supplemented with penicillin-streptomycin, amphotericin B (Fungizone, Gibco, Thermo Fisher Scientific Inc., MA, USA), and fetal bovine serum (FBS, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), at 37°C with 5% CO_2 and incubated for 24 hours until confluence was achieved.

The MC3T3-E1 cells used in this study were at passage 36. Cell suspensions (1.2×10^4 cells/well) were prepared in 24-well plates containing pretreated titanium disks and incubated for 24 hours. The culture medium was then replaced with 0.01 mL of methyl thiazolyl tetrazolium (MTT) solution and incubated for another 4 hours at 37°C until MTT particle reduction occurred, resulting in the formation of opaque, black-colored crystals at the bottom of the well, indicating viable cells. After incubation, 100 µL of MTT assay samples of culture medium containing formazan was transferred to a 96-well plate for measurement. Subsequently, 100 µL of isopropanol with 0.04 N HCl was added to each well and mixed thoroughly.

Cell viability was measured within 1 hour using a spectrophotometer (Multiskan Sky, Thermo Fisher Scientific Inc., MA, USA) at a wavelength of 570 nm and a temperature of 25°C. The percentage of cell viability was calculated using the formula described by Ghasemi et al.[16] Cell viability percentage = (Q-Y)/((Z-Y)) × 100%, where Q represents the mean absorbance of the treatment group, Y represents the mean absorbance of the cell-free medium, and Z represents the mean absorbance of the untreated cell control.

Statistical analyses were performed using GraphPad Prism version 10.6.1 (GraphPad Software Inc., San Diego, CA, USA). Data from each group were assessed for normality using the Shapiro-Wilk test and for homogeneity of variances using the Brown-Forsythe test prior to parametric analysis. As the assumptions of normality and homogeneity of variance were fully met, the effects of surface modification and UV irradiation duration on osteoblast cell viability were analyzed using two-way ANOVA. Statistical significance was set at $p<0.05$ for all analyses.

RESULTS

Figure 1 presents the mean and standard deviation of MC3T3-E1 osteoblast cell viability values on two types of SLA and non-SLA surface modifications, following UV-C treatment.

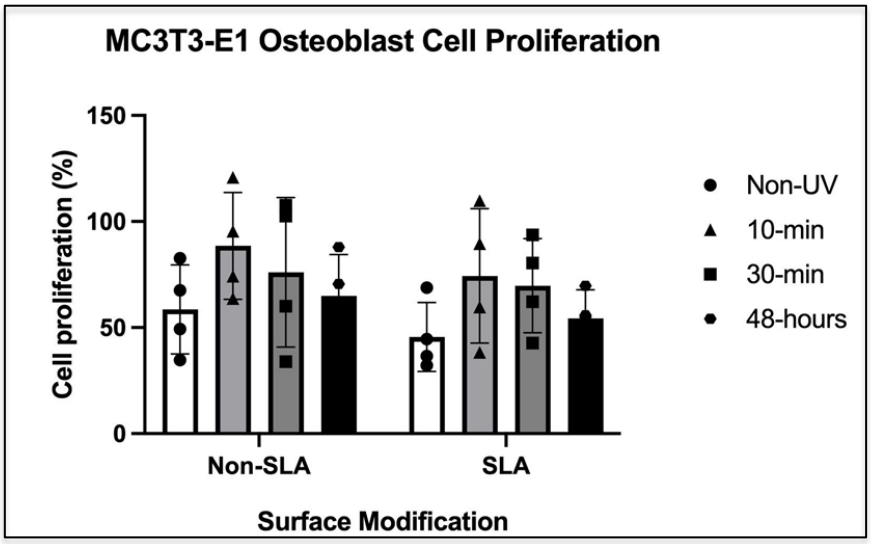

Figure 1. MC3T3-E1 osteoblast cell viability (%) on titanium disk surfaces with two surface modifications (SLA and non-SLA) across four UV-C irradiation durations (non-UV, 10 minutes, 30 minutes, and 48 hours). Data are presented as mean ± SD (n = 4 biological replicates per group).

A 10-minute UV-C exposure produced the highest mean cell viability for both surface types, reaching 88.53 ± 25.22% in the non-SLA group and 74.36 ± 31.65% in the SLA group. In contrast, the non-UV groups demonstrated the lowest viability values, particularly on the SLA surface (45.55 ± 16.29%), whereas the corresponding non-SLA surface showed a mean viability of 58.58 ± 20.96%.

