Original Article

Effect of UV-Photofunctionalization on Bioactivity of Titanium to Promote Human Mesenchymal Stem Cells

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ABSTRACT

Background and Aim: The present study introduces photofunctionalization as a technique for tackling biological aging and increasing the bioactivity of titanium. This in-vitro study evaluated the effects of ultraviolet (UVC) light treatment of titanium surfaces with different time-related changes on the behavior and function of human mesenchymal stem cells (MSCs).

Materials and Methods: MSCs were cultured on nine untreated titanium surfaces (four-week-old surfaces; group 1), nine fresh UVC-treated surfaces (immediately after UV treatment; group 2), and nine three-day-old UVC-treated surfaces (group 3). Cellular proliferation and attachment were measured by MTT. Alkaline phosphatase (ALP) was assessed by total protein extraction, and the enzyme activity was evaluated using a special ALP kit. One-way analysis of variance (ANOVA) and post-hoc Tukey's test were used to examine the effect of UVC on titanium surfaces.

Results: The mean attachment of MSCs to titanium disks in groups 1 to 3 was 0.118±0.003, 0.103±0.007, and 0.155±0.009, respectively. The mean proliferation of MSCs in groups 1 to 3 was 0.229±0.004, 0.189±0.023, and 0.298±0.020, respectively. The proliferation and attachment in group 3 were significantly higher compared to other groups ($P<0.05$). Speeds of MSCs growth in groups 1 to 3 were 94%, 81%, and 92%, respectively. The ALP activity of MSCs in groups 1 to 3 was 0.153±0.003, 0.187±0.003, and 0.161±0.003, respectively. The ALP activity in group 2 was significantly higher compared to other groups ($P<0.05$).

Conclusion: UVC pretreatment of titanium surfaces increases the ALP activity. However, cellular attachment and proliferation were not increased in the present study due to the high probability of laboratory error.

Keywords: Ultraviolet Rays, Cell Adhesion, Cell Proliferation, Mesenchymal Stem Cells, Photochemistry, Titanium, Radiation Effects

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Introduction:

Improving the osseointegration capability of dental implants remains a persistent challenge in oral implantology. The characteristics of titanium surfaces are a critical factor for achieving optimal bone-titanium integration.[1] The average bone to implant contact (BIC) has been reported to be between 50% and 70% in previous research.[1,2] However, four-week-old titanium surfaces (stored for four weeks after manufacture preparation process to allow sufficient aging) had less than 50% of BIC with decreased osteogenic cell attachment and proliferation compared to new surfaces.[3] The time-dependent biological degradation of titanium surfaces has been defined as the biological aging of titanium.[1]

The lack of hydrophilicity, the progressive accumulation of hydrocarbons, and the electrostatic positivity were considered as factors that induce this biological aging.[2,3]

Ultraviolet (UV) photofunctionalization (UV-C and UV-A) has been introduced as a technique to enhance the biocompatibility of titanium and to overcome the biological aging by removing hydrocarbon contaminants.[4,5] UV-A irradiation (Wavelength: 315-400 nm) induces decontamination and hydrophilization.[4] UV-C irradiation (wavelength: 100-280 nm) increases the BIC, implant stability, bone growth, initial attachment of osteoblast-like cells and super-hydrophilic surfaces and decrease healing time for UV-C-treated titanium compared to untreated surfaces.[4-11]

Some studies have focused on the influence of UV light treatment on bioactivity and osteoconductivity of titanium surfaces. Aita et al assessed the influence of UV light pretreatment (UV-C and UV-A) of titanium surfaces (without different time-related changes) on the behaviors of human mesenchymal stem cells (MSCs).[12]

This study evaluated the effects of UVC light treatment of titanium surfaces with different time-related changes (untreated surfaces (four-week-old surfaces), fresh UVC-treated surfaces (immediately after UV treatment), and three-day-old UVC-treated surfaces) on the behavior and function of human MSCs.

Materials and Methods

Preparation of titanium surfaces:

Commercial grade 5 pure titanium disks with SLA surfaces (12 mm in diameter and 1 mm in thickness) were supplied by Medentika Co., Germany. All disks were kept in dark ambient conditions for four weeks for appropriate aging. Twenty-seven titanium samples were randomly allocated to three groups using a balanced block randomization list. Untreated titanium surfaces (four-week-old surfaces) in group 1 were considered as control. The disks in groups 2 and 3 were UVC photofunctionalized using a 15-W lamp (Philips, Eindhoven, Netherlands) at 1 mW/cm2 (360±20 nm) and 2 mW/cm2 (250±20 nm) for 60 minutes under atmospheric conditions.

The samples in group 2 (fresh UVC-treated surfaces) were used immediately for cell culture, and the disks in group 3 (three-day-old UVC-treated surfaces) were stored in the dark for three days before cell culture.[3]

Mesenchymal stem cell culture:

Human MSCs (Pasteur Institute, Tehran, Iran) were cultured using MSC growth medium containing MSC basal medium and growth supplements. The growth supplements consisted of fetal bovine serum (FBS), L-glutamine, and penicillin/streptomycin. The cells were put for incubation in a humidified atmosphere of 95% air and 5% carbon dioxide (CO2) at 37°C. After 2-4 passages, the cells were cultured using MSC osteogenic induction medium containing MSC basal medium and an osteogenic induction supplement consisting of FBS, L-glutamine, penicillin/streptomycin, ascorbate, and β-Glycerophosphate. At 80% confluency of the last passage, cells with and without osteogenic induction were separated using Trypsin 0.25 %/ 1 mM EDTA 4Na and were seeded onto titanium disks at a density of 3×104 cells/cm2. The culture medium was refreshed every three days.[13,14]