Furthermore, longer UV-C irradiation durations (30 minutes and 48 hours) showed a declining trend, although these values remained higher than those of the non-UV group. Overall, osteoblast cell viability appeared descriptively higher in the UV-irradiated groups than in the non-UV (control) groups for both non-SLA and SLA surfaces, although these differences were not statistically significant. These findings suggest that, in the absence of UV treatment, non-SLA surfaces are more conducive to osteoblast cell viability. In contrast, SLA surfaces without UV exposure exhibited the lowest viability levels among all experimental groups.

The Shapiro–Wilk normality test performed on the residuals indicated that the data were normally distributed. Brown-Forsythe's test for homogeneity of variances also revealed that the variances among groups were homogeneous ($F = 1.621$; p-value = 0.177; p-value > 0.05), thus fulfilling the assumptions for the application of parametric statistical tests of two-way ANOVA as presented in the following Figure 2.

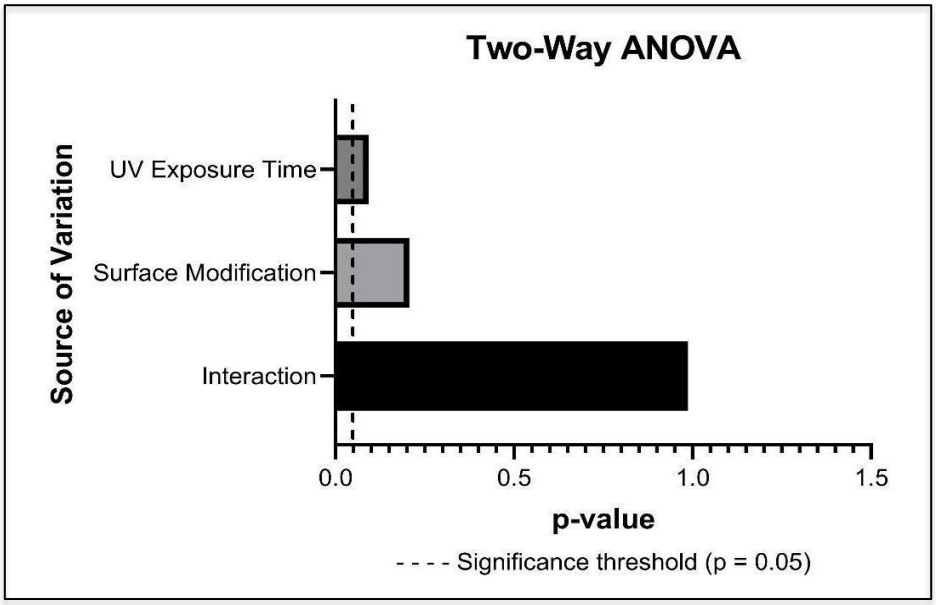

Figure 2. Two-way ANOVA results for MC3T3-E1 osteoblast cell viability, showing p-values for each source of variation: UV-C irradiation duration, surface modification, and their interaction. The dashed line indicates the significance threshold (p = 0.05). None of the tested sources of variation reached statistical significance.

The two-way ANOVA results (Figure 2) demonstrated no statistically significant differences in osteoblast viability among treatment groups with respect to UV-C irradiation duration (p-value = 0.093; p-value > 0.05), surface modification (p-value = 0.207; p-value > 0.05), or their interaction (p-value = 0.988; p-value > 0.05). There was considerable variability in the data, as reflected by the high standard deviations, which may have contributed to the absence of statistically significant differences among the variables examined in this study.

DISCUSSION

Figure 1 illustrates the findings of the present study, which showed a trend toward increased osteoblast cell viability with increasing UV-C irradiation duration. Separately, the Shapiro–Wilk test confirmed that the viability data in each group were normally distributed (Figure 2), satisfying the assumption required for parametric analysis. However, the two-way ANOVA results indicated that UV-C irradiation duration, surface modification type, and their interaction did not have statistically significant effects on MC3T3-E1 osteoblast cell viability. These findings are consistent with those of Jeon et al., who reported that the proliferation of MG-63 human osteoblast-like cells was unaffected by UV treatment of titanium surfaces.[17]

The absence of statistical significance for UV-C irradiation duration ($p = 0.093$), as shown in Figure 2, may be attributed to the non-linear biological effects of UV treatment on titanium disk surfaces. Cell viability increased from the non-UV groups to 10 minutes of UV-C exposure but subsequently declined at 30 minutes and 48 hours of exposure, suggesting that the biological response of osteoblasts to UV-C does not increase proportionally with longer exposure duration.

During the early stages of healing, osteoblast migration and viability significantly influence the outcome of titanium bone integration. The chemical composition and surface topography of biomaterials play a critical role in determining the extent of bone–implant contact. The morphology and initial attachment of primary human alveolar osteoblasts are not significantly affected by bioactivation through UV photofunctionalization.

The hydrophilic status of the material surface is considered a marker of surface energy and has been shown to affect protein adsorption and facilitate cellular attachment and interaction. An optimal biological response across the spectrum of water–protein–cell interactions is therefore highly dependent on these surface characteristics.[18]

UV photofunctionalization is known to reduce carbon or hydrocarbon contamination, enhance surface hydrophilicity, and improve the bioactivity of titanium.[7] While these mechanisms are conducive to cellular responses, their effects may reach a saturation point, beyond which further increases in irradiation time (e.g., from 10 minutes to 30 minutes or 48 hours) do not produce sufficiently distinct or statistically significant differences.

There is a potential for re-adsorption of carbon contaminants, which can re-deposit from the surrounding air onto the surface of the titanium disk and restore its hydrophobic characteristics.[19] In addition, limited light penetration and incomplete surface coverage of specimens may make UV-C treatment less effective in achieving uniform and long-lasting hydrophilicity, which may, in turn, adversely affect cell viability.[20]

The results of this study also indicate that the SLA treatment alone did not produce a statistically significant difference in the viability of MC3T3-E1 osteoblast cells compared with the non-SLA group ($p = 0.207$) (Figure 2). This finding is consistent with that reported by Cho et al., who evaluated the same MC3T3-E1 mouse preosteoblast cell line. They observed only slightly higher cell viability and proliferation on machined titanium surfaces than on SLA-treated surfaces.[21]

At first glance, the finding appears to contradict a growing body of literature reporting the superiority of SLA surfaces in promoting osteoblast responses. However, this discrepancy may be explained through several perspectives. First, osteoblast cell viability is a hierarchically regulated process that is influenced more by surface physicochemical properties such as surface charge and hydrophilicity than by topographic roughness alone.[22]

In the context of the present study, although descriptively, UV-C irradiation appeared to influence surface physicochemical properties more than SLA treatment, this observation was not supported by statistically significant differences in cell viability. As a result, the additional contribution of SLA treatment may have become statistically undetectable.

Second, in short-term (24-hour) in vitro studies, SLA-induced surface roughness appears to play a more prominent role during the early stages of cell adhesion than in cell viability.[22] This finding is in line with Lee et al., who reported that the machined + UV group demonstrated greater cell proliferation than the SLA without UV group.[23]

Another comparative study evaluating four levels of titanium surface roughness likewise found no statistically significant differences in osteoblast viability or cytotoxicity among the different roughness levels during the early culture period. Instead, cell viability was influenced primarily by culture duration rather than surface roughness itself.[24]

The absence of a significant interaction between SLA treatment and UV-C irradiation (p=0.988), as shown in Figure 2, indicates that the effects of these two factors were independent, with no evidence of a synergistic effect under the conditions of this study. This finding is supported by Roy et al., who evaluated UV-C photofunctionalization on both machined and SLA titanium surfaces. They reported that the titanium surface topographies remained remarkably similar following the modification processes.[25] Although the combination of SLA and UV-C irradiation is theoretically expected to produce an optimal surface (topographically rough and physicochemically active), the present findings suggest that UV-C irradiation exerts a greater influence on 24-hour osteoblast cell viability, whereas SLA treatment does not provide significant additional benefit.