Cell attachment assay:

The initial attachment of the cells was evaluated by measuring the quantity of MSCs attached to titanium substrates after 24 hours of incubation. These quantifications were performed using MTT colorimetric assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) in which the culture wells were put for incubation at 37°C for four hours with 100 µl of tetrazolium salt WST-1 reagent. The optical density of formazan dissolved in dimethyl sulfoxide (DMSO) was determined using an enzyme-linked immunosorbent assay (ELISA) reader set at 420 nm.[14]

Proliferation assay:

The proliferation potential of the stem cells was evaluated using MTT assay incorporated during DNA synthesis after 72 hours. Following a three-day cell seeding, 100 µl of MTT solution was placed in each well and incubation was performed for 10 hours. For dissolution of formazan, DMSO solvent was added. The ELISA reader was used to read the optical density at 420 nm.[14,15]

ALP assay:

Through total protein extraction at the 14th day, using RIPA lysis buffer, the ALP activity of human MSCs cultured in the osteogenic induction medium was studied. For colorimetry, ALP activity was evaluated as the amount of p -nitrophenyl phosphate released through the enzymatic reaction. The 20-µl protein solution was added to 100 µl of ALP kit, and the optical density of the solution was measured using the ELISA reader at 405 nm after 30 minutes of enzymatic activity.

Statistical analysis:

The sample size was calculated based on a previous trial using one-way analysis of variance (ANOVA) and by considering α=0.5, β=2, standard deviation (SD)=2, and effect size=1.58.[16] The minimum sample size was estimated to be 27 samples (nine disks for each group and three disks for each assay). One-way ANOVA was used to examine the effect of UVC treatment on titanium surfaces. Post-hoc Tukey's test (HSD) was used for multiple comparisons. P-values lower than 0.05 were considered statistically significant.

Results:

The mean attachment of MSCs on titanium disks of groups 1 to 3 was 0.118±0.003, 0.103±0.007, and 0.155±0.009, respectively. The attachment rate of cells in group 3 (three-day-old UVC-treated surfaces) was significantly higher compared to other groups (P<0.05). However, these values in groups 1 and 2 were not significantly different (P=0.160).

The mean proliferation of human MSCs in groups 1 to 3 was 0.229±0.004, 0.189±0.023, and 0.298±0.020, respectively. The proliferation in group 3 was significantly higher compared to the control group (30%) and group 2 (57%; P<0.05). However, these values in groups 1 and 2 were not significantly different (P=0.109).

The ALP activity of human MSCs in groups 1 to 3 was 0.153±0.003, 0.187±0.003, and 0.161±0.003, respectively. The ALP activity in group 2 (fresh UVC-treated surfaces) was significantly higher compared to group 3 (16%) and group 1 (22%; P<0.05). However, these values in groups 1 and 3 were not significantly different (P=0.07).

Human MSC growth rates in groups 1 to 3 were 94%, 81%, and 92%, respectively. This rate was calculated according to the number of cells after 24 hours of culturing (attachment) and at three days after culturing (proliferation).

Discussion

This study demonstrated that the attachment and proliferation of human MSCs on UVC-treated surfaces (stored for three days) were significantly higher compared to the other two groups. Speed of MSC growth on UVC-treated surfaces (immediately) was significantly lower compared to the other groups. However, MSC growth rates in groups 1 and 3 were not significantly different. The ALP activity on UVC-treated surfaces (immediately) was significantly higher compared to the other groups.

The present outcomes showed that UVC light treatment did not increase cellular attachment, proliferation, and speed of human MSC growth. Enhanced cellular attachment and proliferation in three-day-old UVC-treated surfaces may be related to human and laboratory errors related to the number of cells placed on the disks. However, the MSCs adherent to the fresh UVC-treated surface have been considered to differentiate faster than the other groups. The angulation and the distance of the pipette from the titanium disk as well as the speed of pouring cells on the disk were important factors associated with the number of cells seeded on the disks. Therefore, reliable and reproducible techniques such as an automated method for pouring MSCs on the titanium disk are recommended for future research.

Previous studies have evaluated the effect of UV light treatment on the bioactivity and osteoconductivity of titanium surfaces.[12,15-17] The following outcomes are in agreement with our results regarding cell differentiation (ALP activity) and in disagreement with the results related to cell attachment and proliferation. Aita et al stated that UV light treatment significantly improved the titanium surface affinity to MSCs and enhanced cellular migration, attachment, and spread.[12]

Also, proliferation and differentiation of MSCs were significantly increased compared to untreated surfaces.[12,15-17] Minamikawa et al demonstrated that the number of cells attached to UV-treated surfaces after three hours of incubation was not different compared to untreated surfaces after 24 hours of incubation (an 8-fold acceleration in the attachment of cells).The biological and osteoconductive enhancements were also shown in their study.[15,16]

Att et al demonstrated that the attachment capacity, proliferation, and ALP activity of bone marrow-derived osteoblastic cells were higher on UVC-treated four-week-old surfaces compared to new surfaces (immediately after sandblasting or acid-etching).[17] Also, Shen et al have shown that UV irradiation could efficiently remove hydrocarbon contamination and improve the bioactivity of titanium surfaces regardless of the storage medium (air or water).[18] However, this time-dependent biological degradation of titanium was not shown in the present study, which could be due to experimental errors. For future research, an automated method for pouring MSCs on titanium disks is recommended to avoid this error.

Conclusion

UV-C irradiation caused considerable improvement in osteoblastic differentiation of human MSCs. However, cellular attachment and proliferation did not increase in the present study due to human and laboratory errors.

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