Descriptively, the most notable observation of this study was the time-dependent effect of UV-C irradiation on cell viability. Although this effect was not statistically significant, it is consistent with the findings of Guo et al., who specifically investigated the impact of UV treatment duration on MC3T3-E1 cells. They reported that growth factor expression and cellular viability peaked after 12 minutes of UV exposure, whereas prolonged exposure did not provide additional benefits. These findings support the concept that the biological response of osteoblasts to UV irradiation is time-dependent rather than linear.[26] Similarly, Suzumura et al. reported that methylene blue decomposition reached its maximum after 20 to 30 minutes of UV-C exposure.[27]

Furthermore, as illustrated in Figure 1, UV-C irradiation for 10 and 30 minutes descriptively appeared to increase MC3T3-E1 cell viability compared with the non-UV control, although these differences were not statistically significant. Likewise, the 48-hour UV-C group demonstrated higher cell viability than the non-UV group. This pattern is consistent with the dose-dependent mechanism of UV-C photolysis. During short-duration exposures of 10 and 30 minutes, UV-C effectively degrades the hydrocarbon layer on the TiO_2 surface and induces a shift in surface charge from electronegative to electropositive, thereby enhancing the adsorption of osteogenic proteins and facilitating cell proliferation.[18]

The reduced cell viability observed after 48 hours of UV-C exposure may be explained by several mechanisms. First, prolonged UV-C irradiation has the potential to induce oxidative stress in cells through the excessive generation of reactive oxygen species (ROS), which become cytotoxic at high concentrations. ROS contribute to bone loss by promoting osteoclastogenesis through activation of RANKL-dependent signaling pathways while simultaneously suppressing osteoblast differentiation and mineralization.[28] ROS-induced bone resorption may occur either directly or indirectly through the modification of kinase activity and transcription factors in both osteoclasts and osteoblasts.[29]

Second, 48 hours of UV-C exposure may cause photodegradation of culture medium components (FBS and MEM), which are essential for maintaining cell viability. Third, the rebound effect, namely the re-adsorption of hydrocarbons from the atmosphere onto titanium surfaces that have been photofunctionalized for an extended period before cell testing, may also reduce surface bioactivity. It is inevitably formed on titanium surfaces over time.[3] These findings align with those of Huang et al., who reported that shorter UV-C exposure durations produced improvements in osseointegration comparable to those achieved with longer exposures.[10]

These findings have several significant implications for the development of more effective and clinically applicable dental implant surface modification protocols. From a clinical standpoint, chairside UV treatment for 10 to 30 minutes is a realistic and time-efficient approach that can be incorporated into standard implant placement procedures without requiring substantial modifications.

In contrast, 48-hour UV treatment, which is more commonly used in laboratory settings, is impractical for clinical application and, according to the findings of the present study, does not provide additional proliferative benefits over shorter exposure durations. In addition, both SLA-treated and non-SLA

titanium surfaces may benefit from UV-C photofunctionalization without requiring a specific topographic precondition. Because of its flexibility, short-duration UV-C photofunctionalization may be applied to a greater variety of implant surface types that are currently used in clinical settings.

Further studies evaluating other cellular parameters, such as osteoblastic differentiation and mineralization, are needed to further validate these findings. Third, the MTT assay employed in this study is used to assess cell viability; this limited the ability of the present findings to directly reflect osteoblast proliferative activity. A recent study demonstrated that the MTT assay may yield increased metabolic activity readouts without a corresponding increase in actual cell proliferation.[30] Future studies are therefore recommended to employ proliferation-specific assays, such as BrdU, together with multiple time points (e.g., 20 minutes, 1 hour, and 6 hours) to evaluate cell attachment, as well as 24, 48, and 72 hours to more accurately assess osteoblast cell proliferation.

Nevertheless, several limitations of this study should be acknowledged. First, the relatively high within-group variability of the data, reflected by the large standard deviations and the small sample size (n=4 per group), likely reduced the statistical power of the analysis and limited the ability to detect significant differences among UV irradiation durations. Future studies should therefore include larger sample sizes to improve reliability and reproducibility of the findings. Second, in vitro studies do not fully represent the in vivo biological conditions involving factors such as vascularization, immune responses, and three-dimensional bone-matrix interactions.

CONCLUSION

Neither UV-C irradiation duration, surface modification treatment, nor their interaction had a statistically significant effect on MC3T3-E1 osteoblast cell viability. Descriptively, cell viability tended to be highest following 10 minutes of UV-C exposure, although this trend was not statistically significant. The findings of this study suggest that, from a clinical standpoint, chairside UV-C treatment for 10 to 30 minutes is a realistic and time-efficient approach that can be incorporated into standard implant placement procedures, as it produced the highest descriptive cell viability without requiring prolonged exposure. Furthermore, this study provides a preliminary scientific basis that both SLA-treated and non-SLA titanium surfaces obtain similar benefits from UV-C photofunctionalization without requiring a particular topographic precondition.

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